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Ruthenium(II) diimine complexes for luminescence-based oxygen sensors and impedance spectroscopy of nitrogen dioxide-sensitive polymeric membranes

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Ruthenium(II) diimine complexes for luminescence-based oxygen sensors and Impedance spectroscopy of nitrogen dioxide-sensitive polymeric membranes

A dissertation submitted to the SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH for the degree of Doctor of Natural Sciences

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Summary

In the first part of the present thesis, ruthenium(II) diimine complexes used in luminescence-based oxygen sensors are described. The work focusses on those physico-chemical aspects which determine the sensors’ long-term behavior. The second part deals with the combined impedance/absorption characterization of nitrogen dioxide-sensitive polymeric membranes.

One of the major degradation processes of luminescence-based oxygen sensors is dye photobleaching, but so far, it has been little studied. In this thesis, I describe a clear mechanism for the photobleaching of highly luminescent ruthenium(II) diimine dyes in the presence of oxygen. According to this mechanism, the first, rate-limiting step is a partial decoordination of a diimine ligand. In a second step, singlet oxygen oxidizes the diimine ligand to the N-oxide. The oxidized ligand recoordinates and forms a fairly stable complex which quenches luminescence. In a polymer, the reaction stops here. In solution, the photoproduct is preferentially degraded to volatile products.

This mechanism was deduced from photobleaching experiments in plasticized polystyrene and ethanol, which were monitored with absorption, luminescence and IR spectroscopy. Photoproduts in the polymer were investigated with Matrix-Assisted Laser Desorption/Ionization (MALDI) and in solution with Electrospray Ionization (ESI) mass spectroscopy. Initial quantum efficiencies for photobleaching in the plasticized polystyrene membrane were $3 \cdot 10^{-14}$ in absorbance and $1.4 \cdot 10^{-13}$ in luminescence. These rates were confirmed by an independent LED experiment and a literature search. Photobleaching was found to be 25 times faster in the polymer than in ethanol. Evidence from the literature, as well as the different kinetics in solution and polymer, are discussed in detail.

Furthermore, two lipophilic Ru(II) diimine complexes synthesized in our group were characterized: tris(4,7-bis(4-propylphenyl)-1,10-phenanthroline)-ruthenium(II) perchlorate (ETHT 3001) and tris(4,7-bis(4-octylphenyl)-1,10-phenanthroline)-ruthenium(II) perchlorate monohydrate (ETHT 3003). Most of their properties agree with the popular 4,7-diphenyl-1,10-phenanthroline analogue. However, ETHT 3003 shows a higher lipophilicity with a slight improvement in the luminescence quantum yield.

The luminescence quantum yield of ETHT 3003 was $0.56 \pm 0.05$ in deoxygenated and $0.03 \pm 0.01$ in aerated ethanol; in polystyrene it was $0.19 \pm 0.05$ in oxygen-free atmosphere. Note that the luminescence quantum yield of 4,7-diphenyl-1,10-phenanthroline analogue was determined in this study as $0.52 \pm 0.05$ in deoxygenated ethanol. The procedures for a proper characterization of sensor membranes, the
determination of the luminescence quantum yields in solution and in membranes, as well as the necessary refractive index corrections, are discussed in detail.

The sensor performance of ETH\textsuperscript{T} 3003 for measurements in gas and water is reported. The polymers used for the sensor membranes were polystyrene (PS), polystyrene plasticized with \textit{o-}nitrophenyl octyl ether (\textit{o-}NPOE) and \textit{o-}cyanophenyl octyl ether (\textit{o-}CPOE), poly(\textit{\alpha-}methylstyrrene), poly(\textit{p-}tert-butylstyrrene), poly(4-methoxystyrene), poly(2,4,6,-tribromostyrene) as well as poly(2,6-dimethyl-\textit{p-}phenylene oxide) and poly(bisphenol-A-carbonate) (PC). Membranes were characterized by their relative quantum yield, oxygen sensitivity in air and in oxygenated water in terms of Stern-Volmer constants, absorption and emission maxima wavelength. A set of membranes was subdued to tempering at 135°C with two subsequent steam sterilizations at 125°C. Even though oxygen sensitivity in some membranes changed only by a few percent, there is evidence for systematic ETHT 3003 precipitation within the polymer.

Finally, the electrochemistry of ETH\textsuperscript{T} 3001 was characterized by cyclic voltammetry and by spectroelectrochemical experiments in absorbance and luminescence.

In the second part, a nitrogen dioxide sensor for the gas phase with sensitivities in the ppb-range is discussed. It is based on an aquacyanocobalt(III)-cobyrrinate which reacts selectively with nitrite. In a coextraction mechanism, the Nile Blue derivative ETH 5418 is protonated, and its change in absorbance around 665 nm was monitored.

Transparent interdigitated microelectrodes allowed combined, simultaneous impedance/absorbance measurements with a time resolution down to 10 s. Blank poly(vinyl chloride) (PVC)/bis(2-ethylhexyl) sebacate (DOS) membranes and those containing one or both reactive compounds were characterized at different relative humidities (RH) and in the presence of nitrogen dioxide at ppm-levels. The obtained impedance spectra can be well described by an equivalent circuit with a resistor and a Warburg impedance in parallel with a capacity. This model holds for up to RH > 60%, but a more complicated model is needed for higher RH levels. Impedance spectroscopy was found to be very suitable for monitoring “invisible” membrane processes, such as changes in water content or disproportionation reactions. A transduction of a nitrogen dioxide response with impedance spectroscopy is feasible. Relative humidity-induced changes, however, are the norm.
Zusammenfassung

Der erste Teil dieser Arbeit beschäftigt sich mit Ruthenium(II)-diimin-Komplexen, die für optische Sauerstoffsensoren nach dem Prinzip der Lumineszenzlösung verwendet werden. Der Schwerpunkt dieses Teils der Arbeit liegt dabei auf den physikalisch-chemischen Eigenschaften dieser Komplexe, die die Langzeitstabilität der Sensoren bestimmen. Im zweiten Teil werden Stickoxid-sensitive Polymermembranen behandelt, die mittels kombinierter Absorptions- und Impedanzspektroskopie untersucht werden.


Desweiteren haben wir zwei neue Ru(II)-Diimin-Komplexe in unserer Gruppe synthetisiert und charakterisiert: Ruthenium(II)-tris(4,7-bis(4-propylphenyl)-1,10-phenanthroline)-perchlorat (ETH₇ 3001) und Ruthenium(II)-tris(4,7-bis(4-octylphenyl)-1,10-phenanthroline)-perchlorat Hydrat (ETH₇ 3003). Die meisten ihrer Eigenschaften stimmen mit denen des häufig verwendeten 4,7-Diphenyl-1,10-phenanthroline-Gegenstücks überein. Sie haben jedoch eine höher Lipophilie sowie eine leicht erhöhte Lumineszenzquantenausbeute.

Die Lumineszenzquantenausbeute von ETH₇ 3003 war 0.56 ± 0.05 in entgastem und 0.03 ± 0.01 in aerobem Ethanol; in Polystyrol war sie 0.19 ± 0.05 in der Abwe-
senheit von Sauerstoff. Die Quantenausbeute des 4,7-Diphenyl-1,10-phenanthrolin-Komplexes wurde in dieser Arbeit zu $0.52 \pm 0.05$ in entgastem Ethanol bestimmt. Das Vorgehen für die Charakterisierung der Sensormembranen, die Bestimmung der Lumineszenzquantenausbeuten in Lösung und in Membranen sowie die hierzu nötigen Brechungsindexkorrekturen werden im Detail besprochen.

Die Verwendung von ETH^T 3003 für Messungen in wässrigen und gasförmigen Medien wurde untersucht. Die für die Sensormembranen verwendeten Polymere waren Polystyrol (PS) mit und ohne die Weichmacher $o$-Nitrophenyl-octylether ($o$-NPOE) und $o$-Cyanophenyl-octylether ($o$-CPOE), Poly($\alpha$-methylstyrrol), Poly($p$-tert.-butylstyrrol), Poly(4-methoxystyrrol), Poly(2,4,6,-tribromstyrrol) sowie Poly(2,6-dimethyl-$p$-phenylenoxid) und Poly(bisphenol-A-carbonat) (PC). Die Membranen wurden durch ihre relative Quantenausbeute, Sauerstoffansprechverhalten (Stern-Volmer-Konstanten) sowie durch die Wellenlänge ihrer Absorptions- und Emissionsmaxima charakterisiert. Ein Teil der Membranen wurde bei 135°C konditioniert sowie zweimal bei 125°C dampfsterilisiert. Obwohl das Sauerstoffansprechverhalten bei einigen Membranen sich nur um ein paar Prozent änderte, gibt es Hinweise, dass ETH^T 3003 in allen Polymeren ausfiel.

Schliesslich wurde die Elektrochemie von ETH^T 3001 durch Cyclische Voltammetrie sowie durch spektroelektrochemische Experimente in Absorption und Lumineszenz untersucht.


Die Impedanzspektroskopie erwies sich als geeignete Methode, um “unsichtbare” Membranprozesse wie Aenderungen des Wassergehalts oder Disproportionierungsreaktionen zu beobachten. Eine Transduktion des Ansprechens auf Stickoxid ist mit der Impedanzspektroskopie möglich; Aenderungen der Luftfeuchte wirken sich aber wesentlich stärker aus.
# Table of contents

Acknowledgements ................................................. iii

Summary ........................................................... v

Zusammenfassung ................................................ vii

Table of contents ................................................ ix

1 Introduction ...................................................... 1

2 ETH\textsuperscript{T} 300x dyes ................................. 7

2.1 Introduction to ruthenium(II) diimine dyes ................. 7

2.2 Synthesis of ETH\textsuperscript{T} 3001 and 3003 ............... 11

2.2.1 Synthesis strategy ........................................ 11

2.2.2 Materials .................................................. 12

2.2.3 (2-Chloroethyl)-(4-octylphenyl)ketone (3a) ............... 12

2.2.4 4,7-Bis(4-octylphenyl)-1,10-phenanthroline (5a) ........ 13

2.2.5 ETH\textsuperscript{T} 3003 (6a) ................................ 13

2.2.6 (2-Chloroethyl)-(4-propylphenyl)ketone (3b) ............... 14

2.2.7 4,7-Bis(4-propylphenyl)-1,10-phenanthroline (5b) ........ 14

2.2.8 ETH\textsuperscript{T} 3001 (6b) ................................ 14

2.3 Chemical characterization of ETH\textsuperscript{T} 300x dyes .... 16

2.3.1 Crystal structure ........................................ 16

2.3.2 Solubility .................................................. 17

2.3.3 Lipophilicity ............................................. 17

2.3.4 IR spectra ................................................ 18

2.4 Spectroscopic properties of ETH\textsuperscript{T} 300x dyes .... 20

2.4.1 Absorption and luminescence spectra ...................... 20

2.4.2 Luminescence quantum yields ............................ 22

2.4.3 Luminescence lifetimes .................................. 24

2.5 Discussion .................................................... 24

2.6 Summary and Perspectives ................................. 26

2.7 References .................................................. 27

3 Determining luminescence quantum yields ................... 31

3.1 Introduction ................................................ 31

3.2 Characterizing oxygen optode membranes .................. 32

3.2.1 Measuring luminescence in membranes .................... 32

3.2.2 Spectrometer calibration .................................. 35

3.2.3 Refractive index corrections ............................. 41

3.2.4 Methodology of luminescence and absorbance measurements 46
3.3 Quantum yield determination in solution .......................... 51
3.4 Quantum yield determination in membranes ....................... 59
3.5 Summary and Perspectives .......................................................... 63
3.6 References ..................................................................................... 64

4 Luminescence-based oxygen optodes .......................................... 67
4.1 Introduction .................................................................................... 67
4.2 Theory of Stern-Volmer kinetics .................................................. 71
  4.2.1 Simple Stern-Volmer kinetics ..................................................... 71
  4.2.2 Distribution-based Stern-Volmer kinetics .................................... 75
4.3 Polymer materials for sterilization ............................................... 80
  4.3.1 Glass transition temperature .................................................... 80
  4.3.2 Suitable thermoplastic polymer membranes ............................. 81
  4.3.3 Polysiloxane membranes ......................................................... 83
4.4 Evaluation of suitable polymers .................................................. 85
  4.4.1 Plasticized polystyrene membranes ......................................... 85
  4.4.2 Thermoplastic polymer membranes ........................................ 89
4.5 Sterilization ..................................................................................... 99
  4.5.1 Dye leaching .............................................................................. 99
  4.5.2 Phosphate buffer sterilization of polystyrene membranes .......... 100
  4.5.3 Phosphate buffer sterilization of polysiloxane membranes ....... 101
  4.5.4 Steam sterilization of thermoplastic polymers ......................... 103
4.6 Discussion ...................................................................................... 109
4.7 Summary and Perspectives .......................................................... 112
4.8 References ..................................................................................... 114

5 Photobleaching of ruthenium(II) diimine dyes .............................. 119
5.1 Introduction ..................................................................................... 119
5.2 Methods and materials .............................................................. 122
  5.2.1 Bleaching light source ............................................................. 122
  5.2.2 Membrane bleaching set-up ..................................................... 123
  5.2.3 Solution bleaching set-up ......................................................... 124
  5.2.4 Bleaching monitored with ATR FT-IR ....................................... 125
  5.2.5 Mass spectrometry .................................................................. 125
5.3 Bleaching under measuring conditions ....................................... 126
  5.3.1 Bleaching in the luminescence spectrophotometer ..................... 126
  5.3.2 Bleaching with a blue LED ....................................................... 126
5.4 Bleaching of ETH\textsuperscript{T} 3001 in the membrane .................. 128
5.5 Bleaching of ETH\textsuperscript{T} 3001 in solution ............................. 131
5.6 Investigation of bleaching by ATR FT-IR spectroscopy .................. 135
### 5.7 Analysis of ETH\(^T\) 3001 photoproducts

- Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry
- Electrospray Ionization Mass Spectrometry

### 5.8 Bleaching mechanism

- Influence of the polymer matrix
- How does photobleaching work?

### 5.9 Summary and Perspectives

### 5.10 References

### 6 Electrochemical investigation of ETH\(^T\) 3001

- **Introduction**
- **Methods and materials**
  - Materials
  - Cyclic voltammetry
  - Spectroelectrochemical cell (Changchun)
  - Spectroelectrochemistry in luminescence
  - Spectroelectrochemistry in absorbance
- **Designing a spectroelectrochemical cell**
- **Cyclic voltammetry experiments**
- **Spectroelectrochemical experiments**
  - Cyclic voltammetry
  - Luminescence
  - Absorbance
  - Experiments in absorbance with the new cell
  - Discussion
- **Summary and Perspectives**
- **References**

### 7 Impedance measurements with NO\(_2\)-sensitive membranes

- **Introduction to NO\(_2\)-sensitive polymeric membranes**
- **Theory of impedance spectroscopy**
  - Introduction to impedance spectroscopy
  - Fitting an equivalent circuit to impedance data
- **Methods and materials**
  - Materials
  - Sandwich electrodes
  - Interdigitated microelectrodes
  - Measuring set-up
- **Measurements with sandwich electrodes**
7.5 Measurements with transparent interdigitated microelectrodes ........................................... 214
  7.5.1 Steady-state measurements with NO₂-sensitive membranes ........................................... 214
  7.5.2 Timedrive measurements with NO₂-sensitive membranes ........................................... 220
7.6 Discussion ................................................................. 225
  7.6.1 Experimental issues .......................................................... 225
  7.6.2 NO₂-sensitive polymeric membranes .......................................................... 227
7.7 Summary and Perspectives .................................................. 231
7.8 References ................................................................. 232
8 Appendix ........................................................................ 235
  8.1 Materials ...................................................................... 235
  8.2 Membrane preparation and characterization ........................................ 235
  8.3 Instruments .................................................................... 237
    8.3.1 Luminescence spectrophotometer PE LS-50B ........................................... 237
    8.3.2 Life time measurements .......................................................... 239
    8.3.3 Self-referenced two-photodiode set-up ........................................... 239
  8.4 Flow-through cells .......................................................... 243
    8.4.1 Flow-through cell design .......................................................... 243
    8.4.2 Flow-through cell performance .................................................. 244
  8.5 Oxygen measurement set-up .............................................. 246
    8.5.1 Media handling .............................................................. 246
    8.5.2 Measurements in oxygenated water ........................................... 250
    8.5.3 Experimental limitations .......................................................... 253
  8.6 Data treatment ............................................................... 254
  8.7 References ................................................................. 254
Glossary ........................................................................... 257
Chemical structures .......................................................... 260
Index ................................................................................ 263
Curriculum vitae .............................................................. 275
1 Introduction

Oxygen is vital in many industrial processes, in medicine and biotechnology. Therefore, oxygen concentration is one of the major parameters besides pH which is routinely determined with chemical sensors [1]. The de facto standard on-line determination of oxygen relies on the electrochemical reduction of oxygen in a Clark electrode. Clark electrodes have fairly good sensitivities, very good stability and reproducibility and are available for almost any special application. Other techniques, such as paramagnetic oxygen sensors, have until now played a minor role.

Like any analytical technique, Clark electrodes also have their disadvantages, including oxygen consumption, and flow sensitivity, relatively long response times and technical difficulties in miniaturizing them. For applications where these considerations are paramount, luminescence-based oxygen sensors have been proposed as alternative. These sensors rely on a luminescent indicator dye whose luminescence is effectively quenched by molecular oxygen; the decrease in luminescence can be directly related to the oxygen partial pressure. More details on the description of the quenching process can be found in section 4.2 on p. 71.

For sensor purposes, the luminescent dye is embedded in a thin membrane, usually a polymer. Such optical chemical sensors have frequently been called optodes [2], in analogy to “electrode”. What is striking about these oxygen sensors is their very high selectivity, which is unparalleled among most chemical sensors. Known interferences include Cl₂, SO₂ and H₂O₂, but they must be present at least in ‰-concentrations (v/v) [3]. One limitation of these sensors is that the quenching process is very temperature-dependent.

Apart from polycyclic aromatic hydrocarbons (PAHs), it is mostly transition metal complexes that have been used for luminescence-based oxygen sensing [4, 5]. These include ruthenium(II), osmium(II), palladium(II), platinum(II) or more exotic, iridium(III) and copper(I) complexes. Ruthenium(II) diimine complexes are, however, unrivalled favorites due to their strong absorption in the visible range, their high quantum yield, long excited-state lifetimes and large apparent Stokes shift (wavelength difference between excitation and emission wavelength). A detailed discussion of the photophysics of Ru(II) diimine dyes is given in section 2.1 on p. 7. A wide range of ruthenium(II) diimine dyes have been synthesized and characterized in the literature [6-9].

Much of the literature on luminescence-based oxygen sensors deals with secondary improvements which could have been demonstrated for any optical sensor, but are exemplified using oxygen sensors because they are widely known. Examples are three papers using radioluminescent light sources for excitation of such sensors in the same issue of Analytical Chemistry [10-12]. Other efforts were directed towards
extending the analytes which can be measured with ruthenium(II) diimine dyes. They have been used, for instance, in energy transfer schemes to sense pH directly [13], or to measure pH, CO₂, K⁺, Na⁺, Ca²⁺, Cl⁻, NH₄⁺, urea and glucose indirectly via energy-transfer [14].

The field of luminescence-based oxygen sensors must be considered interdisciplinary since improvements can come from fields as varied as inorganic chemistry, organic synthesis, polymer chemistry, modelling, optics or electronics. Since so much research has been done in this area, both in the past or currently, it was necessary to focus on few selected topics. Our research group is particularly interested in the chemical aspects of chemical sensors, and especially in the sensors’ long-term performance. For oxygen optodes, one topic of interest is the photobleaching of ruthenium(II) diimine dyes, which had previously only been investigated empirically. For many applications, photobleaching limits the lifetime of an oxygen optode.

Photochemical stability is also of upmost importance for other ruthenium(II) diimine dye applications, such as in solar energy conversion or fluorescent solar collectors [15]. Their use in fluorescent solar collectors was eventually limited by the lightfastness of the dyes. In the 1970s and 1980s, intensive efforts were made to find a way using photocatalysis in decomposing water into hydrogen and oxygen. Even though the whole reaction is thermodynamically feasible, reducing water to molecular hydrogen and hydroxide remains a dream [6].

Another topic considered is the sterilizability of oxygen optodes, which is a requirement for many commercial applications. To date, this has hardly been investigated, and so far no clear-cut strategy has been developed to make oxygen optodes sterilizable.

Luminescence-based oxygen sensors make up only a small fraction of the whole fascinating world of chemical sensors. For many interesting analytes, there is still no sensor with which they can be determined or, if there is, its development is in its infancy. An example of one that has been recently developed in our group is a nitrogen dioxide optode [16, 17]. It is based on the reactivity of nitrite with an aquacyanocobalt(III)cobyrinate. Its sensitivity in the ppb-range makes it very attractive for use in early fire detection and it is currently being further pursued together with industrial partners. Very little, however, is known about the mechanism of the nitrogen dioxide response. A promising method used in the research described here to find out more about optodes in the gas phase is combined absorption/impedance spectroscopy.

We chose to use two lipophilic ruthenium(II) diimine dyes, which are an evolutionary improvement of the commonly used tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) complex ([(Ru(dpp)₃]²⁺). The new dyes are ETH T 3001 (tris(4,7-bis(4-propylphenyl)-1,10-phenanthroline) ruthenium(II) perchlorate) and
**ETH** 3003 (tris(4,7-bis(4-octylphenyl)-1,10-phenanthroline) ruthenium(II) perchlorate monohydrate). The intention was two-fold: to improve the lipophilicity of the dyes whilst at the same time retaining or even improving their photophysical properties. Both goals were achieved. More lipophilic dyes are less prone to dye leaching into the sample media and help using new polymer combinations. Their synthesis, chemical characterization and spectroscopic properties, such as spectra, luminescence quantum yields and lifetimes, are given in the chapter 2 ETH** 300x dyes on p. 7.

Their electrochemical behavior in the absence and presence of oxygen is thoroughly explored in chapter 6 Electrochemical investigation of ETH** 3001 on p. 161. Cyclic voltammetry and spectroelectrochemical methods were used in this research. Worthy of note are especially the characterization of a new spectroelectrochemical cell and the use of spectroelectrochemical experiments in luminescence, both of which have been rarely described in the literature.

Much emphasis has been put in this thesis on the proper characterization of the spectrometer, oxygen optode membranes and the proper determination of quantum yields. These are described in chapter 3 Determining luminescence quantum yields on p. 31. Additionally, procedures for refractive index corrections and the determination of quantum yields in polymeric membranes are discussed.

Chapter 4 Luminescence-based oxygen optodes on p. 67 describes the application of ETH** 300x dyes in luminescence-based oxygen sensors. Thermoplastic polymers were screened for suitable polymer matrices for sterilizable oxygen optodes. Two subsequent steam sterilizations were carried out with a prior tempering at 135°C. The performance of ETH** 3003 in polystyrene (PS) and its derivatives with a high glass transition temperature poly(α-methylstyrene), poly(p-tert-butylstyrrene), poly(4-methoxystyrene), poly(2,4,6-tribromostyrene), as well as the two polymers poly(2,6-dimethyl-p-phenylene oxide) and poly(bisphenol-A-carbonate), were evaluated. Quantum yields, quenching characteristics, absorption and emission maxima wavelengths were determined and compared. Furthermore, the characteristics of [Ru(dpp)$_3$]$^{2+}$, ETH** 3001 and ETH** 3003 in PS were compared and the influence of the plasticizers o-nitrophenyl octyl ether (o-NPOE) and o-cyanophenyl octyl ether (o-CPOE) were studied.

In chapter 5 Photobleaching of ruthenium(II) diimine dyes on p. 119, the photodecomposition of ETH** 3001 is elucidated in great detail with data from luminescence and absorption spectroscopy, as well as with data from on-line Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) spectroscopy monitoring, Matrix-Assisted Laser Desorption/Ionization (MALDI) and Electrospray Ionization (ESI) mass spectrometry studies. Based on the experimental evidence and the literature, the first mechanism for photobleaching of highly luminescent ruthenium(II) diimine complexes in the presence of oxygen is proposed.
The characterization of the nitrogen dioxide optodes on interdigitated microelectrodes by simultaneous impedance/absorption spectroscopy is treated in *Impedance measurements with NO2-sensitive membranes* on p. 189. The study focussed on whether a nitrogen dioxide response could be monitored by impedance changes alone. A further concern was to understand the underlying mechanism better, which was suggested to be based on a disproportionation mechanism.

The chapters in this thesis have been structured as consolidated as possible because most readers will probably be only interested in some aspects of this widely ranging work. To help the reader, summaries and outlooks, as well as literature references, are given at the end of each chapter. Experimental details which are relevant for several chapters are given in the Appendix. These include, in particular, aspects of the membrane preparation (8.2 on p. 235), the flow-through cells employed (8.4 on p. 243) and the set-up used for the oxygen measurements (8.5 on p. 246).

The experimental details for the chapters 5, 6 and 7 are contained within each of these chapters.

Please note that there is an index (p. 263) and a glossary (p. 257) at the back of the thesis. The glossary includes the chemical structures of frequently mentioned components (p. 260).


2 ETH$^T$ 300x dyes

2.1 Introduction to ruthenium(II) diimine dyes

The photophysics of ruthenium(II) diimine complexes and, more generally, those of platinum metal complexes has been well described in several review articles [1, 2]. Ruthenium(II) diimine complexes belong to the d$^6$ (i.e. 6 electrons in d orbitals) transition metal complexes with a strong crystal (or ligand) field. The octahedral crystal field generated by the 6 imine ligands splits the 5 d orbitals by an energy amount $\Delta$ into a set of 3 degenerate low-lying t and 2 high-lying e orbitals. Ligands, such as diimine, create a strong crystal field. Therefore, it is energetically favorable to distribute electrons into the low-lying orbitals in pairs rather than distribute them unpaired over all orbitals (Hund’s rules). For a d$^6$ ruthenium(II) diimine complex all low-lying orbitals are occupied. Since all 6 electrons are paired, the ground state must be a singlet.

Apart from the metal d-orbitals, the $\pi$ bonding and $\pi$ antibonding ($\pi^*$) orbitals of the ligands are also of spectroscopic importance.

In Fig. 2.1 a simplified state diagram of a symmetric ruthenium(II) diimine complex is shown. The state classifications are determined by the original and the final orbital.

![Simplified state diagram for [RuL$_3$]$^{2+}$ complexes](image)

The different rate constants are: $k_f$ for fluorescence, $k_{ic}$ internal conversion, $k_{isc}$ intersystem crossing, $k_r$ radiative decay (phosphorescence or more general, luminescence), $k_{nr}$ non-radiative decay, according to Jablonski diagram terminology. Energies are not to scale. States are more degenerated than shown. $\Delta E$ denotes the energy difference between $^3$MLCT and $^3$MC, which is important for the photochemistry.
There are metal-centered (MC) d-d states, ligand-centered (LC) $\pi$-$\pi^*$ states and charge-transfer states (MLCT for Metal Ligand Charge Transfer). The name charge transfer is derived from the fact that an electron from a metal-centered $\pi$ orbital is promoted to a ligand-centered $\pi^*$ orbital, which corresponds to a formal oxidation of the metal center and a reduction of the ligand. The intense absorption band in the visible range at 460 nm is one such MLCT band, see the absorption spectra in Fig. 2.8 on p. 20.

Ligand-centered transitions are spectroscopically very similar to those of the free ligand. High-energy absorptions around 300 nm are assigned to these LC transitions. As always, the triplet states are lower than the corresponding singlet states. The MC state is formally forbidden, which explains why there is no corresponding band in the absorption spectra. MC states have long radiative lifetimes, which makes them very prone to environmental quenching. Furthermore, their anti-bonding character makes them very reactive in reactions, such as substitution or racemization.

In Fig. 2.1, we can follow those individual processes which happen after absorption of a photon. Irradiation into the strongly absorbing MLCT band results in one of the $^1$MLCT states. There is no direct fluorescence when reverting from this state to the ground state since the intersystem crossing to the lowest excited state, the $^3$MLCT state, occurs far too fast ($k_{isc} > k_f$). Experimental evidence suggests that $\Phi_{isc} \approx 1$. This process is favored in inorganic complexes because of the increasing spin-orbit coupling of the heavier transition metals. There is also no direct radiationless relaxation to the ground state (internal conversion) because the energy gap between the $^1$MLCT and the ground state ($k_{ic} << k_{isc}$) is very large (energy gap law).

From the $^3$MLCT to the ground state we find there is formally forbidden phosphorescence ($k_{r}$ for radiative) and non-radiative decay ($k_{nr}$). Due to the partially allowed character of this transition from the long-lived triplet state, the more general term luminescence (which compromises both phosphorescence and fluorescence) is used.

A very important quantity is the luminescence quantum yield, which is the ratio of emitted photons per absorbed photons. For the ruthenium(II) dimine complexes, this is equal to the contribution of both radiative and non-radiative decays

$$\Phi_L = \frac{k_r}{k_{nr} + k_r}.$$  \hspace{1cm} (2.1)

It is important that any $^3$MC state is well above the emitting level; otherwise it can be thermally excited from the $^1$MLCT state and will give rise to fast excited-state decay and photochemical instability. This is one of the main reasons for the pro-
nounced temperature dependence of the luminescence quantum yield because \( k_{nr} \) also includes deactivation via the \(^3\text{MC}\) state across the energy barrier \( \Delta E \).

Most other transition metal diimine complexes have a much lower luminescence quantum yield than the corresponding ruthenium(II) complexes. This can be easily rationalized by their lowest triplet-state ordering. For complexes, such as \( \text{FeL}_3^{2+} \), the \(^3\text{MC}\) state is lowest, which effectively curbs luminescence. Other complexes, such as \( \text{RhL}_3^{2+} \), have either the same problem or a \(^3\text{LC}\) state as the lowest triplet state. The long lifetime of the pure phosphorescence from the \(^3\text{LC}\) state results in a measurable luminescence only at very low temperatures; at room temperature radiationless decay prevails.

The energy levels of the MLCT, LC and MC states can be adjusted to some extent by suitably combining the central metal ion and the ligands, which is frequently referred to as tuning.

One of the important additional quenching processes of the \(^3\text{MLCT}\) state occurs with molecular oxygen. The \(^3\text{MLCT}\) state of a ruthenium(II) diimine complex is both a stronger oxidizing agent and a stronger reducing agent than the original complex. This explains why both energy and electron-transfer quenching takes place. Many examples of oxidative and - to a lesser extent - of reductive quenching have been reported [3].

Exhaustive experiments have been made with \([\text{Ru(bpy)}_3]^{2+}\) (bpy = 2,2'-bipyridine) in solution, which showed that approximately equal amounts of energy and electron transfer to oxygen occurred [4]. The corresponding products are singlet oxygen and a superoxide radical anion, but the latter quickly recombines with the oxidized \([\text{Ru(bpy)}_3]^{3+}\).

Countless luminescent transition metal complexes have been reported [3], as have increasingly more complicated polynuclear compounds, among them many ruthenium(II) complexes [5]. Some of them can be used as redox or photo switches [6]. The Thomson group have synthesized and systematically investigated almost 50 Ru(II) diimine complexes [7-11]. Despite this potentially large selection, there are still only a few, namely, \([\text{Ru(bpy)}_3]^{2+}\), \([\text{Ru(phen)}_3]^{2+}\) (phen = 1,10-phenanthroline) and especially \([\text{Ru(dpp)}_3]^{2+}\) (dpp = 4,7-diphenyl-1,10-phenanthroline), which are almost unrivalled in luminescence-based oxygen sensing. The photophysics of these ruthenium(II) diimine complexes has been thoroughly investigated, e.g. [11-13].

We recommend a lipophilization to provide a better incorporation of \([\text{Ru(dpp)}_3]^{2+}\) into polymers. This generally improves the solubility and retention in such very apolar polymers as those used for chemical sensing. Furthermore, there was evidence that this would improve the photophysical parameters based on the electron-donating character of the alkyl groups (+I effect) [7, 9, 10]. The structures of the
new, lipophilized dyes ETH\textsuperscript{T} 3001 and ETH\textsuperscript{T} 3003 are given in Fig. 2.2. The dyes follow the nomenclature ETH\textsuperscript{T}, with the superscript “T” for Technopark, the home of the Centre for Chemical Sensors (CCS). ETH\textsuperscript{T} 3001 is tris(4,7-bis(4-propylphenyl)-1,10-phenanthroline)-ruthenium(II) perchlorate and ETH\textsuperscript{T} 3003 is tris(4,7-bis(4-octylphenyl)-1,10-phenanthroline)-ruthenium(II) perchlorate monohydrate. The lipophilization corresponds to a formal alkylation at the 4’-position of the phenyl groups of dpp.

The three ruthenium(II) diimine dyes, which have been used in this work, are given in Table 2.1.

<table>
<thead>
<tr>
<th>Name</th>
<th>Composition</th>
<th>$M_r$</th>
<th>Synthesis by</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="ClO$_4$">Ru(dpp)$_3$</a>$_2$</td>
<td><a href="ClO$_4$">Ru(II)(dpp)$_3$</a>$_2$</td>
<td>1297.20</td>
<td>Daniel Freiner</td>
</tr>
<tr>
<td>ETH\textsuperscript{T} 3001</td>
<td><a href="ClO$_4$">Ru(II)(3-dpp)$_3$</a>$_2$</td>
<td>1549.66</td>
<td>Stefan Rásonyi</td>
</tr>
<tr>
<td>ETH\textsuperscript{T} 3003</td>
<td><a href="ClO$_4$">Ru(II)(8-dpp)$_3$</a>$_2$·H$_2$O</td>
<td>1988.49</td>
<td>Luzi Jenny</td>
</tr>
</tbody>
</table>

Table 2.1 Synthesized ruthenium(II) dyes

[Ru(II)(dpp)$_3$](ClO$_4$)$_2$ could possibly be a dihydrate with $M_r$ 1333.23. 3-dpp is 4,7-bis(4-propylphenyl)-1,10-phenanthroline and 8-dpp is 4,7-bis(4-octylphenyl)-1,10-phenanthroline.

In section 2.2 the synthesis of the ETH\textsuperscript{T} 300x dyes is reported. Standard chemical characteristics, such as solubility, lipophilicity as well as the IR spectra and a crystal structure of [Ru(dpp)$_3$](ClO$_4$)$_2$, are given in 2.3. In section 2.4 on p. 20, the absorption and emission spectra, and the luminescence quantum yields and lifetimes are discussed compared to other ruthenium(II) diimine dyes in section 2.5 on p. 24.
For details about the electrochemical characterization of ETH\textsuperscript{T} 3001 see 6 Electrochemical investigation of ETH\textsuperscript{T} 3001 on p. 161, for its photochemistry see 5 Photobleaching of ruthenium(II) diimine dyes on p. 119 and for the discussion of the use of ETH\textsuperscript{T} 3003 in luminescence-based oxygen sensors see 4 Luminescence-based oxygen optodes on p. 67.

The determination of luminescence quantum yields of the ruthenium(II) diimine dyes is described in great detail in chapter 3 on p. 31.

### 2.2 Synthesis of ETH\textsuperscript{T} 3001 and 3003

#### 2.2.1 Synthesis strategy

The three-step synthesis of the new ruthenium(II) diimine dyes ETH\textsuperscript{T} 300x is sketched in Fig. 2.3. The procedure is strictly valid for ETH\textsuperscript{T} 3003 only (R = \textit{n}-octyl, denoted as a-products). ETH\textsuperscript{T} 3001 with R = \textit{n}-propyl (denoted as b-products) was obtained with an analogue reaction sequence.

---

**Fig. 2.3 Synthesis of the ETH\textsuperscript{T} 300x dyes**

For the ETH\textsuperscript{T} 3003 (a-products) R = \textit{n}-octyl, for ETH\textsuperscript{T} 3001 (b-products) R = \textit{n}-propyl.
The first step is a *Friedel-Crafts* reaction to obtain (2-chloroethyl)-(4-octylphenyl)ketone (3a) from octylbenzene (1a) and 3-chloropropionylchloride (2) similar to the procedure in [14]. The ligand 4,7-bis(4-octylphenyl)-1,10-phenanthroline (5a) was synthesized according to *Skraup* [15] by a reaction of (3a) with 1,2-phenylenediamine (4) similar to the method in [16, 17]. The ruthenium complex was prepared with standard procedures [3], the challenging part being its purification. Eventually a very pure product resulted as was deduced from elementary analysis and $^1$H and $^{13}$C NMR spectra. $^{13}$C NMR data and assignments for phenanthroline ligands and the respective Ru(II) complexes have been published [18].

The propyl-analogue, ETH$^T$ 3001 (6b), was synthesized accordingly with (2-chloroethyl)-(4-propylphenyl)ketone (3b) and 4,7-bis(4-propylphenyl)-1,10-phenanthroline (5b). The synthesis for ETH$^T$ 3001 is only given here because the dye has been used extensively in this work; the synthesis is not optimized, the procedures for ETH$^T$ 3003 should be used instead. No literature procedures exist for (3a), (3b), (5a), (5b), (6a) and (6b).

### 2.2.2 Materials

The following chemicals were used: sodium $m$-nitrobenzenesulfonate (Fluka, pract.) ruthenium trichloride monohydrate, 3-chloropropionylchloride (both from Fluka, purum), $n$-propylbenzene, $n$-octylbenzene, aluminium trichloride, anhydrous zinc chloride, 1,2-phenylenediamine, carbon disulfide, ethanol and hexamethyolphosphoramide (all from Fluka, puriss.).

### 2.2.3 (2-Chloroethyl)-(4-octylphenyl)ketone (3a)

To a well stirred solution of 29.80 g (156.6 mmol, 34.8 ml, $M_r$ 190.32) $n$-octylbenzene (1a) in 90 g (71.3 ml) of carbon disulfide 21.9 g (164.1 mmol, $M_r$ 133.34) of aluminium trichloride were added; the resulting suspension was put under argon. During 20' a solution of 22.62 g (178.8 mmol, $M_r$ 126.97) 3-chloropropionylchloride (2) in 5 ml carbon disulfide was added dropwise. The resulting yellow solution was refluxed during 70' at 75°C. The mixture was cooled and poured onto 150 g ice in 150 ml concentrated hydrochloric acid. The organic phases from three subsequent extractions with ethylacetate were joined and washed twice with 200 ml 2 M NaOH each and once with saturated brine, dried over MgSO$_4$ and the solvent evaporated under vacuum. Upon standing the residue quickly solidified to afford 40.81 g (0.16 mol, 100%) of (2-chloroethyl)-(4-octylphenyl)ketone (3) as a pale yellow powder, $R_f$ 0.84 (hexane/ethylacetate, 6:4; developed with sulfuric acid/vanillin, yielding olive-green spots), m.p. 51-52°C (Found: C, 72.65; H, 8.91. $C_{17}H_{25}OCl$ with $M_r$ 280.83 requires C, 72.71; H, 8.97%).
2.2.4 4,7-Bis(4-octylphenyl)-1,10-phenanthroline (5a)

To a nearly white suspension of 1.40 g (13.0 mmol, \( M_r 108.14 \)) 1,2-phenylenediamine (4), 7.42 g (33.0 mmol, \( M_r 225.15 \)) sodium \( m \)-nitrobenzenesulfonate and 492 mg (3.6 mmol, \( M_r 136.28 \)) anhydrous zinc chloride in 50 ml ethanol at 60°C, half of a solution of 6.68 g (23.8 mmol, \( M_r 280.83 \)) (3a) in 30 ml EtOH was added, resulting in a yellow color. Through a second funnel 9.8 ml of concentrated hydrochloric acid were added to the reaction mixture which then turned orange-red. After adding the other half of (3a) the solution was refluxed for 12 h. After 24 h of further stirring at room temperature the solvent was removed \textit{in vacuo} and the residue suspended in a mixture of 100 ml concentrated aqueous ammonia and 75 ml dichloromethane. The organic phase was separated and the remaining aqueous phase extracted a second time with dichloromethane. The pooled organic phases were washed with saturated brine and concentrated at reduced pressure. An orange-brown oil resulted which was chromatographed on silica gel (gradient of hexane/pyrrolidine, 9:1 to hexane/ethylacetate/pyrrolidine, 6:3:1). After fraction collection and five-fold recrystallization from pure hexane 0.67 g (1.2 mmol, 5% with respect to (3a)) of 4,7-bis(4-octylphenyl)-1,10-phenanthroline (5a) were afforded as a beige, amorphous mass, \( R_f 0.10 \) (hexane/pyrrolidine, 9:1 on UV\textsubscript{254}), m.p. 71-72°C (Found: C, 86.26; H, 8.63; N, 5.10. \( C_{40}H_{48}N_2 \) with \( M_r 556.83 \) requires C, 86.28; H, 8.69; N, 5.03%).

\( ^1H \) NMR (CDCl\textsubscript{3}, 200 MHz): 9.22 (d, \( J = 5 \) Hz, 2 H), 7.90 (s, 2 H), 7.58 (d, \( J = 5 \) Hz, 2 H), 7.45 (d, \( J = 8 \) Hz, 4 H), 7.34 (d, \( J = 8 \) Hz, 4 H), 2.72 (t, \( J = 7.7 \) Hz, 4 H), 1.78-1.60 (m, 4 H), 1.44-1.24 (m, 20 H), 0.98 (t, \( J = 6.6 \) Hz, 6 H).

\( ^13C \) NMR (CDCl\textsubscript{3}, 50 MHz): 149.9 (d), 148.6 (s), 147.1 (s), 143.6 (s), 135.3 (s), 129.7 (d), 128.8 (d), 126.6 (s), 124.1 (d), 123.6 (d), 35.7 (t), 31.9 (t), 31.4 (t), 29.4 (t), 29.3 (t), 29.2 (t), 22.6 (t), 14.1 (q).

2.2.5 ETH\textsuperscript{T} 3003 (6a)

A suspension of 429 mg (0.77 mmol, \( M_r 556.83 \)) (5a) and 88 mg (0.39 mmol, \( M_r 225.44 \)) ruthenium trichloride monohydrate in 6 g (6.3 ml) dimethylformamide was stirred overnight at 120-150 °C after which a intensely orange-colored solution resulted. After the mixture was allowed to cool to ambient temperature, it was dissolved in 50 ml acetone and 7 ml of 70% perchloric acid were added. After 12 h of standing the solvent was evaporated, leaving the crude product as a dark, viscous oil. Three subsequent chromatographic separations on aluminium oxide (neutral, activity III, with at least a 100-fold excess of aluminium oxide with respect to the raw product, eluant: ethylacetate/hexane/triethylamine, 9:1:1, UV\textsubscript{254}) afforded 116 mg (0.18 mmol, 21-25% with respect to (5a)) of ETH\textsuperscript{T} 3003 (6a) as an orange powder, \( R_f 0.15-0.3 \) (hexane/ethylacetate/triethylamine, 2:9:1.7 at UV\textsubscript{366} smears,
orange luminescence) (Found: C, 72.47; H, 7.34; N, 4.18; O, 7.29. C_{120}H_{144}N_6O_8RuCl_2 with M_r 1970.47 requires C, 72.48; H, 7.40; N, 4.23; O, 7.24%).

\(^1\)H NMR (CDCl\(_3\), 300 MHz): 8.47 (d, J = 5.6 Hz, 6 H); 8.24 (s, 6 H); 7.82 (d, J = 5.6 Hz, 6 H); 7.55 (d, J = 8.1 Hz, 12 H); 7.35 (d, J = 8.1 Hz, 12 H); 2.69 (t, J = 7.7 Hz, 12 H); 1.67 (m, 12 H); 1.42 - 1.20 (m, 60 H); 0.88 (t, J = 7 Hz, 18 H).

\(^{13}\)C NMR (CDCl\(_3\), 75 MHz): 153.2 (d); 149.1 (s); 148.5 (s); 145.0 (s); 133.1 (s); 130.2 (d); 129.3 (d); 128.8 (s); 127.2 (d); 126.1 (d); 35.8 (t); 31.9 (t); 31.4 (t); 29.5 (t); 29.4 (t); 29.3 (t); 22.7 (t); 14.1 (q).

Please note that the reaction mixture assumes a deep orange color only with pure educt (5). With educt (5) of insufficient purity a violet color resulted from a by-product (2-3%, R\(_f\) = 0.57, hexane/ethylacetate/triethylamine, 2:9:2). \(^1\)H-NMR and \(^{13}\)C-NMR spectra suggest that (5) had been oxidized to give a biketone at either positions 4 and 5 or 2 and 9.

### 2.2.6 (2-Chloroethyl)-(4-propylphenyl)ketone (3b)

(2-Chloroethyl)-(4-propylphenyl)ketone (3b) was synthesized by the above procedure for (3a) using the following quantities: 20 g (166 mmol, M\(_r\) 120.20) of \(n\)-propylbenzene, 20.1 g (158 mmol) 3-chloropropionylchloride, 22.2 g (166 mmol) aluminium trichloride and 50 ml carbon disulfide. Recrystallization from hexane afforded 26 g (123 mmol, 78%) of the product (3b), m.p. 51-52°C (C\(_{12}\)H\(_{15}\)OCl with M\(_r\) 210.71).

### 2.2.7 4,7-Bis(4-propylphenyl)-1,10-phenanthroline (5b)

4,7-Bis(4-propylphenyl)-1,10-phenanthroline (5b) had been synthesized by a similar procedure as for (5a). Rather than adding (3b) in two aliquots it was added at once; the crude product had been suspended in freshly distilled diethylether and 100 ml of 5 M sodium hydroxide solution. The product had been chromatographed on aluminium oxide (neutral, activity III, eluant ethylacetate) rather than silica gel. The following quantities had been used: 4.8 g (45 mmol) 1,2-phenylenediamine in 228 ml ethanol, 1.88 g (13.2 mmol) anhydrous zinc chloride, 25.3 g (112 mmol) sodium \(m\)-nitrobenzenesulfonate, 36 ml concentrated hydrochloric acid, 18 g (85.4 mmol) (3b) dissolved in 150 ml ethanol. Purification afforded 2.5 g (6 mmol, 14%) of product (5b) (C\(_{30}\)H\(_{28}\)N\(_2\) with M\(_r\) 416.56).

### 2.2.8 ETH\(^T\) 3001 (6b)

ETH\(^T\) 3001 (6b) had been synthesized by a similar procedure as for (6a). Instead of dimethylformamide a mixture of ethyleneglycol, water and hexamethylphosphora-
mide had been used for the initial suspension. The following quantities had been used: 0.49 g rutheniumtrichloride monohydrate in 14 ml ethyleneglycol containing 10% water, 2.34 g (5.6 mmol) (5b) in 6 ml ethylene glycol with 10% water and 6 ml hexamethylphosphoramide. The reaction solution was filtered and the precipitate was filtered with water and hexane. The crude product was recrystallized from acetone/water (4:1), washed with acetone/water (1:1) and hexane and dried \textit{in vacuo} which afforded 2.2 g (1.5 mmol) of a reddish orange powder (6b) \((\text{C}_{120}\text{H}_{144}\text{N}_6\text{O}_8\text{RuCl}_2\text{ with }M_r\text{ 1549.67})\).

Please note that its purity had only been checked by thin-layer chromatography (dichloromethane/acetone, 8:2 on aluminium oxide), no NMR or elementary analysis data is available.

According to the thin-layer chromatogram ETH\textsuperscript{T} 3001 \((R_f = 0.9)\) must have one or probably two absorbing \((R_f = 0.31\text{ and 0})\), non-fluorescent impurities, see Fig. 2.4. Two-dimensional thin layer chromatography with an identical eluant proved that the brown spot at the starting point is identical to the one at \(R_f = 0.31\). ESI-MS spectra (see 5.7.2 Electrospray Ionization Mass Spectrometry on p. 142) suggest they have a mass which deviates by +14 or -28 with the expected double charge. This has also been found by MALDI mass spectra (see 5.7.1 Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry on p. 140).

A plausible explanation for the impurity would be a small amount of oxidized ligand with a ketone function (M +14) as detected as by-product for (6a), see above.
2.3 Chemical characterization of ETH\textsuperscript{T} 300x dyes

2.3.1 Crystal structure

The crystal structure [Ru(dpp)\textsubscript{3}](ClO\textsubscript{4})\textsubscript{2}\cdot2H\textsubscript{2}O had been determined by Volker Gramlich of the Laboratorium für Kristallographie at ETH Zürich on a SYNTEX P21 diffractometer. The structure determined from a 0.7 x 0.7 x 0.7 mm purple needle crystal is shown in Fig. 2.5.

[Ru(dpp)\textsubscript{3}](ClO\textsubscript{4})\textsubscript{2}\cdot2H\textsubscript{2}O forms an orthorombic system of the space group \textit{Pbca} with the dimensions \(a = 13.395(5)\) Å, \(b = 29.526(9)\) Å and \(c = 30.483(7)\) Å, \(V = 12056(6)\) Å\(^3\) with \(Z = 8\). Structure has been determined from 2645 reflections (\(F > 2.0\ \sigma(F)\)), \(R = 4.83\%\). The Ru-N bond lengths are 2.06 ± 0.02 Å.

The structure of ETH\textsuperscript{T} 3001 in Fig. 2.4 has been force-field optimized \textit{in vacuo} with the software package Sybyl 6.3 (TRIPOS Inc., St. Louis, USA). Since no ruthenium
parameter set was available the phenanthroline moieties were fixed in space based on the crystal structure of \([\text{Ru(dpp)}_3]\)(\text{ClO}_4)_2\) in Fig. 2.5.

![Fig. 2.6 Calculated structure of ETH\textsuperscript{T} 3001](image)

**Fig. 2.6 Calculated structure of ETH\textsuperscript{T} 3001**
Calculated by the software package Sybyl based on the crystal structure of \([\text{Ru(II)(dpp)}_3]\)(\text{ClO}_4)_2\):
ETH\textsuperscript{T} 3001 is the propyl-derivative of \([\text{Ru(II)(dpp)}_3]\)(\text{ClO}_4)_2\). Perchlorate ions are shown at their original position in the crystal structure.

### 2.3.2 Solubility

ETH\textsuperscript{T} 3001 and 3003 are insoluble in water. The solubility of ETH\textsuperscript{T} 3001 in ethanol at room temperature had been determined as 0.48 mM based on the concentration determined by the absorbance of a saturated solution. \([\text{Ru(bpy)}_3]\)(\text{ClO}_4)_2\) for instance has a solubility of 1.87 mM in water [3]. It is important to note that the synthesis always yields \textit{rac}-ruthenium(II) diimine complexes. Their solubilities are a single order of magnitude lower than the pure \textit{fac}- or \textit{mer}-isomers. Solubilities can be tuned to some extent by the selection of the counterion.

### 2.3.3 Lipophilicity

A standard method to determine the lipophilicity of a particular compound is by comparison with the retention of standard substances by Reversed Phase Thin Layer Chromatography (TLC). A detailed description can be found in [19]. The method of determination of the lipophilicity described therein with EtOH/water mixtures as eluant on RP-18 WF\textsubscript{245} (Merck, Darmstadt) TLC plates failed. The \([\text{Ru(dpp)}_3]\)(\text{ClO}_4)_2, ETH\textsuperscript{T} 3001 and 3003 essentially stuck on the spot where they were deposited and trailed or fronted. Obviously these substances obey an ion pair distribution chromatography rather than a normal “phase” distribution chromatography which precludes the use of the method.
The non-solubility of the ETH\textsuperscript{T} 300x dyes in water prevents the determination of solubilities in the water/1-octanol system.

A suitable eluant which is used by Prof. Peter Belser’s group at the University of Fribourg, Switzerland, uses a strong salt content for a good separation of a wide variety of ruthenium(II) diimine complexes on silica gel. 1 g potassium nitrate is dissolved in 10 ml water, then 40 ml acetonitrile and subsequently 10 ml ethanol are added. The preparation order is very important because otherwise the potassium nitrate will precipitate. Staining of ligands is done with a Fe\textsuperscript{2+}-solution, which form intensively red-colored spots with most diimine-ligands. The separation gave $R_f = 0.74$ for [Ru(dpp)\textsubscript{3}](ClO\textsubscript{4})\textsubscript{2}, 0.76 for ETH\textsuperscript{T} 3001 and 0.78 for ETH\textsuperscript{T} 3003 with barely remarkable violettish spots at 0.79 and 0.81 respectively for the first two dyes. All the spots showed the usual orange luminescence under the 366 nm UV excitation, though ETH\textsuperscript{T} 3001’s luminescence was remarkably less intense. All three dyes initially had an intense fronting peak which gradually disappeared except for ETH\textsuperscript{T} 3003, where a small remnant had a retention of $R_f = 0.93$ together with a long tail down to the baseline where a considerable portion of ETH\textsuperscript{T} 3003 stuck.

The ligand 8-dpp could not be separated with this mixture, i.e. it tailed from the baseline up to $R_f = 0.68$. It could be clearly identified by its blueish luminescence or with the Fe\textsuperscript{2+}-staining, giving a bordeaux color.

### 2.3.4 IR spectra

IR spectra of the ETH\textsuperscript{T} 300x dyes look essentially like those for [Ru(dpp)\textsubscript{3}](ClO\textsubscript{4})\textsubscript{2}. Fig. 2.7 shows a spectrum of solid ETH\textsuperscript{T} 3001 which has been recorded on a Bruker Vector 33 with a Golden Gate diamond ATR unit. Attribution of the bands observed in the IR spectrum of ETH\textsuperscript{T} 3001 are based on comparison to the spectra of tris(2,2′-bipyridine) nickel dichloride (hydrated), bis(2,2′bipyridine) platinum chloride (yellow form), tris(2,2′-bipyridine) ruthenium dichloride hexahydrate, tris(2,2′-bipyrimidine) iron diperchlorate, tris(5-methyl-1,10-phenanthroline) ruthenium diperchlorate, tris(1,10-phenanthroline) iron diperchlorate [20] and the individual components 2,2′-bipyridine [21], 1,10-phenanthroline [22], 4,7-diphenyl-1,10-phenanthroline [23] and sodium perchlorate [24].

In contrast to spectra in KBr, ATR spectra have less artifacts and a neater baseline, but spectral resolution is worse because of association.
Assignments are based on spectra comparison with literature spectra.

**Figure 2.7** IR spectrum of solid ETHT 3001

- Frequency: $1092 \text{ cm}^{-1}$, $1188 \text{ cm}^{-1}$, $1018 \text{ cm}^{-1}$, $622 \text{ cm}^{-1}$, $740 \text{ cm}^{-1}$, $836 \text{ cm}^{-1}$, $1416 \text{ cm}^{-1}$, $1508 \text{ cm}^{-1}$, $1554 \text{ cm}^{-1}$, $1610 \text{ cm}^{-1}$, $1942 \text{ cm}^{-1}$, $3026 \text{ cm}^{-1}$, $3268 \text{ cm}^{-1}$, $2958 \text{ cm}^{-1}$.

- Important peaks include:
  - $4,7$-dph-1,10-phen
  - $\text{ClO}_4^-$
2.4 Spectroscopic properties of ETH\textsuperscript{T} 300x dyes

2.4.1 Absorption and luminescence spectra

Absorption and luminescence spectra of the investigated ruthenium(II) diimine complexes are shown in Fig. 2.8. All spectra have been recorded with the solutions prepared for the quantum yield determination, see Table 3.2 on p. 56.

![Absorption spectra of ruthenium complexes](image)

**Fig. 2.8** Absorption, emission and excitation spectra of the investigated ruthenium complexes

Emission and excitation spectra have been smoothed with a binomial filter for clarity. Emission spectra have been emission-corrected.

The values of emission and absorption maxima are listed in Table 2.2. The emission spectra are essentially identical except for the very subtle maximum emission wavelength shift of 612.0 to 613.4 nm.

The most striking feature in the absorption spectra is the shoulder of ETH\textsuperscript{T} 3001 around 550 nm which the other ruthenium complexes don’t have, presumably an impurity. A comparison with a spectrum recorded 3 years earlier under identical conditions shows an increase of 20% at 550 nm over time, which means that only a
small fraction of the spectral changes can be attributed to ageing. The excitation spectra only roughly match the corresponding absorption spectra, which might be an artifact of the PE LS-50B’s built-in excitation spectra correction. All the excitation spectra basically look alike down to 350 nm.

<table>
<thead>
<tr>
<th>Substance</th>
<th>( \lambda_{\text{max}}^{\text{em}} / \text{nm} )</th>
<th>( \lambda_{\text{max}}^{\text{abs}} / \text{nm} )</th>
<th>( \varepsilon_{\text{max}} / \text{M}^{-1}\text{cm}^{-1} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>([\text{Ru(dpp)}_3\text{(ClO}_4)_2]</td>
<td>612.0</td>
<td>438, 464</td>
<td>3.303·10^4</td>
</tr>
<tr>
<td>ETH( ^T ) 3001</td>
<td>612.9</td>
<td>442, 467</td>
<td>3.317·10^4</td>
</tr>
<tr>
<td>ETH( ^T ) 3003</td>
<td>613.4</td>
<td>440, 466</td>
<td>3.433·10^4</td>
</tr>
</tbody>
</table>

Table 2.2 Emission/absorption maxima of the ruthenium complexes in ethanol

a. Molar decadic absorption coefficients \( \varepsilon_{\text{max}} \) are for the absorption maximum at longer wavelengths.

Literature values are \( \varepsilon_{\text{max}} = 2.86\cdot10^4 \text{M}^{-1}\text{cm}^{-1} \) in EtOH/MeOH (4:1 v/v) with \( \lambda_{\text{max}}^{\text{abs}} = 438, 463 \text{nm} \) and \( \lambda_{\text{max}}^{\text{em}} = 618 \text{nm} \) [7]. Fig. 2.9 shows the absorption and emission wavelengths of similar compounds, also from Thomson’s research group [7, 9]. There is a 6 nm red-shift in their emission maximum wavelength for \([\text{Ru(dpp)}_3]^{2+}\). Their spectrometer had been emission-corrected based on their own excitation spectra correction which will - together with our potential error in the

**Fig. 2.9 Comparison absorption/emission wavelengths to literature data**

The tags mark radicals in 4,7-position of phenanthroline. Literature data is from the same research group [7, 9], fat markers denote data from this work.
emission calibration - account for the wavelength difference. *Thomson et al.* thought their $\lambda_{\text{max}}^{\text{em}}$ values to be reliable only to ± 5 nm.

### 2.4.2 Luminescence quantum yields

A very detailed description of the quantum yield determination is given in 3.3 *Quantum yield determination in solution* on p. 51. The results of this determinations as well as the emission signal ratio $S_{\text{deareated}}/S_{\text{areated}}$ are listed in Table 2.3. The slightly lower quantum yield of 0.50 for ETH$^T$ 3001 might be explained by the presence of a non-luminescent impurity, see 2.2.8 on p. 14.

<table>
<thead>
<tr>
<th>Substance</th>
<th>$\Phi_L$ in deaerated ethanol</th>
<th>$S_{\text{deareated}}/S_{\text{areated}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="ClO$_4$">Ru(dpp)$_3$</a>$_2$</td>
<td>0.52</td>
<td>16.8</td>
</tr>
<tr>
<td>ETH$^T$ 3001</td>
<td>0.49</td>
<td>18.4</td>
</tr>
<tr>
<td>ETH$^T$ 3003</td>
<td>0.56</td>
<td>20.3</td>
</tr>
</tbody>
</table>

Table 2.3 Luminescence quantum yields in ethanol
Quantum yield standard was Rhodamine 101 in ethanol with $\Phi_L = 1.0$ [25].

*Thomson et al.* have determined the quantum yield of 24 different ruthenium(II) tris-1,10-phenanthroline complexes [7], among them [Ru(dpp)$_3$]$^{2+}$Cl$_2$, which they determined as 0.366. They used Rhodamine B as quantum yield standard for which they assumed a quantum efficiency of 0.73. This value was originally measured relative to quinine bisulfate in dilute H$_2$SO$_4$ when excited at 366 nm. They preferred this value to one of 0.97 measured in ethanol when excited at 535 nm - i.e. in a better solvent and at more suitable wavelength - on the grounds of “more prudent choice for our purposes” as they stated [9]. *Nakamura’s* group found an even lower quantum yield of 0.13 or 0.22 for [Ru(dpp)$_3$]$^{2+}$Cl$_2$, depending on the standard [26]. If we presume that *Karstens* $\Phi_L$ for Rhodamine B of ≤ 50% is correct rather than what *Thomson et al.*’s have chosen, their $\Phi_L$ for [Ru(dpp)$_3$]$^{2+}$Cl$_2$ would be 0.24. Had they taken their rejected higher value they would have obtained 0.48 which is very close to the values reported here.

One might speculate whether the different counter ion (perchlorate) might be responsible for part of the quantum yield difference. For their 28 ruthenium(II) tris-bipyridyl complexes *Thomson et al.* found no significant difference in quantum yields for either Cl$^-$ or I$^-$ counterions, though this applies not necessarily to perchlorate.

*Thomson et al.* confirmed that [Ru(bpy)$_3$]$^{2+}$ has only a slightly lower quantum yield than its perdeuterio-analogue, indicating that the C-H vibrations are not a very important mode of deactivation as in the case of luminescent aromatic hydrocar-
bons. However, changing from solvent H₂O to D₂O lead to an increase in the quantum yield which demonstrates the importance of environmental O-H vibrations in the deactivation process. The quenching effect of water has been confirmed in ethanol, see 3.3 Quantum yield determination in solution on p. 51. Daniel Freiner had observed that dry salts of the ruthenium(II) diimine dyes show luminescence whereas those with crystal water don’t. Solvent differences alone most probably do not explain the luminescence quantum yield differences.

From Hartmann et al.’s quenching data of [Ru(dpp)₃](ClO₄)₂ in ethanol at 25°C we can calculate \( S_{\text{deareated}}/S_{\text{areated}} = 19.1 \) [27]. This is in good agreement with the obtained value 16.8 in Table 2.3. One has to keep in mind that the difference between 1/19.1 and 1/16.8 is only 0.7% of the unquenched luminescence (100%).

Temperature is a very important factor for the luminescence quantum yields [3]. [Ru(4,7-Me₂phen)₃]²⁺ with \( \Phi_L = 0.65 \), [Ru(dpp)₃]²⁺ with \( \Phi_L = 0.682 \) as well as [Ru(phen)₃]²⁺ with \( \Phi_L = 0.584 \), all in EtOH/MeOH (4:1) at 77 K, are all considerably larger than at room temperature. The quenching of Ru(II) diimine complexes in absence of oxygen seems to be equivalent with vibrational deactivation of the excited state. De Cola et al. investigated the temperature-dependence of the luminescence life time of [Ru(bpy)₃]²⁺ in a proprionitrile/butyronitrile (4:5 v/v) matrix [28] and found two subsequent decreases in lifetimes with increasing temperature, the first around 120 K and a much more pronounced second one above 250 K. The first decrease, coincident with the matrix melting, was attributed to increased vibrational relaxation due to higher mobility and the second decrease was due to thermal population of the photoactive \(^3\)MC state at approximately 3000-5000 cm⁻¹, which is known to relax efficiently to the ground state.

Caspar et al. have synthesized a large number of osmium(II) diimine complexes and found that they all share a common deactivation mode for the excited state [1982#83]. Based on vibrational progressions in low-temperature luminescence spectra, which indicate their strong coupling to the emission, a ligand-based skeletal stretching vibration of approximately 1300 cm⁻¹ was identified as the major deactivation mode. According to unpublished results of Thomson et al. they found vibrational progressions of similar frequency in low-temperature luminescence spectra of the Ru(II) bipyridyl complexes [10]. Such vibrational deactivation is especially important for considering suitable polymers for oxygen optodes.

Bard et al. had determined the electrochemiluminescence yield of [Ru(dpp)₃]²⁺ as 0.24 which rose monotonically though not directly proportional to the luminescence quantum yield within the series [Ru(bpy)₃]²⁺, [Ru(dp-bpy)₃]²⁺ (dp-bpy = 4,4’-diphenyl-2,2’-bipyridine) and [Ru(dpp)₃]²⁺ [29]. If this trend continues for the ETH¹ 300x dyes, they probably have an even larger electrochemiluminescence
yield, though the necessary voltage cycling is likely to be difficult regarding their electrochemical (in)activity, compare 6 *Electrochemical investigation of ETH*\textsuperscript{T} 3001 on p. 161.

### 2.4.3 Luminescence lifetimes

All lifetime measurements were carried out by Paul Hartmann in April 1999 at AVL List GmbH in Graz, Austria. The experimental set-up is described in 8.3.2 *Life time measurements* on p. 239. The obtained lifetimes in 10\textsuperscript{-5} M ethanolic solutions are given in Table 2.4.

<table>
<thead>
<tr>
<th>Substance</th>
<th>( \tau_{m(N_2)} / \mu s )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETH\textsuperscript{T} 3001</td>
<td>0.462</td>
</tr>
<tr>
<td>ETH\textsuperscript{T} 3003</td>
<td>0.453</td>
</tr>
</tbody>
</table>

*Table 2.4 Luminescence lifetimes in ethanol*

Experimental errors are ±0.005 µs.

The decays could be well described by a monoexponential decay. The lifetimes in ethanol are a lot lower than expected, usual values would be around 5 µs, compare also Fig. 2.10. Hartmann gave the formation of micelles or aggregation as possible explanation. Hartmann et al. had determined the lifetime of [Ru(dpp)\textsubscript{3}](ClO\textsubscript{4})\textsubscript{2} in ethanol as \( \tau_0 = 5.2 \pm 0.1 \) µs [27]. A determination by Thomson et al. gave 6.4 µs [7], though based on a intensity decay to 1/e of the original intensity rather than a decay fit.

The ETH\textsuperscript{T} 300x dyes and [Ru(dpp)\textsubscript{3}](ClO\textsubscript{4})\textsubscript{2} have a very similar lifetime in a plasticized PS membrane (compare Table 4.4 on p. 86) as well as almost identical quantum yields and oxygen quenching behavior in ethanol. All this evidence does not suggest such low luminescence lifetimes, which will therefore not be discussed any further.

### 2.5 Discussion

A presumably consistent set of literature values is available from Thomson’s group which has synthesized a large number of ruthenium(II) diimine compounds, both based on phen and bpy-moieties [7-10]. The luminescence lifetimes and quantum yields are plotted against each other in Fig. 2.10. Note that no direct relationship is expected.

Different models have been successfully applied in the literature to describe the correlation between several luminescence-related parameters. One of them is the three-state model which reduces the schematics in Fig. 2.1 to the processes characterized
by $\varepsilon_{\text{max}}$, $k_{\text{isc}}$ and $k_r$, $k_{\text{nr}}$. $\Phi_{\text{isc}}$ is generally assumed to be unity [3, 7, 28]. For the $[\text{Ru(bpy)}_3]^{2+}$ derivatives a linear correlation between $\log(\Phi_m)$ and $\log(\varepsilon_{\text{max}} \cdot \tau_0)$ was postulated with a slope of unity and an intercept $\log(c \cdot \Phi_{\text{isc}})$ [10]. $c$ gives the magnitude of spin-orbit coupling which was assumed constant. This constant $c$ in turn can be determined from the linear dependence between $\varepsilon_{\text{max}}$ and $k_r$ ($\Phi_L / \tau_0$).

Another expected correlation is between the non-radiative decay rate $\ln(k_{\text{nr}})$ and the emission energy $E_{\text{max}}^{\text{em}}$. This is based on the energy gap law which predicts that radiationless processes are facilitated by lowering the energy gap between excited and ground states.

Unfortunately, all those models heavily rely on the use of the luminescence lifetime which will not be pursued considering the doubts on the lifetimes of the ETH$^T$ 300x dyes.

Based on the other luminescence properties, such as wavelength of the absorption maximum and emission maximum and the molar decadic absorption coefficient, we can conclude that the ETH$^T$ 300x dyes are indeed an improvement of the photophysics of $[\text{Ru(dpp)}_3]^{2+}$.

To assess their chemical properties is more difficult. Thin layer chromatography showed unambiguously that the ETH$^T$ 300x dyes are more lipophilic than $[\text{Ru(dpp)}_3](\text{ClO}_4)_2$. On the other hand, changes were small and the dyes’ overall behavior was mainly governed by their ionic behavior. What the effects of this dual
character highly lipophilic versus doubly charged complex are is difficult to say. No conclusion can be made on whether ETH T 300x dyes in different polymers (compare 4 Luminescence-based oxygen optodes on p. 67) are better than [Ru(dpp)\textsubscript{3}](ClO\textsubscript{4})\textsubscript{2} with respect to aggregation or solubility due to the lack of comparative experiments. Ruthenium(II) dyes which can be covalently attached to polymers most probably give a better long-term stability, especially for sterilization experiments.

ETH T 3001’s electrochemistry was governed by very slow kinetics and irreversibility (see 6 on p. 161). A very slow electron exchange means that oxidative and reductive quenching can be curbed. This an attractive feature since it means more selectivity in the quenching process of the ETH T 300x dyes without losing oxygen sensitivity or high luminescence quantum yields. Without any comparative quenching experiments in solution this hypothesis cannot be proved since only electron exchange with certain metals could be slow.

The lipophilization strategy definitely is no answer to one of the most important issues for oxygen optodes: photostability. Based on the investigations and the proposed mechanism in 5 Photobleaching of ruthenium(II) diimine dyes on p. 119 the partial ligand detachment must be suppressed. The best strategy is sterical hindrance, such as given in [Ru(cage)]\textsuperscript{2+} compound, see Fig. 5.17 on p. 148.

### 2.6 Summary and Perspectives

We have synthesized and characterized the new lipophilic ruthenium(II) diimine dyes ETH T 3001 and 3003. ETH T 3001 is tris(4,7-bis(4-propylphenyl)-1,10-phenanthroline)-ruthenium(II) perchlorate and ETH T 3003 is tris(4,7-bis(4-octylphenyl)-1,10-phenanthroline)-ruthenium(II) perchlorate monohydrate.

The luminescence quantum yield of ETH T 3003 was found to be 0.56 ± 0.05 in deoxygenated and 0.03 ±0.01 in aerated ethanol (at 25°C vs Rhodamine 101 with $\Phi_L = 1.0$ [25, 30]). These values are probably accurate to ± 0.05. For comparison, the $\Phi_L$ of [Ru(dpp)\textsubscript{3}](ClO\textsubscript{4})\textsubscript{2} was determined under identical conditions as 0.52. Its luminescence quantum yield is thus considerably higher than determinations in other solvents such as EtOH/MeOH (4:1) where it was 0.366 [7]. Thus it is likely that the luminescence quantum yield of [Ru(dpp)\textsubscript{3}](ClO\textsubscript{4})\textsubscript{2} has been previously underestimated.

ETH T 3003 has absorption maxima at 440 and 460 nm (\(\varepsilon = 3.433\times10^4\ \text{M}^{-1}\text{cm}^{-1}\)) and emits at 613 nm in ethanol ([Ru(dpp)\textsubscript{3}](ClO\textsubscript{4})\textsubscript{2} emitted at 612 nm). The luminescence lifetimes and the properties of ETH T 3001 are given only in the full text because we had some reservations.
Thin-layer chromatography showed that, as expected, the lipophilicity of the ETH\textsuperscript{T} 300x dyes was higher than that of [Ru(dpp)\textsubscript{3}](ClO\textsubscript{4})\textsubscript{2}, but only slightly.

For the electrochemistry of ETH\textsuperscript{T} 3001 see chapter 6 on p. 161, for its photobleaching see chapter 5 on p. 119, the determination of the luminescence quantum yields is given in chapter 3 on p. 31) and the uses of the ETH\textsuperscript{T} 300x dyes in luminescence-based oxygen sensors are described in chapter 4 on p. 67.

### 2.7 References


3 Determining luminescence quantum yields

3.1 Introduction

The driving force behind much of the recent synthesis of ruthenium(II) diimine complexes has been mainly to obtain a higher quantum yield. Other parameters have also played a role depending on the intended application. The quantum yield can be considered the most important parameter of any luminescent dye as it is a dye’s high quantum yield $\Phi_L \gg 0$ which makes it a luminescent dye. It is therefore very important to find a way of accurately determining luminescence quantum yields. Extensive reviews of this topic, such as Demas et al. [1], describe experimental procedures in great detail. Demas et al. recommend using a set of 17 questions to help the scientific community to appreciate the quality of quantum yield determinations, but still these determinations nowadays are de facto considered standard laboratory operations. The most experimental detail one can expect in a publication these days is the citation of the quantum yield standard employed.

Some colleagues from the scientific community frankly admit that they use an approximate procedure to determine quantum yields. This has made it more difficult to find reliable data in the literature and explains much of the discrepancy between different literature sources. Comparisons of the quantum yields of different substances determined by the same author are, of course, always more consistent than comparisons of quantum yields of the same substance determined by different authors.

In the case of ruthenium(II) diimine complexes, there is no general agreement on which quantum yield standard to use and very often citations lead to either poorly documented standards or neglect more recent research. Another issue is the need for deoxygenation which further complicates the experimental procedure. Given the complexity involved in choosing a quantum yield standard, the procedure for actually determining it and for assessing of possible error sources are described in great detail in section 3.3. The values obtained are discussed in section 2.4.2 on p. 22.

Modern luminescence spectrophotometers have evolved to make their mark. It is the software that has been improved, but the optical quality of some instruments has decreased. Of upmost importance is a proper correction of excitation and emission spectra. This is rarely provided by the manufacturers when shipping the instrument nor routinely done in research groups, but is probably the most important source of error in determining quantum yields and is therefore extensively discussed in section 3.2.2. The same holds also true for determining emission wavelengths.
Unfortunately, most publications on luminescence-based oxygen-sensors only provide emission data, but rarely data on absorption. As soon as we go from a ruthenium(II) diimine dye in solution to one dissolved in a membrane, characterization becomes very difficult. For this work only polymer membranes that have been spin-coated on glass wafers have been used (compare section 8.2 Membrane preparation and characterization on p. 235) so as to characterize with a wide range of methods. Luminescence and absorption spectroscopy were routinely applied. Since the emphasis of this work is on experimental procedures for testing long-term behavior, I needed to determine as accurately as possible what happens to membranes undergoing different treatments. The determination of artifact-free absorption and emission data as well as its reproducibility is discussed in section 3.2.4.

Although there is considerable interest in the quantum yield of ruthenium(II) diimine dyes, almost no data can be found in the literature on their quantum yield in polymer membranes. One of the few determinations available is that of Thomson et al. for PVC [2]. I have developed the more general concept of a “liquid membrane” standard which allows the absolute quantum yields in polymers to be determined with the same instrumental set-up as for polymer membranes. The whole procedure is outlined in detail in section 3.4. The refractive index corrections necessary for the PE LS-50B luminescence spectrophotometer are presented in section 3.2.3, but can be easily adapted to other instruments.

The quantum yields obtained in the membrane are discussed in 4.4.1 Plasticized polystyrene membranes on p. 85 and 4.4.2 Thermoplastic polymer membranes on p. 92.

3.2 Characterizing oxygen optode membranes

3.2.1 Measuring luminescence in membranes

A more careful evaluation of possible artifacts is needed when measuring luminescence in membranes than when measuring it in solution. Some of these artifacts depend on the instrument used, in this case a PE LS-50B luminescence spectrophotometer (Perkin Elmer, Beaconsfield, UK), but other considerations apply more generally. A brief overview of the PE LS-50B is given in section 8.3.1 on p. 237.

The membrane is a very effective scatterer and introduces new, potentially spectroscopically active compounds (fluorescence, phosphorescence, Raman scattering), via the polymer and its additives. These two major artifact sources mean that the emission-excitation plot of a plasticized polystyrene membrane (containing 38.5% o-NPOE) is not an even distribu-
Fig. 3.1 Emission/excitation plot of polystyrene/o-NPOE membrane
The spectra have been recorded at ambient temperature and atmosphere. Numbers indicate intensities at lines of same intensity (in a.u.). The top part shows different filters that can be used to rule out (most) artifacts. Excitation wavelength was 460 nm, slit widths were 2.5/5 nm. Frequency shifts for alleged Raman bands have been calculated from wavelength differences taken from the spectrum.

...tion, see Fig. 3.1. Similar artifacts are encountered with any other membrane composition and polymer.
There are first and second order grating reflections of scattered excitation light where $\lambda_{em} = n \cdot \lambda_{exc}$, diffuse scattering where $\lambda_{em} = n \cdot \lambda_{exc} + \text{const (in nm)}$, Raman scattering of polystyrene or the grating where $\lambda_{em} = n \cdot \lambda_{exc} + \text{const (in cm}^{-1})$ and possibly (polystyrene) fluorescence where $\lambda_{em} = \text{const (in nm)}$.

If an uncoated glass wafer is used, the artifacts are reduced to reflected excitation light only.

The attribution of Raman bands in Fig. 3.1 is based on the constant energy shift to the excitation wavelength only. The frequency shift is close to the one of aromatic C-H stretch vibrations [3] (around 3100-3000 cm\(^{-1}\)), even though a value of 3300 cm\(^{-1}\) has been calculated from the spectra. Another possibility, though without any further evidence might be the attribution to peroxide stretch vibrations (around 3450-3200 cm\(^{-1}\)) as polystyrene impurity. In normal polystyrene samples there is no such impurity [4], but it can occur in oxidized polystyrene [5]. In any case it is very difficult to explain why the first overtone at 2·3300 cm\(^{-1}\) should be more intense than the fundamental Stokes lines at 3300 cm\(^{-1}\) and why these are absorbed by cut-off filters (compare upper part of Fig. 3.1). The maximum of the alleged Raman band is at approximately 460 nm, which suggests a resonance with an absorption band. However, there is none in this region for polystyrene or o-NPOE. A possible explanation might be Raman bands of some coating on the optical elements of the spectrometer which is only visible due to the high excitation light throughput.

Phosphorescence with a mean lifetime of 8 ms [6], mainly of acetophenone end groups present in the polystyrene [7], could possibly intervene, but only at low temperatures and in the absence of oxygen which neither is the case with measurements reported here. Furthermore, the PE LS-50B takes background measurements immediately after each xenon light pulse (half width approximately 10 µs) which will average out any luminescence on a longer time scale. For these reasons phosphorescence can be ruled out.

Polystyrene fluorescence emission in the near-UV stems from trans-stilbene structures created during polymerization but is of no interest to the emission wavelength range investigated here. Polystyrene can also have an emission at 428 nm and 600 nm which decreases with increasing molecular weight, but with an absolute intensity not specified in the literature [6]. One has to keep in mind that depending on the supplier, polymers contain a wide variety of additives, potentially fluorescent. No detectable luminescence was found in the employed polystyrene sample. Another possible source of fluorescent impurities can be the flow-through cell with contaminations from earlier experiments. Especially the o-rings of the cell frequently have luminescent contaminations from earlier experiments which can be easily detected by an UV lamp at 366 nm or - less suitable - at 254 nm. No detectable luminescence has been found in the glass wafers used in this work.
Several kind of filters have been evaluated to cut down artifacts (see Fig. 3.1, upper part). Most successful was the use of the built-in cut-off filter (515 nm) which eliminated the excitation light peak around 630 nm. The excitation light is reflected on the membrane/glass or membrane/air boundary into the emission monochromator, compare evidence on p. 62. The fact that artifacts could also be eliminated with a horizontal polarizator, but not a vertical polarizator, strongly suggests that this peak is indeed scattered excitation light. The limiting factor eventually are the spectrometer optics, which do not provide sufficient discrimination of the excitation light. An example of an emission/excitation plot recorded with 515 nm cut-off filter is given in Fig. 4.9 on p. 91.

The shape of excitation/emission spectra of a membrane with Ru(II) diimine dyes exposed to nitrogen or oxygen remain identical with the 515 nm cut-off filter on or off, which suggests that the ruthenium(II) diimine complex luminescence is not impaired by the cut-off filter. If luminescence, however, is weak, there seems to be a background as in the upper part of Fig. 3.1. Provided that the difference spectrum taken under nitrogen and oxygen is pure ruthenium(II) diimine luminescence (i.e. other luminescent species, such as additives, were required to be quenched by oxygen too, which is very unlikely), the luminescence spectrum can be decomposed as

\[ I(N_2) = x \cdot (I(N_2) - I(O_2)) + \text{background}. \]  

When \( x \) is maximized for a given emission/excitation plot of ETH\(^T\) 3001 in plasticized polystyrene in order that the calculated background is still positive within the whole 2D-plot the background is everywhere close to zero. This has been shown for other membrane compositions as well. Since it is very improbable that the background assumes exactly the shape of the ETH\(^T\) 3001 we can presume that the background is indeed zero, even though this seems to contradict Fig. 3.1. This is especially important to determine correct intensities for a Stern-Volmer plot because any offset leads to a curved Stern-Volmer plot.

The background is proportional to the excitation light scattered at the membrane boundary. The amount of scattering can be estimated by extending emission measurements down to the excitation light wavelengths where scattered excitation light “leaks” through the 515 nm cut-off filter.

**3.2.2 Spectrometer calibration**

Calibrated spectra are spectra with intensities which reflect actual quanta·s\(^{-1}\)·nm\(^{-1}\) or quanta·s\(^{-1}\)·cm\(^{-1}\) (or energy intensities). Spectrometer normally do not deliver such intensities by default. This is because each optical spectrometer component has certain wavelength transmission characteristics which distort irradiated or emitted photon quanta.
Monochromators usually have an optimum transmission wavelength which decreases towards the UV and the NIR; the same applies to photomultipliers which have a decreased quantum efficiency in the red, although this can be at least partly remedied by the use of a red-sensitive photomultiplier (such as the RS 928 the PE LS-50B), for instance. These effects result in shifted peak wavelengths, distorted spectra and very important for quantum yield considerations, erroneous integrals, compare Fig. 3.2. For ETH 3003, for example, emission wavelength are shifted around 6-8 nm to the red upon calibration, depending on the polymer.

This is why both excitation and emission spectra need to be calibrated. Calibration can be achieved by several means which are more or less workable. A concise and comprehensive survey on the calibration issue is given in [8], pp.39-43. If all the transmission properties of all the elements involved were known, one could easily calculate the transmission/efficiency properties of the whole system and obtain a calibration curve. However, this is hardly ever the case. A simple approach is determining the overall transmission/efficiency factor by comparing a spectral standard (from either a light source or a dye which is excited under well-defined conditions) with the actual recorded spectrum. One should keep in mind that grating monochromators are sensitive to polarized light, which can further complicate the issue in case of polarized luminescence.

*Excitation* spectra correction in the range of 230-600 nm is routinely done for the PE LS-50B prior to shipping; otherwise several peaks around 460 nm from the intensive xenon excitation light source would appear in every excitation spectra which would be hardly acceptable to customers. The correction is based on the quantum counter behavior of Rhodamine B. A quantum counter is a substance

![Fig. 3.2 Effect of calibration on peak shape and on relative intensities](image)
which re-emits every absorbed photon within a given wavelength range. Its expected excitation spectra in the range 230-600 nm is a straight line at a any given emission wavelength. The procedure has been thoroughly discussed by Ostrom et al. [9]. The calibration curve is stored in a non-volatile memory within the instrument and is applied to every spectra, including emission spectra (for which it gives a excitation wavelength-dependent scaling factor).

Emission spectra correction can be achieved by comparison with any known spectrum. The spectra used can either be emission spectra of properly excited reference substances or an emission spectrum of a light source. Because of the uncertainties associated with reference spectra of substances and the inconsistencies that had been encountered (see discussion on p. 39), a more reliable calibration based on a reference light source was done.

A tungsten iodide lamp L-352 from Optronic Laboratories Inc. (Orlando, FL, USA) and a fresh barium sulfate scatterer had been provided by Franz Stocker from Perkin Elmer and Heinz Erni from Novartis, respectively. The lamp was operated at 38 V with a carefully controlled current of 6.50 A to maintain conditions for which the lamp had been calibrated. The light emitted from the lamp was reflected via the scatterer onto the emission spectrometer slit to give a uniform spatial lightning distribution. Great care was taken that the reflected light followed the usual emission beam path and that no light was cast directly from the lamp onto the spectrometer entrance.

The spectrum of the L-352 lamp, as well as the scatterer efficiency and the resulting calibration spectrum recorded by the PE LS-50B, are shown in Fig. 3.3.

The resulting calibration factors in Fig. 3.4 where calculated according to

\[
calibration\ \text{factor}(\lambda) = \frac{\text{measured\ intensity}(\lambda)}{\text{expected\ intensity}(\lambda) \times \text{scattering\\ efficiency}(\lambda)} \tag{3.2}
\]

The relation between energy and quanta intensities is

\[
I(\lambda) / (\text{quanta} \cdot \text{m}^{-2} \cdot \text{s}^{-1}) = \frac{\lambda}{h} \cdot \frac{I(\lambda)}{(\text{J} \cdot \text{m}^{-2} \cdot \text{s}^{-1})}. \tag{3.3}
\]

Assessing the quality of the calibration is not easy. Several peaks appeared in the spectrum whose exact origin is unknown, see Fig. 3.3b. According to the smooth lamp spectrum in Fig. 3.3c, they must have their origin in the spectrometer. A synchronous scan (\(\lambda_{\text{em}} - \lambda_{\text{exc}} = 0 \text{ nm}\)) using the Perkin Elmer diffuser plate in the cuvette holder showed no peaks, even though only the spectral lamp had been exchanged by the spectrometers own light source. This corresponds to a monochromatic light source which enters the emission spectrometer slit at the expected angle. In the case of the calibration, however, neither condition is met. Light which entered the spectrometer slit directly rather than being scattered first showed the same peaks...
3.2 Characterizing oxygen optode membranes

at the same positions with similar intensities, but the continuous light envelope was significantly weaker. This suggests that the peaks derive from a deviation of the light path from the expected emission geometry.

According to Heinz Erni and [8] these peaks are most probably caused by grating imperfections. This coincides well with the observation that peaks were slightly weaker, but still present if either a vertical or horizontal polarization filter was used. This rules out any reflections on optical elements prior to the polarization filter (compare Fig. 8.2 on p. 237) which would absorb reflected light which is polarized.

For the calibration factors which were used in this work, these obvious artifacts have been removed by smoothing. No spectra calibration is done below 300 nm or above 850 nm because lamp intensity or photomultiplier efficiency respectively in this range are too low.

The same calibration procedure was done with the horizontal and vertical polarization filter. In general, intensities were a third of what was obtained without polariza-
tion filters, with up to three orders of magnitude difference in the calibration factor between 300 and 800 nm. The vertical polarization filter transmitted better at lower, the horizontal filter better at higher wavelengths.

For comparison the calibration factors derived from two spectra of standard substances are shown, compare Fig. 3.4. The first one is a spectrum of a $10^{-3}$ M solution of quinine bisulfate in 0.1 N sulfuric acid which was published by Melhuish in 1960 [10]. The second was a spectrum of a solution of 0.3 g/l Rhodamine B in ethanol published by Berlman [11], which had not been intended for spectral calibration. The correlation with the calibration factors based on the L-352 correction for either substance is poor.

A detailed plot of the calibration factors derived from Melhuish’s spectrum is given in Fig. 3.5. The excitation wavelength was 365 nm, with the 2.5/5 nm slit combination using a 10 x10 mm quartz glass cuvette. The quinine sulfate was used as received from Fluka ($10^{-3}$ M in 0.1 N H$_2$SO$_4$) or dried at 100°C ($1.8\cdot10^{-3}$ M in 1 N H$_2$SO$_4$).

Drying during 3h at 100°C, which yields a whiter and flakier quinine sulfate, narrowed the emission peak on the lower wavelength side by 3 to 6 nm. The drying procedure, however, is doubtful because it comes with an almost four-fold loss in intensity with respect to untreated quinine sulfate.

A part of this can be attributed to the method of standard substances itself. When the intensity value is low in either the corrected literature spectrum or in the uncorrected recorded spectrum, the resulting calibration factors are very inaccurate. This

![Fig. 3.4 Correction factors derived from calibration](image)

Calibration factors depicted are for energy intensities.
is reflected in the upward bending of the quinine-derived calibration factors to either side in Fig. 3.4. Furthermore, a series of overlapping reference spectra would be needed for a calibration covering the whole emission range. Another issue are the exact experimental conditions and purity of the substances used. Little useful can be found in the literature. Berlman has its experimental conditions very well documented, but his 0°/45° front face geometry cannot be reproduced with the PE LS-50B. Highly concentrated solution of Rhodamine B exhibit strong self-reabsorption. This is manifested by the large differences in the spectra obtained on the PE LS-50B with a 10 x 10 mm or a 2 x 10 mm quartz cuvette. For the latter’s two possible orientations a red-shift of the peak maximum wavelength of 6 nm resulted for a ~1 mm emission path and 15 nm for a ~5 mm emission path. Lippert et al. have proposed a series of standards [12]. Back in 1959, most of their substances had been synthesized by themselves or purified in a way that cannot be easily reproduced today. Their instrument used components very different from those today, which makes spectral comparison difficult. After all, there is little sense in using standard spectra for calibration which themselves are actually based on an earlier tungsten lamp calibration.
Therefore, it is probably fair to conclude that calibration by reference substances is a historical rather than a practical and efficient option for spectrometer calibration.

3.2.3 Refractive index corrections

A 30°/60° excitation/emission-geometry, as it is used in the PE LS-50B for membranes (in Fig. 8.3 on p. 239), is far more susceptible to refraction effects than a 0°/90°-geometry used for measuring luminescence in cuvettes. Both the excitation and the emission ray are refracted at the planar boundaries membrane/glass and glass/air.

At the planar boundary between two media of refractive indices $n_1$ and $n_2$ an incident ray is either reflected or refracted. The refracted ray obeys Snell’s law:

$$n_1 \cdot \sin \theta_1 = n_2 \cdot \sin \theta_2 \quad (3.4)$$

where $\theta_1$ and $\theta_2$ are the angles between the rays and the normal to the boundary [13], see also Fig. 3.6. In the case of $n_1 > n_2$ $\theta_2$ reaches 90° first as $\theta_1$ increases. This occurs when $\theta_1 = \theta_c$, which is called the critical angle. From Snell’s law it follows that

$$\theta_c = \arcsin \left( \frac{n_2}{n_1} \right). \quad (3.5)$$

If $\theta_1$ goes beyond the critical angle $\theta_c$ we have total internal reflection, the boundary behaves as if it was a perfect mirror.

The variable elements along the optical path include two different glass supports (quartz or soft glass) and a wide range of different polymers, with their respective refractive indices. The geometrical arrangement is always the same because both sorts of glass supports have an identical thickness ($d_g = 2.0$ mm) and the angle of the excitation ray and emission ray is given by the accurate positioning with the flow-through cell. The exact acceptance angle for the emission detection in the PE LS-50B is unknown, but can be roughly estimated. The spectrometer entrance is at a 60 mm distance from the centre of the membrane, the excitation light forms two separate D-shaped sections on the 50 by 50 mm square spectrometer entrance window with a largest diameter of 41 mm. This defines the upper limit for the acceptance angle of 38°.

The situation is depicted in Fig. 3.6, the indices m, g and a denote the media membrane, glass and air, respectively.

In order to visualize these effects, the ray in the excitation/emission plane has been modelled in IgorPro 3.15, with a graphical representation as in Fig. 3.6. Light was
assumed to be emitted from a light spot situated at a $d_m/2$ distance from the membrane/glass interface.

The thickness $d_m$ of the membrane can be assumed to be between 5 to 10 µm according to measurements, i.e. is two orders of magnitude smaller than $d_g$. For all the subsequent calculations we will use the $n_D^{20}$-values, i.e the refractive index at 589.6 nm and at 20°C. This wavelength is reasonably close to the excitation and emission wavelengths of the ruthenium(II) diimine dyes. One should, however, keep in mind that the refractive index increases increasingly with decreasing wavelengths, which means that the refraction at lower wavelengths is underestimated.

The $n_D^{20}$-values for the “membrane” range from 1.362 (ethanol [14]) to 1.61 (PAMS), the refractive index of air is 1.0003 [15].

Snell’s law has an inherent continuity and reversibility because the product

$$n_x \cdot \sin \theta_x = \text{const.}$$  (3.6)

yields a constant for any given ray at a set wavelength, provided that no total internal reflection occurs. Due to the law’s reversibility the ray trace is the same for an incident or an outgoing ray. Continuity means that a ray can cross several parallel boundaries, but the ultimate angle of refraction is determined by the first and last media alone, again provided no internal reflection occurs. In our case this implies that the material of the glass plate is irrelevant for the measurements, though the exact light path, of course, must be different. The calculations with the actual dimensions of $d_m$ and $d_g$ clearly showed that these light path deviations between
quartz and soft glass are below 0.1 mm, i.e. negligible. Therefore, results are not affected by the choice of the membrane support glass material in view of the refractive index of glass.

A very interesting property is the angular emission intensity. Most sensibly this distribution is defined with respect to the emitting light spot. The angles calculated with the model, however, are with respect to a detection position shifted by the shift distance $d_s$:

\[
d_s = d_g \cdot \frac{\sin(\theta_a - \theta_g)}{\cos \theta_g} = d_g \cdot (\sin \theta_a - \cos \theta_a \cdot \tan \theta_g).
\]

Since $d_s$ varies from 0 to maximally $d_g$, the maximum angular error amounts to 1.9° given the spectrometer entrance distance of 60 mm. Another approximation is the zero extension of the light spot of the PE LS-50B’s excitation beam on the membrane which is actually 1.5 mm wide and 8 mm high. This translates into acceptably small deviations of 0.6° horizontally and 3.3° vertically with respect to the spectrometer entrance.

In the actual simulation $\theta_m$ is varied from 0° to $\theta_c$ (equals $0° < \theta_a < 90°$) with identical increments of 0.001°. The angular intensity distribution $I(\theta_m)$ is then calculated as a histogram from the resulting calculated $\theta_a$’s. The identical increments in $\theta_m$ take the isotropic character of the luminescence emission into account, the calculated distribution hence is in $d\theta$.

Intensities, however, are usually measured per surface unit ($dA$) and not per angle unit ($d\theta$). Fig. 3.7 shows the sphere with a radius of unity which is used for the necessary calculations.

![Fig. 3.7 Calculation model for spacial distribution of emission](image-url)
For a sphere with \( r = 1 \) the total surface is \( A = 4\pi \), the surface of a sphere section or a zone is \( 2\pi h \). Since the emitted light is proportional to the covered sphere surface, the corresponding fraction of emitted or received light \( I_{\text{trans}}(\theta)/I_{\text{tot}} \) for a given angle \( \theta \) is

\[
\frac{I_{\text{trans}}(\theta)}{I_{\text{tot}}} = \frac{dA}{A} = \frac{2\pi \cdot dh}{4\pi} = \frac{1}{2} \cdot dh = .
\]

\[
\frac{1}{2} \cdot (\cos \theta - \cos (\theta + d\theta)) = \frac{1}{2} \cdot \sin \theta
\]

The simulated distribution \( I(\theta_m) \) has therefore to be multiplied by \( \sin \theta_m \), remapped to \( \theta_a \) (\( \theta_a \) and \( \theta_m \) form a distinct pair for set of refractive indices) and be divided by \( \sin \theta_a \) to obtain \( I(\theta_a) \) which is renamed as \( I(\theta) \):

\[
I(\theta) = I(\theta_a) = \frac{I(\theta_m) \sin \theta_m}{\sin \theta_a}.
\]

The fraction of the total emitted or received light across the glass plate is

\[
\frac{I_{\text{trans}}}{I_{\text{tot}}} = \frac{2\pi \cdot (1 - \cos \theta_c)}{4\pi} = \frac{1}{2} \cdot (1 - \cos \theta_c)
\]

(3.10)

to which the distribution must be normalized. Some simulated intensity distributions are shown in Fig. 3.8.

Fig. 3.8 Emission intensity distributions for different membrane materials
The gray band indicates the range for the polymers investigated in section 4.4.2. (only those with known refractive index)
No correction is necessary for the excitation situation. The PE LS-50B’s excitation light itself is focused into a spot. Because of the light’s negligible path length within the membrane, refraction has no effect on the spot size, above all in respect to the emission monochromator. Hence, the excitation light intensity is equal, no matter what the membrane media is. “Membrane” media with a lower refractive index, such as EtOH, however, will transmit more of their emission light. Since the shape of the intensity distributions in Fig. 3.8 is very similar though not identical, the emission intensity correction relative to PS is in good approximation

\[
\frac{I}{I(\text{PS})} \approx \frac{1 - \cos \theta_c (\text{PS})}{1 - \cos \theta_c} = \frac{1 - \left[1 - \left(\frac{n_a}{n_{m}}\right)\right]^2}{1 - \left[1 - \left(\frac{n_a}{n_{m}}\right)\right]^2},
\]

(3.11)

which is nothing than the ratio of the distribution’s normalization factors. For EtOH for instance a value of 1.47 instead of 1.51 is obtained. The right part of (3.11) can be easily deducted from the left part based on (3.5) and trigonometry. The refractive index of air $n_a$ can be taken as 1. The simulated relative intensities are listed in Table 3.1. Changes in emission intensity relative to PS range from -3 to +2\% for the polymers investigated in 4.4.2 Thermoplastic polymer membranes on p. 92 with known refractive index, i.e. are relatively small.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$n_D$ a</th>
<th>$\theta_c / ^\circ$</th>
<th>Relative intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>1.5894</td>
<td>38.99</td>
<td>1</td>
</tr>
<tr>
<td>EtOH</td>
<td>1.362</td>
<td>47.24</td>
<td>1.51</td>
</tr>
<tr>
<td>PAMS</td>
<td>1.61</td>
<td>38.40</td>
<td>0.97</td>
</tr>
<tr>
<td>PMOS</td>
<td>1.5967</td>
<td>38.78</td>
<td>0.99</td>
</tr>
<tr>
<td>PC</td>
<td>1.586</td>
<td>39.09</td>
<td>1.01</td>
</tr>
<tr>
<td>PDPO</td>
<td>1.575</td>
<td>39.41</td>
<td>1.02</td>
</tr>
</tbody>
</table>

**Table 3.1 Refractive index correction for different polymers**
Relative intensities are based on the simulation and are given with respect to PS.

a. Refractive indices of pure polymers are from supplier Scientific Polymer Products [16]

The range for refractive indices of polymers, however, goes from 1.3 to 1.7 [16] where corrections can become very substantial. The relative intensity difference between polymers with $n_D$ of 1.3 and 1.7 is a factor of 1.9!
Characterizing oxygen optode membranes

Emission intensities at a 30° or 60°-angle relative to a 0°-angle are 0.91 and 0.59, respectively, as determined from Fig. 3.8. As a matter of consequence, we expect for a 30°/60°-geometry only 0.59 of the emission intensity in a 0°/0°-geometry.

The same reasonings as for the membrane can be applied for the cuvette, where we have a 0°/90°-geometry. The main difference is that the excitation spot becomes in fact a double cone of 10 mm length. Furthermore, refraction of the nearly parallel excitation light beam is negligible because it passes the glass pane of the cuvette at a 0° angle. A refractive index change will manifest itself by shifting the excitation light focus (i.e. the center of the double cone) along the excitation light axis. The amount of absorbed light will always be the same, because the path length within the absorbing solution will vary only slightly due to refraction. If this spatial extension is neglected, we obtain the same situation as in Fig. 3.6, provided that the acceptance angle of the spectrometer is rather small; otherwise the limited size of the cuvette pane facing the spectrometer entrance would matter. As a result we can apply equation (3.11):

\[ \frac{I_1}{I_2} \approx \frac{1 - \sqrt{1 - \frac{1}{n_2^2}}}{1 - \sqrt{1 - \frac{1}{n_1^2}}} \]  

A further simplification results with the refractive index of air \( n_a = 1 \). In contrast to the correction used by Demas in (3.14), which uses the approximation \( \sin \theta \approx \theta \) for small angles, the correction factors are systematically higher for a given \( \frac{n_X}{n_r} \). They furthermore depend on the absolute values of \( n_X \) and \( n_r \) for a given \( \frac{n_X}{n_r} \) with larger deviations at lower refractive indices. Given the simplicity of the correction in (3.14), the errors are very small. They are at most 1.5% for the usual refractive index range of solvents (methanol, water, EtOH) between 1.33 and 1.36. If, however, a slightly exotic solvent, such as chloroform \( n_D^{20} = 1.446 \) [14]), is used, the error amounts already to 3.8%.

For the couple EtOH/H_2O, as it was used for the quantum yield determination with quinine sulfate in section 3.3, a ratio of 1.054 is obtained, i.e. an error of -1% in the quantum yield.

3.2.4 Methodology of luminescence and absorbance measurements

Like the discussion on artifacts in 3.2.1 on p. 32, this section will mainly focus on the instruments available in our lab to elaborate a more generally applicable procedure. Luminescence measurements are taken from a very small spot on the mem-

\[ \approx \]
brane. In the case of the PE LS-50B the excited area is approximately 8 by 1.5 mm. The excitation light spot is not exactly at the centre of the membrane, where the membrane is expected to be most homogenous.

Measurements in different orientations of two identically prepared membranes (1.5% ETH\textsuperscript{\textregistered} 3001, 17.3% \textit{o}-NPOE and 81.2% polystyrene) clearly show that membranes cannot be assumed to be homogenous. Fig. 3.9 shows that spectra depend on the membrane’s relative orientation in its plane.

![Fig. 3.9 Luminescence spectra reproducibility between two identical membranes](image)

The relative emission intensity variation for the two identical membranes is as big as 14% for the peak section if no special concern is given to orientation. It is obvious that membranes cannot be compared under these conditions.

If the membrane is not positioned as intended (i.e. facing the sample channel as shown in Fig. 8.3 on p. 239), but reversed (i.e. facing the spectrometer side), the existing inhomogeneities observed in the normal geometry seem to be averaged out. The signal is slightly less intense because the membrane is no longer in focus of the intended emission beam geometry meaning that less emission light actually reaches the emission slit. The defocussing of the excitation beam results in a larger spot size which produces the averaging effect. This reduced the relative emission error to less than 2.5% no matter which orientation was assumed.

The reversed positioning of the membrane of course is not an interesting option because it no longer allows any membrane conditioning other than ambient air. Tests have shown that approximately \( \leq 2\% \) error can be attributed to the positioning of the whole block containing the flow-through cell with the membrane. This will ultimately limit the positioning reproducibility. Different flow-through cell designs also differ in the apparent measured emission. The directly emitted portion depends
heavily on exact positioning of the membrane relative to the emission slit. The reflected portion of the isotropic luminescence, on the other hand, depends to a lesser extent on the distance and much more on the reflectivity of the back-side of the flow-through cell. Changing from a 3rd generation to a 2nd generation flow-through cell (compare Fig. 8.9 on p. 244) for instance increases the signal by 5%. Little can be said on the effect of different membrane surface topologies, e.g. a rough surface.

The emission maxima wavelengths have been determined from emission-corrected spectra by fitting a third-order polynomial to the peak section and taking the polynomial’s maximum. This procedure gives emission maxima wavelengths which are 6-10 nm red-shifted with up to 6% in relative emission intensity change in comparison to simply take the data point with the highest intensity value of uncorrected spectra.

Additionally, luminescence spectra of the two identical membranes were measured on a calibrated Spex Fluorolog Spectrometer (Metuchen, NJ, USA) and compared with the spectra obtained on the Perkin Elmer LS-50 B, see Fig. 3.10.

The membrane in the Spex Fluorolog had to be mounted in an inconvenient 45°/45° geometry which causes high amounts of reflected light. Slit widths chosen were 0.2/0.5 (no nm, instrument units) with an integration time of 0.5 s. The spot size on the membrane was 1 by 8 mm, i.e. smaller than for the absorption spectra or the PE LS-
50B. To compare spectra with those recorded with a cut-off filter on the PE LS-50B, the spectra of a blank membrane (i.e. containing no ETH\textsuperscript{T} 3001) were subtracted. The apparently higher noise level for the Spex Fluorolog, which has a cooled photomultiplier, is due to the default spectra smoothing on the PE LS-50B. The close agreement between the spectra shapes for the two spectrometers is an evidence for the quality of the emission calibration of the PE LS-50B described in 3.2.2 Spectrometer calibration on p. 35.

![Fig. 3.11 Typical membrane with marks for reproducible positioning](image)

The marks are made together with a membrane ID on the back-side of the glass wafer. The picture shows the membrane side up.

An immediate consequence of these findings on membrane inhomogeneity was that every membrane described in this work was position-marked on its back-side (i.e. on the glass wafer) as depicted in Fig. 3.11. All sensor membranes used in this work have received an arbitrary absolute position mark after production which guaranteed an identical orientation in all spectrophotometers. For all measurements (except those in water) the same 3rd generation flow-through cell had been used.

The same reproducibility considerations as for the luminescence spectroscopy are valid for absorption spectroscopy. Therefore, the absorption spectra of the two membranes mentioned above had been measured on three different absorption dual-beam spectrometers. These included the \textit{Uvikon 942} (Kontron Instruments, Zürich) and a \textit{Hitachi U-3210} (Hitachi, Tokio, Japan) available in our lab as well as a \textit{PE UV/VIS/NIR Spectrometer Lamda 900} (Perkin Elmer, Beaconsfield, UK). The major difference of the Lamda 900 to the two others is its comparably large spot size of 2 by 11 mm. Reference for all measurements was air, the glass wafer was held by a securely mounted optode cell perpendicular to the light beam. It is expected that the spectra should be the same for all spectrometers since identical membranes in the same position had been scrutinized.
A difficult question is with respect to which baseline the absorbance of the dye in the membrane should be measured. The absorbance background in the wavelength range of 500 to 800 nm depends on the nature of the glass support and is a mixture of reflection, scattering and absorption. For quartz glass, there is essentially a flat baseline, whereas for soft glass the absorption is increasing from 550 nm onwards by ~0.02.

For quartz glass supports the flat range around 700 nm can be safely taken as zero-line. Absorption spectra of membranes on soft glass usually have a local minima around 600 nm, which was taken as zero reference. This can diminish the dye’s absorbance by an amount in the order of 3% because there is still some residual absorbance of the ETH$^T$ 300x dyes. The two absorption maxima of ETH$^T$ 300x dyes smudge and cannot be clearly discerned. Therefore, only one maximum wavelength is reported for absorbance measurements of membranes.

Absorbances of the two membranes were determined as $0.270 \pm 0.007$ and $0.259 \pm 0.007$, respectively. The error of the determination on different absorption spectrophotometers is ~3%~. i.e. in the same order as the emission determination error. The same difference is also obtained for comparing the absorbance of the two membranes on the same absorption spectrophotometer which means that they most probably have a 3% difference in both absorbance and emission. Please note that comparisons on the same absorption spectrophotometer are better than 3%. Absorbance deviations for solutions were usually below 0.001.

![Absorption spectra of two identical membranes recorded on three different absorption spectrometers](image-url)
It is interesting to note that the absolute absorbance difference of the two membranes on all three spectrometers is minimal at the absorption maximum of 460 nm (ca. 0.005) and rises to either side to a maximum around the absorption minima of ~600 nm and ~370 nm (ca. 0.014).

An oblique position of the glass wafer relative to the measuring light beam can largely increase the background. There has been no noticeable difference between the two possible membrane orientations, i.e. the membrane or the glass side facing the detector.

The Hitachi U-3210 has the poorest optical quality of all the absorption spectrophotometer investigated, especially discontinuities in the spectra seriously interfere. Its effectively usable wavelength range is 250 to 800 nm, whereas the Uvikon 942 can be used from 200 to 900 nm. The Uvikon 942 has therefore been used for all absorbance measurements of membranes. Absorption spectra reported here have not been background-corrected.

### 3.3 Quantum yield determination in solution

The determination of quantum yields is dominated by the review of Demas and Crosby in 1971 [1], little has been published on this subject since. Rhodamine 101 was eventually chosen as quantum yield standard for this work for several reasons. It is easily soluble in ethanol and other solvents which dissolve the ETH T 300x dyes, which precludes uncertainties associated with refractive index corrections [17]. Its emission around 590 nm is very close to the one of the ETH T 300x dyes, which will minimize any errors due to the emission calibration. Its quantum yield of unity has been mentioned by Drexhage [18], who is usually cited as reference for the use of Rhodamine 101 as a quantum yield standard. He had measured its quantum yield with respect to fluorescein, for which he assumed a quantum yield of 90%. Independent measurements carried out by Karstens et al. seem to strongly support the quantum yield of 1.00 for Rhodamine 101 [19]. They had investigated the dye’s fluorescence at several excitation wavelengths as well as temperatures down to 150 K and found virtually no temperature dependence of the quantum yield, whereas Rhodamine B’s quantum yield was increasing up to the one of Rhodamine 101 at temperatures below 200 K. It is very reasonable to assume that this quantum yield level at below 150 K is indeed unity. They stated that the quantum yield is not influenced for concentrations up to $3 \times 10^{-6}$ M, i.e. self-absorption is negligible.

There are other quantum yield standards outside this spectral range, especially quinine bisulfate [1, 10] and 9,10-diphenylanthracene [17] which have been investigated in depth and frequently applied; the former has also been used in this work. Demas and Crosby recommend Melhuish’s $\Phi_L$-value of 0.546 of quinine sulfate in
1.0 N sulfuric acid for 365 nm excitation at 25°C. This quantum yield is constant to 
±5% for excitation between 200 and 400 nm for quinine sulfate concentrations 
below 10⁻⁴ M, with no overlap between excitation and emission. The solution 
becomes photoinstable if excited below 300 nm. The quantum yield is dependent on 
c(H₂SO₄) with a +6 to 8% increase in 1N with respect to 0.1N H₂SO₄, is quenched 
by halides and shows a -0.25%/°C temperature dependence.

The greatest experimental challenge for the quantum yield determination of oxy-
gen-quenched compounds is the degassing procedure. Degassing is the only way to 
have a controlled oxygen concentration in a solution, i.e. to have no oxygen at all 
present. If degassing lasts too long, the solvent will evaporate which will increase 
the dye concentration and decrease temperature. Another undesired side effect of 
prolonged degassing may be water condensation. On the other hand, if degassing is 
not complete, meaningless results are obtained. A 0.8 mm syringe needle was put 
vertically into the cuvette to guide the gas flow. The argon (or nitrogen) flow was 
adjusted to slightly agitate the solution. Fig. 3.13 shows the temporal evolution of 
active degassing and passive oxygen uptake of an approx. 10⁻⁶ M solution of 
ETH T 3001 in ethanol.

Degassing in ethanol seems to be complete after 2 to 3 minutes. With no further 
measures, oxygen uptake will resume immediately leading to a very fast (t₁/₂ = 
1.8 min) signal decay down to 5% of the signal of the deoxygenated solution. Note 
that this is in slight disagreement with the value determined later in Table 3.3 on 
p. 56. Fluctuations within the decay are very large, probably because the oxygen 
concentration at the site of the excitation beam is governed by convection rather 
than diffusion. If a slight nitrogen flow in the headspace of the cuvette was main-
tained after degassing of the solution a stable emission signal resulted for the half an
hour-duration of the experiment, i.e. the zero oxygen concentration could be maintained.

The cuvette holder was thermostated at 25 ± 0.1°C which was confirmed in-site within 0 to -0.7°C with a GREISINGER electronic GTH 1200A digital thermometer with a NiCr-Ni thermoelement. Degassing led to a rather linear temperature decrease of -1°C in 2 min after which it remained stable. When degassing stopped, temperature recovered within 3 to 4 min.

The effect of possible water condensation has been tested by adding water to an aerated 10⁻⁵ M solution of ETH T 3001 in ethanol (absolute, pro analysi) which had been deoxygenated earlier. Adding consecutively two drops of water (corresponds to ~1 and 2% v/v) gave an emission red-shift of 4.5 and 6 nm and an emission change of -6% and -9%, respectively. However, no \( \lambda_{\text{max,em}} \) change has been observed during the whole quantum yield determination procedure, indicating that no water condensation had taken place.

Solution level measurements have shown that 5-10% of the ethanol have evaporated during the described degassing procedure. This gives an apparently higher quantum yield.
yield and has to be corrected for. The following procedure has been established. The emission is recorded in a timedrive with the initially aerated solution. After about 100 s, the syringe needle is placed into the solution and deoxygenation with the inert gas is started. The emission increase is monitored and as soon as the emission reaches a constant level (within 2-3 min), the syringe needle is replaced by a small tube which purges only the cuvette headspace. After the desired spectra is recorded under deaerated conditions, a new timedrive is started. The headspace purging is maintained for another 100 s to check whether the signal level is stable. Furthermore, this makes it possible to accurately determine the luminescence level in the degassed solution. The solution is then gently aerated by air blown from a Pasteur pipette until the solution is air-saturated. The timedrive is continued for another 100 s to determine the signal level of the aerated solution. The signal ratio $S_{after}/S_{before}$ in the aerated solution is proportional to $c_{after}/c_{before}$ and allows to determine the effective concentration at the time of the deoxygenated conditions.

If one is not bound to a particular solvent because of the quantum yield standard, ethanol is not a very suitable choice. Oxygen solubility in ethanol at 20°C is 2.1 mM, which is for instance ten times the one in water [20, 21]. A lower oxygen solubility and a lower vapor pressure would facilitate the degassing procedure.

To take advantage of the full sensitivity of the luminescence measurements the standard and sample solution should have a similar luminescence level. For the PE LS-50B’s standard slit combination 2.5/5 nm this is an approx. $10^{-7}$ M solution for a substance with a quantum yield of unity and an absorption coefficient of $\varepsilon \approx 10^5 \text{ M}^{-1}\text{cm}^{-1}$. These solutions have a too small absorbance to give accurate absorbance values, so they should be prepared from mother solutions with absorbances in the range of 0.1 to 0.5. Mother solutions for the absorbance measurements were $10^{-5}$ M for the ruthenium(II) diimine complexes (3.34, 3.84 and 5.03 mg/250 ml) from which gravimetrically diluted $5 \times 10^{-7}$ M solutions for the luminescence measurements were made, all in ethanol from Merck (absolute, pro analyti). For Rhodamine 101 (Fluka Biochemika) a $5.01 \times 10^{-5}$ M (6.15 mg/250 ml) mother solution was freshly prepared, from which a $6.52 \times 10^{-6}$ M solution for absorbance and from which a $1.195 \times 10^{-7}$ M solution for luminescence measurements was made. All solutions were freshly prepared.

Bard et al. found adsorption of $[\text{Ru(dpp)}_3]^{2+}$ at $\mu$M-concentrations in acetonitrile to both polyethylene and glass [22]. No evidence for a similar phenomena was found for the more concentrated solutions of ETH’ 300x dyes.

Absorption spectra have been recorded on the Uvikon 942 absorption spectrophotometer with a bandpass of 2 nm in 10 x 10 mm Suprasil™ cuvettes, reference was ethanol. Luminescence spectra have been recorded on the PE LS-50B luminescence spectrophotometer with a 2.5/5 nm slit combination with no cut-off filters in place and identical photomultiplier voltage. The 515 nm cut-off filter gave a signal
decrease (-9.7% at $\lambda_{\text{max}} = 589$ nm) for the Rhodamine 101 spectrum and was therefore not used. The same 10 x 10 mm Suprasil™ cuvette in a black cuvette holder has been used for all luminescence measurements. The resulting luminescence spectra are shown in Fig. 3.15. The small difference in the bandpasses of the two spectrometers does not matter because of the very broad-banded spectra.

![Graph](image)

**Fig. 3.15 Quantum yield determination of different ruthenium complexes**
Arrows mark the respective excitation wavelengths.

Very important is a proper post-processing of the obtained data. The luminescence spectra have to be transformed from the wavelength scale to an energy scale according to

$$I(\tilde{\nu}) = \nu^2 I(\lambda) \tag{3.13}$$

to obtain $[I(\tilde{\nu})/\Delta\tilde{\nu}]$ from $[I(\lambda)/\Delta\lambda]$ intensities. This accounts for the constant bandpass in nm (2.5 nm in the case of the PE LS-50B) which decreases in cm$^{-1}$ as the square of the exciting wavelength [8].

According to Demas et al. the quantum yield $\Phi_{L,x}$ of a substance $X$ is calculated as

$$\Phi_{L,x} = \Phi_{L,r} \cdot \left( \frac{A_r(\lambda_r)}{A_x(\lambda_x)} \right) \left( \frac{I(\lambda_r)}{I(\lambda_x)} \right) \left( \frac{n_x^2}{n_r^2} \right) \left( \frac{D_x}{D_r} \right), \tag{3.14}$$

where the subscripts $r$ denote the reference and $x$ the substance whose quantum yield is determined [1]. $A(\lambda)$ is the absorbance at the exciting wavelength, $I(\lambda)$ is the relative intensity of the exciting light, $n$ is the average refractive index of the solu-
tion to the luminescence and $D$ is the integrated area under the corrected emission spectra. $\Phi_{L,r}$ of Rhodamine 101 is assumed to be 1.0 as stated above. Both the $I(\lambda)$ and the $n$-term can be set to 1. The standard excitation correction for the PE-LS 50B already performs the necessary quanta correction, whereas the effect on the refractive index of the very diluted and weakly absorbing solutions in the same solvent (ethanol) can be neglected.

Table 3.2 contains all the relevant parameter for the quantum yield determination. $c_{\text{abs}}$ and $c_{\text{lum}}$ are the concentrations of the solutions which have been used for the absorbance and luminescence determination, respectively. $c_{\text{abs}}$ is calculated based on the molar masses in Table 2.1. $A_{\text{abs}}(\lambda_{\text{exc}})$ is the absorbance of the solution for absorbance measurements, with $\lambda_{\text{exc}} = 460$ nm for the ruthenium complexes and 565 nm for Rhodamine 101. $c_{\text{lum}}/c_{\text{abs}}$ is the gravimetric dilution factor used to calculate the absorbance $A_{\text{lum}}(\lambda_{\text{exc}})$ of the solutions used for the luminescence measurements which cannot be directly measured. $S_{\text{after}}/S_{\text{before}}$ is the emission signal ratio of the aerated solution before and after degassing with argon which accounts for the evaporation of ethanol. D finally denotes the integral below the emission-corrected, bandpass-corrected emission spectra plotted against wavenumbers. The 2.7% of the Rhodamine 101 luminescence spectrum integral which are below the excitation wavelength (565 nm) have been considered. Measurements with excitation wavelengths shifted to lower wavelengths or with a blank ethanol solution indicate that the scattering peak at 565 nm does not contribute more than 1% to the total integral of Rhodamine 101.

<table>
<thead>
<tr>
<th>Substance</th>
<th>$c_{\text{abs}} / M$</th>
<th>$c_{\text{lum}} / c_{\text{abs}}$</th>
<th>$A_{\text{abs}}(\lambda_{\text{exc}})$</th>
<th>$D$ / a.u.</th>
<th>$S_{\text{after}}/S_{\text{before}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="ClO$_4$">Ru(dpp)$_3$</a>$_2$</td>
<td>1.03·10$^{-5}$</td>
<td>4.9579·10$^{-2}$</td>
<td>0.3379</td>
<td>1.2485·10$^6$</td>
<td>1.203</td>
</tr>
<tr>
<td>ETH$^T$ 3001</td>
<td>9.91·10$^{-6}$</td>
<td>4.9447·10$^{-2}$</td>
<td>0.3238</td>
<td>1.1245·10$^6$</td>
<td>1.206</td>
</tr>
<tr>
<td>ETH$^T$ 3003</td>
<td>1.01·10$^{-5}$</td>
<td>4.8735·10$^{-2}$</td>
<td>0.3430</td>
<td>1.2359·10$^6$</td>
<td>1.104</td>
</tr>
<tr>
<td>Rhodamine 101</td>
<td>6.52·10$^{-6}$</td>
<td>1.8328·10$^{-2}$</td>
<td>0.5249</td>
<td>1.1465·10$^6$</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3.3 Results of the luminescence quantum yield determinations in ethanol

<table>
<thead>
<tr>
<th>Substance</th>
<th>$\Phi_{L}$ in deaerated ethanol</th>
<th>$S_{\text{deareated}}/S_{\text{areated}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><a href="ClO$_4$">Ru(dpp)$_3$</a>$_2$</td>
<td>0.52</td>
<td>16.8</td>
</tr>
<tr>
<td>ETH$^T$ 3001</td>
<td>0.49</td>
<td>18.4</td>
</tr>
<tr>
<td>ETH$^T$ 3003</td>
<td>0.56</td>
<td>20.3</td>
</tr>
</tbody>
</table>
The modified formula for the quantum yield determination with the mentioned parameters hence becomes

\[
\Phi_{L,x} = \Phi_{L,r} \left( \frac{A_{r,\text{abs}}(\lambda_{r,\text{exc}})}{A_{x,\text{abs}}(\lambda_{x,\text{exc}})} \right) \left( \frac{c_{\text{lum}}/c_{\text{abs}}}{c_{\text{lum}}/c_{\text{abs}}}_r \right) \left( \frac{D_x}{(S_{\text{after}}/S_{\text{before}})_x} \right). \tag{3.15}
\]

The results of this calculations as well as the emission signal ratio \(S_{\text{after}}/S_{\text{before}}\) are listed in Table 3.3. A discussion of the values is given in 2.4.2 Luminescence quantum yields on p. 22.

An earlier, completely independent investigation with the same procedure had resulted in a \(\Phi_L\)-value for ETH\(^T\) 3001 of 0.64, when considering the newer Rhodamine 101 reference data a value of 0.56. The main differences to the newer determination is the lack of an evaporation correction and the use of the 515 nm cut-off filter. Both effects decrease the calculated quantum yield. Considering a decrease of -9.7% due to the use of the cut-off filter and assuming a very reasonable \(S_{\text{after}}/S_{\text{before}}\) of 1.18, one would obtain 0.49 as for the newer determination. If one takes the newer Rhodamine 101 reference data a \(S_{\text{after}}/S_{\text{before}}\)-value of 1.14 results if an identical quantum yield is assumed. The corresponding data used for the determination is listed in Table 3.4

<table>
<thead>
<tr>
<th>Substance</th>
<th>(c_{\text{abs}}/M)</th>
<th>(c_{\text{lum}}/c_{\text{abs}})</th>
<th>(A_{\text{abs}}(\lambda_{\text{exc}}))</th>
<th>(D/\text{a.u.})</th>
<th>(S_{\text{after}}/S_{\text{before}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>ETH(^T) 3001</td>
<td>1.25(\times)10(^{-5})</td>
<td>7.12(\times)10(^{-2})</td>
<td>0.4211</td>
<td>2.018(\times)10(^{6})</td>
<td>n.d.</td>
</tr>
<tr>
<td>Quinine sulfate</td>
<td>1.004(\times)10(^{-4})</td>
<td>3.401(\times)10(^{-2})</td>
<td>0.3564</td>
<td>1.581(\times)10(^{6})</td>
<td>1</td>
</tr>
<tr>
<td>Rhodamine 101</td>
<td>9.43(\times)10(^{-7})</td>
<td>8.667(\times)10(^{-2})</td>
<td>0.0769</td>
<td>7.002(\times)10(^{5})</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 3.4 Parameters for earlier quantum yield determinations of ETH\(^T\) 3001

Another earlier determination for ETH\(^T\) 3001 using the same procedure with quinine sulfate dihydrate (Fluka, purum) in 1.0 N H\(_2\)SO\(_4\) (Fluka, puriss p.a., Cl\(^-\) \(\leq 0.00001\%) gave a quantum yield of 0.29 with the older and 0.25 with the newer ETH\(^T\) 3001 data based on Melhuish’s \(\Phi_L\)-value of 0.546 for 365 nm excitation.

This values are with the refractive index correction in (3.14). The validity of the correction given in (3.14) has been discussed in section 3.2.3. If we assume that the debated \((n_x/n_p)^2\)-factor correction is correct, we need the refractive index of either solution averaged of the emission peaks. The Kramers-Kronig transformation allows to calculate refractive indices based on absorption spectra. The problem rests
3.3 Quantum yield determination in solution

in the integration from 0 to $\infty$ nm, because no absorbance data is available from 0 to 200 nm. Nevertheless the estimates are usually good, especially if boundary values are close to zero. Michael Linhoff has written the C program kram2dn1.exe which calculates the estimated relative refractive index $(n_1/n_0)$, where $n_0$ is the refractive index of the pure solvent and $n_1$ the refractive index of the solvent with an absorbing compound dissolved. Based on literature values of the solvents at a given wavelength, one can calculate an estimate for the refractive index correction. $n_D$ for ethanol is 1.3611 and for water 1.333 [15], the index D denotes the standard 589 nm line.

Based on the absorption spectra of the mother solutions (as listed in Table 3.4), the program yields $(n_1/n_0)_D = 1.0417$ for ETH$^T$ 3001 and 1.0107 for the quinine sulfate as anchor values at 589 nm with a $(n_1/n_0)_{em}$ weighted average over the emission peak of 1.0322 and 1.0085, respectively. This estimate of the absorption effect modifies the expression $(n_x/n_r)^2$ only by a factor of 1.0015. This value needs to be corrected for the more diluted solutions which have actually been used for the luminescence measurements based on the linearity of the Kramers-Kronig transformation. Considering the dilution factor $c_{lum} / c_{abs}$ the true $(n_1/n_0)$-factors are even a single order of magnitude smaller and hence completely negligible. What remains is $(n_x/n_r)^2$ which is 1.043, i.e. a rather small increase in quantum yield.

The procedure is rather complicated and is best avoided by choosing a quantum yield standard in a solvent which is compatible with the investigated dye. The low quantum yields determined with quinine sulfate should be regarded with great care. Emission calibration factors (compare Fig. 3.4 on p. 39) differ by a factor of 3.5 for the emission maximum of quinine sulfate and ETH$^T$ 3001 and even up to a factor of 8.5 within the 50% intensity range of both spectra! The emission correction within that range can be easily several 10% off the actual value with respective errors in the quantum yields. Furthermore, experimental conditions at that time had not been as optimized as for later determinations with Rhodamine 101.

What are the effects if the outlined procedure for the emission spectra treatment is not accurately observed? The worst case is if spectra, which have not been emission-corrected, are integrated against the wavelength. For the Rhodamine 101 standard 84% of the correct quantum yield are obtained, for the quinine sulfate standard only 38%! The same order of magnitude of error applies if the energy conversion and bandpass correction have been properly done, but without initial emission correction. On the other hand, if emission-corrected spectra are integrated against the wavelength for either standard, the results deviate less than 2‰ from the correct result. This illustrates the importance of a proper emission correction and the selection of a quantum yield standard in the same spectral region as the dye whose quantum yield is measured. If spectra are correctly emission-corrected and integrated
against wavenumbers, but not bandpass-corrected, 89% of the correct quantum yield is obtained.

We realize that errors in emission spectra treatment can drastically decrease the calculated quantum yield, but in no case increase it. The values given in Table 3.3 on p. 56 are probably good to ±0.05 and should be taken as the true quantum yields of the ETHT 300x dyes.

### 3.4 Quantum yield determination in membranes

The quantum yield of ruthenium(II) diimine dyes in any polymer is likely to be different from those in solution. Unfortunately, there is no solid quantum yield standard available for measurements in polymers. Rhodamine 101 for instance is a poor choice as a standard in a polymer. It can form a non-fluorescing lactone in an apolar media to an uncontrollable extent, like it has been shown for Rhodamine B [23]. The protonated or the zwitterion form alone fluoresce. Preliminary experiments with a membrane containing 1.6‰ Rhodamine 101 in polystyrene (ca. 3.5 mM) excited at 565 nm gave still a reasonable intensity, but hard-to-correct scattering. Going to excitation at lower wavelengths partly remedies the scattering problem, but drastically reduces the signal level. This illustrates that really dilute “solutions” are not feasible, because scattering prevails even with very good optics, not to mention the problems to accurately measure the absorbance. The issue is in any case complex, because the dye distribution within a polymer remains largely unknown. One would have to resort to higher dye concentrations in the polymer, with self-absorption distorting the result.

Thomson et al. have measured the quantum yield in a membrane by comparison with a reference solution [2]. They used a 1 mm path cell filled with a standard solution of Rhodamine B in a linear excitation-emission geometry. Preliminary experiments with a 1 mm cuvette filled with a 1.8·10^{-5} M Rhodamine 101 solution in ethanol have shown that this is far too inaccurate, at least for a 30°/60°- geometry as it is available in the PE LS-50B. A 1 mm 110-QS Suprasil™ cuvette (total thickness 3.5 mm) had been scotched to the backside of the optical window of the flow-through cell holder, with the cell removed, compare Fig. 8.3. A 565 nm-excitation gave for both an empty or filled cuvette a far more intense scattering than the 0°/90°-geometry for the quantum yield determination in solution. Therefore, a 500 nm or 460 nm-excitation combined with a 515 nm cut-off filter was used. This has no influence on the quantum yield which is constant for any excitation wavelength between 450 and 565 nm [19]. Shifting the cell block with the cuvette perpendicular to the cuvette’s surface gave extreme variations in emission intensity as can be seen in Fig. 3.16. The relative position marks the centre of the cuvette (front wall thickness 1.25 mm) relative to
the usual membrane position (thickness of membrane support glass is 1.95 mm). Shifting only 1 mm in either direction can cut down luminescence to 10%. It is noteworthy that the usual position is not optimized for maximum intensity, but should be placed a 1 mm further away from the instrument.

Another interesting experiment was simply covering the back-side of the cuvette with brown cardboard to imitate the flow-through cell, thereby tripling the apparent luminescence emission, accompanied by a more than six-fold increase in scattered light. Even though the geometry was obviously identical, the missing scattering plane behind the cuvette would have underestimated the emission intensity with respect to the membrane. Considering these findings such determinations using membranes and cuvettes must be regarded with great care.

The approach in this work has been to compare the polystyrene membrane emission with one of a “liquid” membrane, a concept which is illustrated in Fig. 3.17.

The upper part of the illustration shows the quantum yield determination as it is commonly known. The ratio of the integrated emission intensity divided by the absorption at the excitation wavelength ($D/A_{exc}$) of both the sample and a reference with a known quantum yield is compared, which allows to calculate the sample’s quantum yield. This has been done in 3.3 Quantum yield determination in solution on p. 51.

For obvious reasons we need a different set-up to measure the emission of the polymer membrane and a standard in an identical geometrical setting. In our case the task of the cuvette has been assumed by a reproducibly placed flow-through cell, compare Fig. 8.3 on p. 239 and Fig. 8.10 on p. 245.

For the above-mentioned reasons there is no reliable solid quantum yield standard which is why I have turned to Rhodamine 101 in ethanol, as for the quantum yield
determinations in ethanol. The special construction of our flow-through cell with is very shallow lumen of 0.20 mm, sealed of by a glass wafer as used for the polymer membrane, enabled us to have the standard solution in almost identical position and dimensions as the polymer membrane; that is, a \textit{liquid membrane}.

Going from the standard solution to the liquid membrane involves only geometrical changes, because the chemical nature of the solution and hence Rhodamine 101’s quantum yield will remain unchanged. We can calculate a geometrical factor

\[
k_{\text{geom}} = \frac{(D/A_{\text{exc}})_{\text{Rhodamine 101 in cuvette}}}{(D/A_{\text{exc}})_{\text{liquid membrane}}}
\]  

(3.16)

which reflects this change in geometry. The experimental value is 1.90, i.e. there is more emission than for a cuvette even though calculations in 3.2.3 \textit{Refractive index corrections} on p. 41 suggested a factor of 0.59. This factor 3 discrepancy can be easily explained by a position slightly out of focus and above all by the reflecting power of the flow-through cell behind the membrane, compare the brown cardboard experiment above.

The interesting step is going from the liquid membrane to the polystyrene membrane which involves the quantum yield in polystyrene \( \Phi_{\text{L,PS}} \) and an optical factor \( k_{\text{opt}} \):

\[
\Phi_{\text{L,PS}} \cdot k_{\text{opt}} = \frac{(D/A_{\text{exc}})_{\text{PS}}}{(D/A_{\text{exc}})_{\text{liquid membrane}}}. 
\]  

(3.17)
The optical factor stems from the considerably higher refractive index of the membrane \( n_D(\text{PS}) = 1.5894 \) compared to the liquid membrane, i.e. ethanol \( n_D(\text{ethanol}) = 1.3611 \). This refractive index correction has also been calculated in section 3.2.3 and amounts to 0.661. Note that no provisions were made for light scattering of the colored membrane, which is described for instance in [24].

A membrane pair with 1.45\% ETH T 3003 in PS with an absorbance of \( \sim 0.11 \) had been used for the investigation. Measurements were carried out in nitrogen atmosphere. Polystyrene was used as membrane reference for all subsequent measurements in nitrogen atmosphere. In polystyrene a quantum yield of 0.19 was obtained in nitrogen atmosphere. This value is probably good to \( \pm 0.05 \). This value is not discussed at this point, but in the sections 4.4.1 Plasticized polystyrene membranes on p. 85 and 4.4.2 Thermoplastic polymer membranes on p. 92 where individual values are compared.

A problem of this approach is that the liquid membrane’s absorption is calculated based on the flow-through channel’s depth and Rhodamine 101’s absorption coefficient. It is not directly measured as the PS membrane’s absorbance, which is obviously not as accurate. Having the results in Fig. 3.16 in mind, it is difficult to estimate the effect of the liquid membrane’s larger spatial extension of 0.20 mm, but these changes in the excitation/emission light collection efficiency in comparison to the ca. 30 times thinner membrane are likely to be negligible. Although the Rhodamine 101 solution used is too concentrated for usual quantum yield determinations, reabsorption and to a lesser degree re-emission is negligible because of the very thin solution thickness.

The liquid membrane was a \( 2.25 \cdot 10^{-6} \) M Rhodamine 101 solution in ethanol, which was pumped into a latest generation flow-through cell (see Fig. 8.10). This was covered with a soft glass wafer identical to the one used for the PS membrane support. Visual observation guaranteed that the lumen was bubble-free. The CNC-fabricated flow-through channel for the liquid membrane had the expected depth of 0.20 mm at the centre, but gave readings of up to 0.35 mm towards the edges with a vernier caliper. Given the small lateral extension of the excitation beam of 1.5 mm compared to the channel’s width of 10 mm, the 0.20 mm-value is probably accurate to 0.01 mm. Based on an experimentally determined absorption coefficient for Rhodamine 101 of \( 8.05 \cdot 10^4 \) M·cm\(^{-1}\) at 565 nm, an absorbance of \( 3.62 \cdot 10^{-3} \) was calculated.

The spectra of the liquid membrane corrected for scattered light showed no qualitative difference to the solution spectrum of Rhodamine 101.

Noteworthy is that scattering is identical for the \( 2.25 \cdot 10^{-6} \) M Rhodamine 101 solution in ethanol and for ethanol alone. This suggests that scattering mainly takes place at the solution/glass interface and not at the flow-through cell or in the solu-
tion itself. Moreover, very little scattering occurs in an empty flow-through cell with an uncoated glass wafer. This makes it possible to use consistently a 565 nm excitation for the Rhodamine 101 solutions in cuvette and the “liquid membrane”. A smaller excitation wavelength would give a better excitation peak separation, but would also yield a much smaller signal and introduce another unnecessary difference between experiments.

3.5 Summary and Perspectives

Rhodamine 101 in ethanol ($\Phi_L = 1.0$ [18, 19]) is proposed as the most credible quantum yield standard for the quantum yield determination of ruthenium(II) diimine complexes. The quantum yield of 1.0 is supported by its temperature dependence [19] and can be plausibly explained as due to the rigid structure of Rhodamine 101, which contrasts with that of other xanthene dyes [18]. Ethanol is a solvent in which all Ru(II) complexes are readily soluble, so there is no need for refractive index corrections [1]. The emission of Rhodamine 101 ($\lambda_{max} = 590$ nm) is very close to those of Ru(II) complexes, which means that the error arising from emission spectra calibration is minimized.

The quantum yields of $[\text{Ru}($dpp$)_3](\text{ClO}_4)_2$, ETH$^\text{T}$ 3001 and ETH$^\text{T}$ 3003 in deoxygenated ethanol have been determined as 0.52, 0.49 and 0.56, respectively. These estimates are reliable within ±0.05 and have been confirmed by two individual determinations, with the largest single error contribution coming from the emission spectra calibration. Even small amounts of water significantly changed the emission of ETH$^\text{T}$ 3001. ~1% v/v water lead to an emission decrease of -6% and a red-shift of $\lambda_{max}$ of 4.5 nm.

The importance of a proper degassing procedure has been demonstrated by errors as large as +21% of the quantum yield if uncorrected. Systematic errors in spectra treatment, such as poor spectra emission calibration errors or missing bandpass corrections, are shown to decrease the calculated quantum yield.

A procedure to determine quantum yields of dyes in a polymer membrane has been presented which uses a virtually identical arrangement of a Rhodamine 101 standard solution and the polymer membrane spin-coated on a glass wafer in a custom-made flow-through cell. The effect of refractive index corrections has been simulated and can amount to a factor of 1.9 for polymers. For polystyrene derivatives, however, corrections account only for a few percent. Since the method is based on the integrated emission intensity obtained in a flow-through cell for the luminescence spectrometer, quantum yields of other membranes can be easily determined by comparing the ratios of integrated emission intensity and absorbance with the quantum yield of ETH$^\text{T}$ 3003 in polystyrene. The latter has been determined as 0.19 in a nitrogen atmosphere, which is probably reliable within ±0.05.
Similar procedures using cuvettes showed experimental errors of a single order of magnitude because of different reflection properties, as well as positioning errors. These procedures are, therefore, not recommended.

Spin-coated polymer membranes are inhomogeneous on a mm-scale and need an absolute position mark to allow *intra* and *inter* membrane comparisons. With this position mark, the absorbance and luminescence intensity of embedded Ru(II) dyes can be determined with a least 2% accuracy.

### 3.6 References


4 Luminescence-based oxygen optodes

4.1 Introduction

Luminescence-based oxygen optodes are among the best-investigated class of chemical sensors [1, 2]. Unfortunately, this considerable research effort has not yet resulted in many actual applications, where these optodes are routinely used. In this chapter mostly chemical aspects of oxygen optodes are discussed, but it is important to understand the technical realization of such a sensor as well.

Luminescence quenching can be measured either based on the luminescence intensities $I$ or on the luminescence lifetimes $\tau$ (see also section 2.1 on p. 7 for the photo-physics of ruthenium(II) diimine dyes). Either the intensity or the lifetime will decrease upon quenching. This process is usually described by modified Stern-Volmer kinetics which are discussed in section 4.2 on p. 71. The main difference between the two measurement methods is that luminescence intensity is an extensive quantity whereas lifetime is an intensive quantity. Extensive quantities, such as volume or energy, depend on the size of the sample whereas intensive quantities, such as temperature or concentration, do not. The advantage of using lifetimes to determine luminescence quenching is obvious. Intensity fluctuations of the excitation light source, changing sample reflectance, dye loss and the like all have no influence on the lifetime. This means that using the luminescence lifetime method is the only way of achieving a reasonable batch-to-batch reproducibility.

Lifetimes can be determined either by pulsed or modulated methods [3]. A short light pulse is used to excite the dye, and then the transient luminescence decay is recorded. Fitting exponential decay curves to the transient allows the luminescence lifetimes to be extracted. This is mostly used for laboratory determinations because it reveals much information about the nature of the decay; however, the experimental effort is considerable. Most sensor set-ups determine the lifetime by phase measurements. A sinusoidally modulated excitation light source, usually a LED, excites the dye. After a delay given by the dye’s luminescence lifetime, the dye will emit. The result is also a sinus-modulated intensity, but with a phase shift with respect to the excitation light source which is proportional to the luminescence lifetime.

For sensor purposes, the luminescent indicator dyes need to be immobilized. This is usually done by co-dissolving the dye with the polymer or polymer precursors in which it is embedded, adsorbed or covalently linked.

Ruthenium(II) diimine dyes have been used in a wide range of polymers, such as polystyrene (PS) [4-6], poly(methyl methacrylate) (PMMA) [5], cellulose acetate
and cellulose acetate butyrate [5], poly(acrylamide) [7], and poly(vinyl chloride) (PVC) [5]. Very popular are polysiloxanes of all kind, especially sol-gels [8, 9] and silicone rubbers [10, 11].

The construction of a luminescence-based oxygen sensor with ruthenium(II) diimine dyes is greatly facilitated by the dyes’ properties which explains much of their popularity, for examples see [12, 13]. Irradiation in the main absorption band around 450 nm can be done with inexpensive blue light-emitting diodes (LED) whose emission spectra matches the dyes’ excitation spectra very well (compare Fig. 8.8 on p. 243). The dyes’ high quantum yield gives rise to reasonably large signal levels, which help to ensure a good signal-to-noise (S/N) ratio. Finally, the large Stokes shift, i.e. the large spectral separation of dye excitation and emission, allows the use of inexpensive optical filters to discriminate the emission light from residual excitation light.

The Oxygen MicroOptode combined with the MICROX I oxygen meter (Precision Sensing GmbH, Neuburg a. d. Donau, Germany) is one of the few successful commercial applications of ruthenium-based oxygen-sensing. Originally developed for oxygen gradient measurements in marine sediments, it is useful for any oxygen measurement with a high spatial resolution [14, 15]. Its relative success most probably stems from the fact that it was tailor-made for a niche application. It uses \([\text{Ru(dpp)}_3\text{ClO}_4\)]^2 in different polymers which are not disclosed, with a yellow LED as a reference and a blue LED as an excitation source, silica fibers, optical filters and a photo multiplier tube as a detector.

There are a few oxygen optodes based on absorbance, but they are practically irrelevant because of their low sensitivity. A fairly recent example is cobalt(II) porphyrin which can bind oxygen reversibly to form oxo-adducts to the cobalt(II) centers [16].

Unfortunately, most researchers in this area have not been very interested in the long-term behavior of the luminescence-based oxygen sensors they describe. Of crucial importance here is, for instance, the photobleaching of the indicator dye which, given its importance, is discussed in the separate chapter 5 Photobleaching of ruthenium(II) diimine dyes on p. 119.

Another issue is the undisputed need for sterilizable sensors for almost all industrial applications, especially those in biotechnology and medicine, which are potential application fields which have been frequently advocated in papers. Few sensor membranes are actually sterilizable or have been investigated with respect to sterilization, at least, as far as we know from the literature.

If the ruthenium(II) diimine dye is dissolved in a polymer, the polymer has to meet two conditions. First, it must be structurally stable to support mechanical stress during sterilization at 121°C or higher, depending on the sterilization conditions. Second, it has to effectively prevent dye migration within the membrane, which would
be likely to stop both dye aggregation and leaching. One parameter which describes quite well when a change in the morphology of polymers takes place is the so-called glass transition temperature $T_g$, which is introduced in section 4.3.1 on p. 80. A glass transition temperature which is well beyond the sterilization temperature indicates that the polymer is suitable for sterilization.

Suitable thermoplastic polymers, keeping this requirement in mind, are discussed in 4.3.2 on p. 81 and polysiloxanes are considered briefly in section 4.3.3 on p. 83. Based on the very favorable properties of polystyrene known from polymer dyeing, a series of polystyrene derivatives was investigated for their use in luminescence-based oxygen sensors with ETH$^T$ 3003 (tris(4,7-bis(4-octylphenyl)-1,10-phenanthroline)-ruthenium(II) perchlorate monohydrate). Ruthenium(II) diimine dyes had already been investigated in polystyrene (PS) [4, 6].

Finally, polystyrene and its derivatives poly($\alpha$-methylstyrene), poly($p$-tert-butylstyrene), poly(4-methoxystyrene), poly(2,4,6,-tribromostyrene), as well as two other polymers with a high $T_g$, namely, poly(2,6-dimethyl-$p$-phenylene oxide) and poly(bisphenol-A-carbonate), were investigated in depth. The relative quantum yield with respect to polystyrene, with the Stern-Volmer constants determined with the one- or two-site model, the absorption and emission wavelengths were each determined from three different membranes with the same compositions. The absolute quantum yield of ETH$^T$ 3003 in PS was determined as described in 3.4 Quantum yield determination in membranes on p. 59 as 0.19 ± 0.05.

Note that relative quantum yield data is very rare in the literature even though it represents a key parameter for assessing indicator dye performance in a polymer. The same holds true for parameters determined from more than one single membrane, which is a rather questionable practice in the literature.

As a first step in investigating the sterilizability of oxygen optodes, dye leaching from a PS membrane into sodium sulfite solutions was monitored at room temperature over a month. There was no evidence of dye loss (see section 4.5.1 on p. 99), which is a precondition for sterilizations. The above-mentioned polymers discussed in 4.4.2 on p. 92 were subdued to tempering at 135°C and two subsequent steam sterilizations at 121°C and 2 bar. The results of this study are presented in 4.5.4 Steam sterilization of thermoplastic polymers on p. 103. The evolution of optical aspect, emission intensities, emission maximum wavelength and absorbance, as well as the oxygen sensitivities derived from one- and two-site model data, are discussed below. The characterization was supplemented with measurements in oxygenated water. In section 4.5.2 on p. 100 and section 4.5.3 on p. 101, the results of sterilizing polysiloxane and plasticized polystyrene membranes in a phosphate buffer at 135°C are given.

Based on polystyrene membranes, ETH$^T$ 3003 was compared with ETH$^T$ 3001 and the commonly used indicator dye [Ru(dpp)$_3$]$^{2+}$. Furthermore, the influence of the
two popular plasticizers, \(o\)-nitrophenyl octyl ether (\(o\)-NPOE) and \(o\)-cyanophenyl octyl ether (\(o\)-CPOE), on sensor performance was evaluated. Again, the relative quantum yields, Stern-Volmer constants and the emission and absorption maxima wavelengths are given and analyzed.

The discussion in 4.6 on p. 109 compares the performance of the investigated polymers with other indicator dyes and polymers reported in the literature and summarizes the conclusions from the experiments.

Theoretical descriptions of luminescence-based oxygen sensors are based on the Stern-Volmer kinetics originally established for quenching processes in solution. Since the very first studies there has been considerable effort devoted to explaining the non-linearities frequently encountered in the Stern-Volmer plots of polymer-based sensors, both empirically and causally. Whilst the theories tend to fit the data well empirically, causal explanations usually fail to address defined polymer properties which could have predictive value. Another issue is that the differences encountered between lifetime- and intensity-based data have up to now, not been explained. An introduction to the theoretical description of luminescence-based oxygen optodes and a discussion of the above-mentioned topics are given in 4.2.1 Simple Stern-Volmer kinetics on p. 71.

Attempts have been made to model polymer membrane heterogeneity using distribution-based models [17, 18]. These are discussed in 4.2.2 Distribution-based Stern-Volmer kinetics on p. 75 according to how they fit real data rather than using simulated data. Of special interest is the continuity of the fitting parameters obtained upon membrane treatments, such as tempering or sterilization, which would allow such changes to be described consistently in one model.

Since detection limits and measuring ranges tend to be very arbitrary quantities, they are not singled out for attention in this thesis, even though they are usually referred to in the literature. The research described here focuses on the physicochemical aspects of oxygen optodes and not on applied measurements or on instrumentation, see 8.5.3 Experimental limitations on p. 253. Great care, however, was taken to avoid artifacts arising from the instrumentation.

The procedures used to characterize the oxygen sensor membranes are outlined in great detail in 3.2.1 Measuring luminescence in membranes on p. 32, in 3.2.4 Methodology of luminescence and absorbance measurements on p. 46 as well as in 3.4 Quantum yield determination in membranes on p. 59.

Membrane preparation is described in 8.2 on p. 235 and the measurement set-up in 8.5 on p. 246.
4.2 Theory of Stern-Volmer kinetics

4.2.1 Simple Stern-Volmer kinetics

Luminescence quenching processes are usually described by a Stern-Volmer kinetics. These have been developed for quenching processes in solution [19], but also provide the sole theoretical basis for luminescence-based oxygen sensors in the gas and aqueous phase and will therefore be thoroughly discussed. Fig. 4.1 shows the basic principle of luminescence quenching.

**Dynamic (or collisional) quenching** is a process where a quencher molecule Q deactivates an excited fluorophore $M^*$ in a bimolecular reaction with the reaction constant $k_Q$:

$$\frac{d[M^*]}{dt} = -(k_r + \sum k_i \neq r + k_Q [Q])[M^*]$$  \hspace{1cm} (4.1)

When the luminescence is quenched, the luminophore returns to the ground state without the emission of light. The first term with $k_r$ denotes radiative decay by luminescence emission. The second term summarizes all radiationless processes and the $k_Q [Q]$-term describes the quenching contribution. The luminescence quantum yield $\Phi_L$ in absence of the quencher is

$$\Phi_L = \frac{k_r}{k_r + \sum k_i \neq r} = k_r \cdot \tau_0$$  \hspace{1cm} (4.2)
and in presence of the quencher

$$\Phi_{LQ} = \frac{k_r}{k_r + \sum_{i \neq r} k_i + k_Q[Q]} = \frac{\Phi_L}{1 + \tau_0 \cdot k_Q[Q]}.$$  \hspace{1cm} (4.3)

$\tau_0$ denotes the luminescence lifetime in absence of the quencher. If we form the ratio of the two luminescence quantum yields we obtain

$$\frac{\Phi_L}{\Phi_{LQ}} = 1 + \tau_0 \cdot k_Q[Q] = 1 + K_{SV}[Q]$$  \hspace{1cm} (4.4)

which is linear in $[Q]$. $K_{SV}$ denotes the so-called *Stern-Volmer constant* which is a measure of the sensitivity of the quenching process. All ruthenium(II) dimine complexes exhibit purely dynamic quenching.

There is a different quenching reaction mechanism called *static* quenching. It assumes that the fluorophore $M$ forms a non-luminescent complex $MQ$ with the quencher $Q$ with the stability constant

$$K_S = \frac{[MQ]c_0}{[M][Q]}.$$  \hspace{1cm} (4.5)

c_0 is the standard concentration (usually 1 M) which is needed to make $K_S$ dimensionless. A similar linearization as in (4.4) yields

$$\frac{\Phi_F}{\Phi_{FQ}} = 1 + K_S \cdot \frac{[Q]}{c_0}.$$  \hspace{1cm} (4.6)

Please note that dynamic and static quenching are *a priori* not discernible. Dynamic quenching increases with increasing temperature whereas static quenching decreases. Luminescence quenching monitoring at different temperatures allows to determine the nature of quenching.

Oxygen optodes are usually characterized by one of several models based on the Stern-Volmer kinetics. The simplest is the original linear model based on (4.4), where the ratio of the two quantum yields is replaced by the ratio of the emission intensities in absence ($I_0$) and presence of oxygen ($I$):

$$\frac{I_0}{I} = 1 + K_{SV} \cdot pO_2.$$  \hspace{1cm} (4.7)

The linear representation of $I_0/I$ versus $pO_2$ is called a *Stern-Volmer plot*.

$K_{SV}$ has the corresponding units of the oxygen partial pressure $pO_2$, in this work always [bar$^{-1}$]. The higher $K_{SV}$, the lower the detection limit and the higher the sen-
Luminescence-based oxygen optodes

Sensitivity. Large $K_{SV}$-values, however, limit the dynamic range of the sensor to low oxygen partial pressures.

One has to keep in mind that $K_{SV}$ stems from $k_Q [Q]$, i.e. is proportional to the bimolecular reaction rate $k_Q$ and the concentration $[Q]$ of the quencher in the observed media. $pO_2$ for oxygen sensors always denotes the oxygen concentration outside the membrane, which is linked to $[Q]$ by the oxygen solubility in the membrane. For most gases the solubility in polymers is not proportional to their partial pressure, but downward-curved [20]. $k_Q$, on the other hand, reflects basically the diffusion constant of oxygen in the membrane, because the large luminescent dye molecules can be considered immobile.

It is obvious that a curved Stern-Volmer plot cannot be described by the linear equation in (4.7). Therefore, a multi-component model [21] has been developed which assumes several, usually two (two-site model), quenching sites, which is sufficient to obtain a satisfactory fit. Its general form is

$$\frac{I_0}{I} = \left[ \sum_{i=0}^{\infty} \frac{f_i}{1 + K_{SV,i} \cdot pO_2} \right]^{-1}. \quad (4.8)$$

The index $i$ enumerates the different quenching sites and $f_i$ the fraction of the molecules which experience the site’s Stern-Volmer quenching constant $K_{SV,i}$. The implicit condition that

$$\sum_i f_i = 1 \quad (4.9)$$

reduces the actual number of parameters for the two-site model to three. This model is capable to fit virtually any downward-curved Stern-Volmer curves, though with little physical meaning of the obtained parameters, compare Fig. 4.17 on p. 106.

A popular variation of the two-site model assumes that one site is not dynamically quenched (i.e. one $K_{SV}$-value = 0). This reduces the number of parameters to two:

$$\frac{I_0}{I} = \left[ \frac{f_0}{1 + K_{SV} \cdot pO_2} + (1 - f_0) \right]^{-1}. \quad (4.10)$$

This model makes sense if it can be assumed that there are two domains in a polymer, in which one is e.g. crystalline or in another way oxygen-impermeable.

It is important to note that all quenching data reported here is based on intensity and not lifetime measurements. $I_0/I$ and $\tau_0/\tau$ Stern-Volmer plots should in theory be equal, but in practice, they are often different, compare also data of this work in Fig. 4.6 on p. 86 or e.g. in [10]. Usually lifetime data is below the intensity data (i.e.
$I_0/I > \tau_0/\tau$ in a Stern-Volmer plot.

Hendrick et al. calculated for a two-site model with empirically chosen $K_{SV,i}$’s and $j_i$’s the $I_0/I$ as well as $\tau_0/\tau$-ratios [22]. They found that if the strongly quenched site (the one with the larger $K_{SV}$) had the longer lifetime $\tau_0/\tau$ was above $I_0/I$ for all pO$_2$, and vice versa, if the strongly quenched site had the shorter lifetime. The latter case is especially interesting, because $\tau_0/\tau$ no longer rose monotonically, but showed a local minima. This was a case, however, which they did not support by experimental evidence. If both lifetimes were approximately equal, the two curves intersected at some pO$_2$ with $\tau_0/\tau$ above $I_0/I$ at low oxygen pressures and vice versa at high oxygen pressures. From the limited number of their calculations one cannot tell whether their findings hold true for all possible parameter selections.

An important consideration is that the Stern-Volmer equation per se is only valid for a $I_0/I$-ratio, but many authors use it also for a $\tau_0/\tau$-ratio. The emission intensity, which is compromised in a monoexponential decay curve intensity, corresponds to its integral between 0 and infinity

$$\int_0^{\infty} e^{-t/\tau} dt = -\tau \cdot e^{-t/\tau} \bigg|_0^{\infty} = \tau$$

and is therefore directly proportional to the measured lifetime, i.e. $\tau_0/\tau = I_0/I$. This of course holds only true if a true monoexponential decay is modelled, which is hardly ever the case in heterogeneous media such as polymers used for oxygen optodes.

In the case of a multi-exponential model composed of $n$ different monoexponential decay curves with the lifetime $\tau_i$ and amplitude $B_i$

$$i(t) = \sum_{i=1}^{n} B_i e^{-t/\tau_i}$$

a preexponential weighted lifetime

$$\tau_m = \sum_{i=1}^{n} B_i \tau_i / \sum_{i=1}^{n} B_i$$

must be introduced for each point in a Stern-Volmer plot in order to comply with (4.11). The subscript m in $\tau_m$ stands for modal. If other lifetimes than $\tau_m$ are used for the plot inconsistent $\tau_0/\tau$-ratios are obtained.
4.2.2 Distribution-based Stern-Volmer kinetics

All models discussed so far cannot reasonably fit a linear as well as an explicitly non-linear Stern-Volmer plot with the same number of parameters. Either the model has too few parameters and poorly fits the data or it has too many and the obtained parameters scatter meaninglessly. If it is necessary to apply two different models (e.g. when a Stern-Volmer plot of a membrane has changed from linear to non-linear after sterilization), the parameters obtained with one model cannot be compared to those obtained with the other and allow no continuous assessment.

Most authors stress that their applied one- or two-site model is in fact a simplification of some kind of distribution. Given the complex domain structures of most polymers, a truly isotropic, narrow distribution of the Stern-Volmer constants as in a solution does not seem very probable. Several authors have recently investigated other models, such as a Gaussian (or normal) or a log-Gaussian (or lognormal) model [17, 18], though no universal model with physically plausible parameters has resulted. It is important to note that these papers modelled Stern-Volmer plots based on assumed parameters, rather than fitting these models to real data.

In analogy to the multi-component model in (4.8) the relation with a continuous $K_{SV}$-distribution is

$$\frac{I_0}{I} = \lim_{i \to \infty} \left[ \sum_{i=0}^{\infty} f_i \frac{1}{1 + K_{SV,i} \cdot pO_2} \right]^{-1} = \left[ \int_0^\infty \frac{f(K_{SV})}{1 + K_{SV} \cdot pO_2} dK_{SV} \right]^{-1}$$

(4.14)

with the restriction

$$\int_0^\infty f(K_{SV}) dK_{SV} = 1$$

(4.15)

corresponding to (4.9). It should be noted that negative $K_{SV}$-values are not considered, even though $f(K_{SV})$ can be defined at negative $K_{SV}$-values as in the case of the normal distribution. According to the definition of $K_{SV}$ in (4.4), negative $K_{SV}$-values have no physical meaning. The same applies to the normalization integral in (4.15), where only the positive real part is covered.

All the following functions were fitted in IgorPro 3.1 to some sample experimental data, with one linear (ETH$^T$ 3003 in PAMS) and one very curved (ETH$^T$ 3003 in PDPO after curing at 135°C) Stern-Volmer plot, see sections 4.4.2 and 4.5.4. $f(K_{SV})$ was modelled with 1001 linearly-spaced points in a range determined by the distribution parameters, which always compromised more than 99.999% of the distribution’s integral. In each iteration, $f(K_{SV})$ was numerically integrated to perform the
normalization in (4.15) for the current parameters, \( I_0/I \) calculated by numerically solving the integral in (4.14) and a new iteration started with IgorPro’s optimized parameters. The fitting proceeded until chi-square

\[
\chi^2 = \sum_i \left( \frac{y - y_i}{\sigma_i} \right)^2,
\]

(4.16)

the sum of squared deviations of the original data to the fit, no longer decreased. The standard deviation \( \sigma_i \) of each data point was taken as 1.

Mills had assumed a Gaussian distribution of the lifetimes \( \tau_{0,i} \) at each site in absence of oxygen [18]. The same results would be obtained if a distribution in pO\(_2\), \( \tau_{0,i} \) or \( k_{Q,i} \) was assumed. Note that Mills’ Gaussian distribution is a one-parameter fit, because either \( \tau_{0,i} \) or \( k_{Q,i} \) can be determined at pO\(_2\) = 0. He does not plot \( I_0/I \) against pO\(_2\), but rather \( k_Q \cdot \tau_{0,m} pO_2 \), where \( \tau_{0,m} \) is the modal (center) lifetime and \( k_Q \) the bimolecular quenching constant for all sites. \( k_Q \) therefore corresponds to a second, linear parameter.

Potentially interesting distributions [23, 24], which are examined in the following, are the normal distribution (or Gaussian distribution), exponential distribution, Gamma distribution, lognormal distribution and Max-Boltzmann distribution.

The normal distribution is defined as

\[
f(K_{SV}) = \frac{1}{\sqrt{2\pi} \cdot \sigma} \cdot e^{-\frac{1}{2} \left( \frac{K_{SV} - \mu}{\sigma} \right)^2}
\]

(4.17)

with the center \( \mu \) and the standard deviation \( \sigma \). The preexponential factor normalizes the integral of \( f(K_{SV}) \) to 1 provided that the function is integrated from \(-\infty\) to \(+\infty\) though for our application the negative part has no physical meaning. The Full Width at Half Maximum (FWHM) of the distribution corresponds to \( \sqrt{2} \cdot \sigma \). A variation of the normal distribution was introduced, where a discrete fraction \( f_0 \) at \( K_{SV} = 0 \) (zero-\( K_{SV} \) contribution) is added to compensate for the portion of the distribution at negative \( K_{SV} \) values.

The exponential distribution has been defined with two parameters \( A \) and \( b \):

\[
f(K_{SV}) = A \cdot e^{-b \cdot K_{SV}}.
\]

(4.18)
The lognormal distribution is one of the two standard distributions in statistics for positive values:

\[ f(K_{SV}) = \frac{1}{\sqrt{2\pi} \cdot \sigma} \cdot \frac{1}{K_{SV}} \cdot e^{\frac{1}{2} \left( \frac{\ln(K_{SV}) - \mu}{\sigma} \right)^2} \]  

(4.19)

Its actual fitting in IgorPro proved to be very difficult because of the inaccurate numerical (trapezoidal) integration for the log-spaced \( K_{SV} \)-value distribution. The other distribution which is also intended for absolute values is the Gamma distribution:

\[ \Gamma(K_{SV}) = c \left( \frac{K_{SV}}{\sigma} \right)^{\eta - 1} \cdot e^{\frac{K_{SV}}{\sigma}} \]  

(4.20)

c denotes the normalization constant, the actuarial expectation is \( \eta \sigma \).

The Maxwell-Boltzmann distribution [25] describes the velocity distribution of molecules with the mass \( m \) as a function of the temperature \( T \):

\[ F(v) = 4\pi \cdot \left( \frac{m}{2\pi kT} \right)^{\frac{3}{2}} \cdot v^{\frac{3}{2}} \cdot e^{-\frac{mv^2}{2kT}} \]  

(4.21)

The reasoning behind is that oxygen molecules within a polymer membrane could experience a similar velocity distribution, i.e. also a \( K_{SV} \)-distribution. For the purpose of a \( K_{SV} \)-distribution it is important to note that the Maxwell-Boltzmann distribution is a one-parameter fit in \((m/2kT)\).

Remember that the number of effective parameters in these \( K_{SV} \)-distributions is decreased by one because of the normalization condition (4.15).

The different calculated \( K_{SV} \)-distributions for a curved Stern-Volmer plot (1.54% ETH\(^T\) 3003 in PDPO after heat tempering at 135°C, see bottom right plot in Fig. 4.14 on p. 103) are shown in Fig. 4.2.

If chi-square in (4.16) is taken as a measure of the fit quality, the fits decrease in the order: two-site model \( (\chi^2 = 0.0012, 3 \text{ parameters}) \gg \text{normal distribution with zero-} K_{SV} \text{ fraction} \ (\chi^2 = 0.0251, 3 \text{ parameters}) \gg \text{exponential distribution} \ (\chi^2 = 0.0366, 1 \text{ parameter}) \geq \text{Gamma distribution} \ (0.0374, 2 \text{ parameters}) \gg \text{normal distribution without zero-} K_{SV} \text{ fraction} \ (\chi^2 = 0.08, 2 \text{ parameters}) \gg \text{lognormal distribution} \ (\chi^2 = 0.275, 2 \text{ parameters}) \gg \text{Maxwell-Boltzmann distribution} \ (\chi^2 = 0.326, 1 \text{ parameter}) \gg \text{one-site model} \ (\chi^2 = 0.45, 1 \text{ parameter})\).
4.2 Theory of Stern-Volmer kinetics

The same calculations for a *linear* Stern-Volmer plot (1.82% ETH T 3003 in PAMS, see top left plot in Fig. 4.11 on p. 95) give a different ranking (distributions are not shown): two-site model ($\chi^2 = 0.00014$, 3 parameters) $\geq$ Gamma distribution (0.00025, 2 parameters) $\approx$ Maxwell-Boltzmann distribution ($\chi^2 = 0.00027$, 1 parameter) $\approx$ normal distribution without zero-$K_{SV}$ fraction ($\chi^2 = 0.00308$, 2 parameters) $>$ one-site model ($\chi^2 = 0.0031$, 1 parameter) $>$ exponential distribution ($\chi^2 = 0.0366$, 1 parameter).

It is no surprise that for this over-determined fitting problem, the quality of the fit follows essentially the number of parameters for each distribution. Gamma, Maxwell-Boltzmann and normal distribution look very much alike with a maximum of the $K_{SV}$-distribution at 1.32, 1.66 and 2.16 respectively which scatter around the value of 1.48 obtained for a linear fit. The exponential distribution with one parameter cannot give a straight line, hence the obviously poor fit quality.

The Gamma and the normal distribution have the potential to be a physically more meaningful alternative, however, the fit quality is definitely not as good as the two-site model. Very astonishing is the flexibility of the Maxwell-Boltzmann model, which fits either situations reasonably well with one parameter.

An example for the application of the normal distribution with a zero-$K_{SV}$ contribution to a real data series is given in Fig. 4.3. Except for probably PDPO and PtBuS there is much more continuity than in the same 3-parameter plot obtained with the conventional two-site model, see Fig. 4.17 on p. 106.

As for any other model there is much room for speculation. Take PC for example. Upon tempering at 135°C its average Stern-Volmer constant decreases, but heterogeneity as indicated by the distribution width has increased. With subsequent steril-
Fig. 4.3 Analysis of sterilization effects based on normal $K_{SV}$ distributions with zero-$K_{SV}$ contribution

The distribution width corresponds to the FWHM, $K_{SV}$ to $\mu$ in (4.17). Compare the analyses with the one-site (Fig. 4.15 on p. 104) and two site model (Fig. 4.17 on p. 106). Experiments are described in 4.5.4 Steam sterilization of thermoplastic polymers on p. 103.

izations, heterogeneity and $K_{SV}$ tend towards 0 because the dye increasingly starts to precipitate which reflects a homogenous environment with no or little oxygen quenching capability. Again, what is missing is the correlation between actually measured dye precipitation and any of those parameters. If we take for instance the emission wavelength changes (Fig. 4.20 on p. 108), we see a lot of correlation in the distribution width.

The problem of describing the sensor response based on known polymer properties is a problem of knowing these properties. What is needed are systematic investigations with really well characterized polymers. There is little sense in trying to explain the non-linearity in Stern-Volmer plots by a distribution of quenching sites if one knows that already solubility versus sorption shows the same downward-curvature for many polymers.
A more suitable description could be based for instance on the Dual Mode Sorption Theory [20] which relates solubility $S$ with the partial pressure $p$:

$$ S = S_D + S_H = Kp + \frac{S'_H \cdot bp}{1 + bp}. $$

(4.22)

Two type of sites are postulated: Henry type sites ($S_D$ contribution) and Langmuir sites ($S_H$ contribution). $K$ is Henry’s law dissolution constant, $b$ is the hole affinity constant and $S'_H$ is the hole saturation constant. $S'_H$ is a measure for the sorption capacity of the unrelaxed volume and $b$ characterizes the tendency of a given penetrant to sorb in the Langmuir mode. $S_D$ represents the sorption of diffusible species, $S_H$ the sorption in microvoids or defects. There have been empirical fits of this kind in the literature, but to the author’s knowledge a correlation with polymer properties has never been attempted.

## 4.3 Polymer materials for sterilization

### 4.3.1 Glass transition temperature

For sterilization of polymer membranes a high rigidity of the polymer structure is needed to give mechanical strength to the polymer and to prevent dye migration; the latter comes often with unwanted dye aggregation or precipitation.

An important parameter which describes this structural rigidity is the glass transition temperature $T_g$ [26]. It is determined by Differential Scanning Calorimetry (DSC), where the amount of transferred heat during sample heating is measured as a function of temperature. Glass transitions can be discerned as steps in DSC curves whereas polymer melting results in a peak. Note that different conventions exist about the exact determination of $T_g$ from DSC curves as well as different experimental procedures which result in uncertainties of a few degrees Kelvin.

On a molecular level, the transformations at $T_g$ correspond to the onset of segmental mobility of those parts located in amorphous domains. Amorphous polymers, such as atactic PS, soften at $T_g$. Heating with subsequent cooling reduces the free volume in polystyrene which makes it more brittle [26]. This process is known as physical ageing.

Low molecular additives, such as plasticizers, depress $T_g$. For a weight fraction of 0.16 as used for the plasticized PS membranes (compare Table 4.5 on p. 87), some solvents, such as ethylacetate, methyl acetate or carbon disulfide, may lower $T_g$ to room temperature [27]. Up to a weight fraction of 0.2, the decrease in $T_g$ depends linearly on plasticizer content for most thermoplastic polymers [28].
Diffusion of dye molecules above $T_g$ is greatly facilitated [29]. For the calculation of diffusion coefficients and solubilities exist simplified methods; these are based on the glass transition temperature $T_g$ and the degree of crystallinity $x_c$ [30]. Oxygen for instance would have at 25°C a diffusion coefficient $D$ of $\sim 1.0 \cdot 10^{-7}$ cm$^2$·s$^{-1}$ in a glass polymer, based on a $T_g = 400$ K and a typical value of $x_c$ of 0.6.

4.3.2 Suitable thermoplastic polymer membranes

Experiences in polymer dyeing show that most luminescent dyes can be used in polystyrene [31, 32], but only in few other polymers. This is due to the very good solubility characteristics of polystyrene and the brightness of the luminescence, i.e. the high quantum yield of the dissolved dyes. There is a luminescence maximum with increasing dye concentration in the polymer which is usually at 0.01 to 0.1 weight percent. Please note that these concentrations are a single order of magnitude lower than what is generally used in optodes.

In contrast to various other polymers, dye migration in polystyrene is very limited due to its high glass transition temperature of approximately 100°C. This is a precondition for the use of soluble dyes for conventional polymer dyeing. Only few pigments/dyes can be used for the highest processing temperatures of polystyrene (280-300°C), but a wider choice is available for the usual processing temperatures (220-260°C) [31]. It is evident that temperature stability of dyes is not an important factor when dyes are rather dissolved at room temperature than dispersed in the hot polymer melt. Nevertheless, it should be kept in mind as a possibility for polymers which are not readily soluble.

Usual temperatures for sterilization range from 100°C to 130°C. Polystyrene can no longer be used in this temperature range because its glass transition temperature is at approximately 100°C. To increase the glass transition temperature, the rotational movements have to be hindered. This can be achieved either by a substitution of the polystyrene or by copolymerization.

Table 4.1 lists a choice of commercially available polystyrene derivatives with $T_g > 100^\circ$C. Please note that the abbreviations $PtBuS$ and $PtBrS$ have been introduced by the author.

Among these polymers poly(3-chlorostyrene), poly(4-methylstyrene) and poly(styrene-co-methyl methacrylate) were not pursued because of their relatively low glass transition temperatures. The other mono halogen-substituted polystyrenes were not considered because they were expected to have rather low $T_g$’s, probably poorer chemical stability and potential quenching ability [33].

Most copolymers with styrene actually decrease $T_g$ with the exception of acrylonitrile or acrylic acid. Pure polyacrylonitrile has a $T_g$ of 130°C. Polymers similar to
<table>
<thead>
<tr>
<th>Polymer</th>
<th>$T_g$ / °C</th>
<th>$M_w$ or $M_n$</th>
<th>Further informations</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(styrene) PS</td>
<td>100</td>
<td>280’000</td>
<td>$T_m$ 237.5°, solubility parameter 9.1</td>
<td>Aldrich</td>
</tr>
<tr>
<td>Poly($\alpha$-methylstyrene)* PAMS</td>
<td>n.a.</td>
<td>375’000</td>
<td>Standard, $M_w/M_n = 1.20$</td>
<td>Sp$^2$</td>
</tr>
<tr>
<td>Poly($p$-tert-butylstyrene) PtBuS</td>
<td>132</td>
<td>n.a.</td>
<td>soluble in benzene, THF, toluene</td>
<td>Sp$^2$</td>
</tr>
<tr>
<td>Poly(4-methoxystyrene) PMOS</td>
<td>113</td>
<td>400’000</td>
<td>soluble in MEK, THF, toluene</td>
<td>Sp$^2$</td>
</tr>
<tr>
<td>Poly(4-methylstyrene)</td>
<td>106</td>
<td>100’000</td>
<td>soluble in MEK, THF</td>
<td>Sp$^2$</td>
</tr>
<tr>
<td>Poly(2,4,6-tribromostyrene) PtBrS</td>
<td>195</td>
<td>230’000</td>
<td>soluble in THF; softening point 220°C; minimum 66% Bromine, 1% chlorine</td>
<td>Sp$^2$</td>
</tr>
<tr>
<td>Poly(4-bromostyrene)</td>
<td>118</td>
<td>60’000</td>
<td>soluble in benzene, THF, toluene</td>
<td>Sp$^2$</td>
</tr>
<tr>
<td>Poly(2-bromostyrene)</td>
<td>n.a.</td>
<td>60’000</td>
<td>soluble in benzene, THF, toluene</td>
<td>Sp$^2$</td>
</tr>
<tr>
<td>Poly(3-bromostyrene)</td>
<td>n.a.</td>
<td>60’000</td>
<td>soluble in benzene, THF, toluene</td>
<td>Sp$^2$</td>
</tr>
<tr>
<td>Poly(4-chlorostyrene)</td>
<td>110</td>
<td>n.a.</td>
<td>soluble in benzene, chloroform, dioxane, MEK, THF, toluene, xylene</td>
<td>Sp$^2$</td>
</tr>
<tr>
<td>Poly(2-chlorostyrene)</td>
<td>119</td>
<td>n.a.</td>
<td>soluble in benzene, MEK, THF, toluene</td>
<td>Sp$^2$</td>
</tr>
<tr>
<td>Poly(3-chlorostyrene)</td>
<td>90</td>
<td>n.a.</td>
<td>soluble in benzene, MEK, THF, toluene</td>
<td>Sp$^2$</td>
</tr>
<tr>
<td>Poly(4-iodostyrene)</td>
<td>156</td>
<td>400’000</td>
<td>soluble in benzene, dioxane, THF, toluene; degree of iodination 80%</td>
<td>Sp$^2$</td>
</tr>
<tr>
<td>Poly(styrene-co-methyl methacrylate)</td>
<td>101</td>
<td>100’000 -150’000</td>
<td>ca. 40% styrene,</td>
<td>Aldrich</td>
</tr>
</tbody>
</table>

Table 4.1 Commercially available derivatives of polystyrene with $T_g > 100°C$

Abbreviations used below are given in bold type. Suitable solvents are given to indicate chemical character. Poly(2-bromostyrene) and poly(3-bromostyrene) are expected to have a $T_g > 100°C$. MEK is methyl ethyl ketone.

a. Poly($\alpha$-methylstyrene) with $M_w$ 9600 has a $T_g$ of 76°C, which suggests that this high molecular poly($\alpha$-methylstyrene) has a $T_g > 100°C$ as confirmed by literature.
polystyrene with a high \( T_g \) include polyvinylcarbazol (\( T_g 210^\circ C \)), polyvinylnaphtaline (\( T_g 135^\circ C \)) and polyacenaphtaline (\( T_g 265^\circ C \)) [26].

Most of what has been said about polystyrene also applies to poly(methyl methacrylate) which has a \( T_g \) of 105\(^\circ C \). There are, however, not the substitution/copolymerization possibilities to increase the glass transition temperature that polystyrene offers.

There are also other thermoplastic polymers which are potentially interesting because of their glass transition temperature, Table 4.2 lists a choice of commercially available polymers.

From this selection poly(tetrafluoroethylene) and cellulose acetate (CA) were not investigated because of the former’s opacity, the latter because of its swelling in water.

### 4.3.3 Polysiloxane membranes

A small scope of polysiloxanes has been evaluated for use in oxygen sensor membranes. Polysiloxanes are inert against most of the organic solvents and chemicals, are transparent, thermally stable over a large temperature range, stick well to virtu-
Polymer materials for sterilization

84

ally every support material and feature good oxygen permeability and solubility. Their glass transition temperatures above 121°C makes them suitable candidates for sterilization experiments [34].

The polysiloxanes listed in Table 4.3 were evaluated because they polymerize in addition or condensation reactions. They require comparably small amounts of tin or platinum catalysts which will remain inside the membrane. The approach was part of a more general one in the group which wanted to minimize residual catalysts in the sensor membrane. The question was whether the higher lipophilicity of the ETH[^300] 300x dyes could overcome the known poor solubility of ruthenium(II) diimine complexes in polysiloxanes [36].

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$T_g / ^\circ C$</th>
<th>$M_w$ or $M_n$</th>
<th>Further informations</th>
<th>Supplier</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poly(2,6-dimethyl-1,4-phenylene oxide) PDPO</td>
<td>210</td>
<td>50'000</td>
<td>soluble in benzene, chloroform, pyridine, toluene</td>
<td>Sp²</td>
</tr>
<tr>
<td>Poly(bisphenol-A-carbonate) PC</td>
<td>149</td>
<td>60’000</td>
<td>soluble in chloroform, dioxane, DMF, dichloromethane, pyridine, THF</td>
<td>Sp²</td>
</tr>
<tr>
<td>Cellulose acetate (CA)</td>
<td>120</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly(tetrafluoroethylene) (PTFE)</td>
<td>126</td>
<td></td>
<td>opaque</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.2 Thermoplastic polymers with $T_g > 100^\circ C$

The selection of the polymer is based on a list in [28]. Abbreviations used below are given in bold type.

<table>
<thead>
<tr>
<th>Name</th>
<th>Components</th>
<th>Supplier</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>RTV-2K silicon recipe 36A03</td>
<td>Siloprene U base mixture H 60 Siloprene U Crosslinking Agent 830 Tetramethyl tetravinyl cyclotetrasiloxane</td>
<td>Bayer</td>
<td>mixing, degassing, curing at 200°C</td>
</tr>
<tr>
<td>Siloprene K 1000</td>
<td>Siloprene K 1000 Siloprene Crosslinking Agent K-11 (platinum catalyst in an alcohol)</td>
<td>Fluka Selectophore</td>
<td>mixing, reaction at RT</td>
</tr>
<tr>
<td>Siloprene LSR 2030</td>
<td>Siloprene LSR 2030A Siloprene LSR 2030B</td>
<td>Bayer</td>
<td>mixing, curing at 200°C</td>
</tr>
</tbody>
</table>

Table 4.3 Investigated polysiloxanes

Unfortunately, there is little information about the polymers behind the trade names [35].
4.4 Evaluation of suitable polymers

4.4.1 Plasticized polystyrene membranes

Plasticizers have been used to tailor luminescence-based oxygen sensors to a certain oxygen response, e.g. [6]. Based on the favorable properties of polystyrene outlined in 4.3.2 on p. 81 plasticized polystyrenes have been investigated as well as a comparison of [Ru(dpp)_3](ClO_4)_2, ETH^T 3001 and ETH^T 3003 in polystyrene membranes. Two very similar plasticizers were investigated, namely o-nitrophenyl octyl ether (o-NPOE) and o-cyanophenyl octyl ether (o-CPOE) whose structures are shown in Fig. 4.5.

Papkovsky et al. have suggested to replace the popular plasticizer o-NPOE with o-CPOE because of the latter’s luminescence quenching ability [37]. o-CPOE has a lipophilicity value of logP_{TLC} of 5.0 [37] compared to o-NPOE, which has logP_{TLC} of 5.8 [38]. The dielectric constant of o-CPOE is 23.0 [37], the one for o-NPOE 23.9 [39]. Their chemical similarity was confirmed by these values, both plasticizers are equally compatible with polystyrene (solubility parameter $\delta = 9.1$ (cal·cm$^{-3}$)$^{1/2}$ [28]).

For scientific investigations o-CPOE is preferable because it offers a larger absorption window where the behavior of ruthenium(II) diimine dyes can be investigated; o-NPOE absorbs below 400 nm, o-CPOE below 320 nm (compare Fig. 5.15 on p. 146). Furthermore, absorption maxima determinations of the ruthenium(II) diimine dyes get less accurate because of the residual absorbance of o-NPOE in the visible range which increases the apparent absorbance with respect to the baseline.

The use of plasticizers is limited by mechanical stability. Polystyrene membranes with a high plasticizer content (> 30%) get very soft and sticky and easily detach from their glass support.

Lifetimes for membranes with 1.3% ETH^T 3001 with 20.0% o-CPOE in PS and 1.2% ETH^T 3003 with 19.2% o-CPOE in PS respectively are summarized in
4.4 Evaluation of suitable polymers

Table 4.4. The measurements have been carried out by Paul Hartmann, see 8.3.2 Life time measurements on p. 239 for experimental details.

Table 4.4 Summary of lifetime in plasticized polystyrene

<table>
<thead>
<tr>
<th>lifetimes / µs</th>
<th>membrane ETH\textsuperscript{T} 3001</th>
<th>ETH\textsuperscript{T} 3003</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\tau_m(\text{N}_2))</td>
<td>4.90</td>
<td>4.90</td>
</tr>
<tr>
<td>(\tau_m(\text{air, i.e.20.9}%\  \text{O}_2))</td>
<td>4.13</td>
<td>4.49</td>
</tr>
<tr>
<td>(\tau_m(\text{O}_2))</td>
<td>2.70</td>
<td>2.77</td>
</tr>
</tbody>
</table>

Errors for the lifetime determination are ±0.05 µs. Lifetimes have been determined according to (4.13).

The lifetime data of these cast membranes can be correlated with independently-determined intensity-based quenching data which has been measured with spin-coated membranes of identical composition, see Fig. 4.6. The 4.49 µs lifetime for ETH\textsuperscript{T} 3003 is probably an outlier as suggested by Paul Hartmann. The decay curve could be well described with a monoexponential decay for the nitrogen data, but needed a biexponential fit for the oxygen data.

Stern-Volmer constants \(K_{SV}\) were determined to be 1.00 bar\textsuperscript{-1} for ETH\textsuperscript{T} 3001 (\(A_{\text{max}}\) 0.134) and 0.85 bar\textsuperscript{-1} for ETH\textsuperscript{T} 3003 (\(A_{\text{max}}\) 0.080) membranes. Please note that these \(K_{SV}\)-values are much lower than predicted by the extrapolation of plasticizer content from values in Table 4.6 and those found in later measurements. This is probably an artifact of the oxygen media control set-up, which still evolved at that time.

The lifetime-based Stern-Volmer plots give an apparently lower sensitivity than the...
intensity-based one. This meets the expectations according to Hendrick et al. (see p. 73) that if the major lifetime components are oxygen-quenched, \( I_0 / I \) should be above \( \tau_0 / \tau \).

Note that lifetime-based values would be identical to the intensity-based values if these had an off-set of 10%. Because of the uncertainties associated with the intensity-based data, no thorough discussion of this topic is given.

Lifetimes in the absence of oxygen are slightly higher in the membrane than in ethanol, compare Table 2.4 on p. 24. Furthermore, lifetimes for ETH\( \text{T} \) 3001 and 3003 are identical, which is very surprising considering their different quantum yield in the membrane, see discussion below.

In a systematic fashion the influence of the two plasticizers \( o \)-NPOE and \( o \)-CPOE as well as different ruthenium(II) diimine dyes has been investigated. Table 4.5 lists the composition of the plasticized polystyrene membranes which were investigated.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>( w(\text{plasticizer}) / % )</th>
<th>( w(\text{dye}) / % )</th>
<th>Relative absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>0</td>
<td>1.45</td>
<td>1</td>
</tr>
<tr>
<td>PS (0.1x dye)</td>
<td>0</td>
<td>0.14</td>
<td>1.8</td>
</tr>
<tr>
<td>PS (ETH( \text{T} ) 3001)</td>
<td>0</td>
<td>1.19</td>
<td>1.7</td>
</tr>
<tr>
<td>PS (Ru(dpp)(_3))</td>
<td>0</td>
<td>0.90</td>
<td>1.5</td>
</tr>
<tr>
<td>PS/1x ( o )-CPOE</td>
<td>8</td>
<td>1.31</td>
<td>1.8</td>
</tr>
<tr>
<td>PS/2x ( o )-CPOE</td>
<td>16.2</td>
<td>1.28</td>
<td>2.6</td>
</tr>
<tr>
<td>PS/1x ( o )-NPOE</td>
<td>8.43</td>
<td>1.55</td>
<td>2.1</td>
</tr>
<tr>
<td>PS/2x ( o )-NPOE</td>
<td>16.31</td>
<td>1.28</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Table 4.5 Composition of plasticized PS membranes with ETH\( \text{T} \) 3003

The relative absorbance is the ratio of the measured maximum absorbance of the main band divided by the concentration in weight percent, normalized to ETH\( \text{T} \) 3003 in polystyrene.

The relative absorbance in Table 4.5 gives a measure of membrane thickness provided that absorption coefficients are the same in the polymers and ethanol. For one plasticized PS membrane with ETH\( \text{T} \) 3001 this has been demonstrated, see below.

Membranes for different ruthenium(II) diimine dyes are within 7.8 mM ± 5% equimolar. Two membranes of each composition have been prepared and characterized. Fig. 4.12 summarizes the two most important single parameters, i.e. the quantum yield and the Stern-Volmer constant \( K_{SV} \) according to equation (4.7).
4.4 Evaluation of suitable polymers

All Stern-Volmer plots (not shown) were slightly downward-curved. Fitting with the two-site model regularly gave larger intra than inter membrane differences in the coefficients $K_{SV,1}$, $K_{SV,2}$, $f_1$ and $f_2$ and are therefore not given.

An interesting data triple is PS with $[\text{Ru(dpp)}_3](\text{ClO}_4)_2$, ETH$^T$ 3001 and ETH$^T$ 3003 because the dye alone is varied.

The membranes obviously have similar quenching characteristics ($K_{SV}$ of 1.66, 1.53 and 1.63 bar$^{-1}$ respectively), but widely different quantum yields. The membrane with ETH$^T$ 3003 has a 6.4% higher quantum yield than $[\text{Ru(dpp)}_3](\text{ClO}_4)_2$ which fits well with the quantum yields of the dyes in ethanol which have been determined as 0.52 and 0.56, respectively. Completely off the expected value is ETH$^T$ 3001 with half the quantum yield of ETH$^T$ 3003; the same trend has been found with ETH$^T$ 3001 in plasticized membranes. This is in agreement with observation that ETH$^T$ 3001 has only a faint luminescence on a TLC plate.

The data of ETH$^T$ 3003 with a tenth of the usual dye concentration (0.1x dye) in PS illustrates the limits of the quantum yield determination in the membrane. Lower concentrations mean both lower absorbance and luminescence intensity where background correction problems become predominant. The calculated value has a standard deviation of 0.12, which does not allow to tell whether the quantum yield has increased as expected or not. Remember that in 3.4 Quantum yield determination in membranes on p. 59 the quantum yield of 1.45% ETH$^T$ 3003 in PS had been determined as 0.19, which is considerably smaller than the quantum yield in ethanol of 0.52 (see Table 2.3 on p. 22). A possible explanation could be the orders of mag-
nitude larger concentration in the membrane, caused by energy transfer between individual ETH\textsuperscript{T} 300x dyes or solubility problems.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$\Phi_{rel}$</th>
<th>$K_{SV} / \text{bar}^{-1}$</th>
<th>$\lambda_{\text{abs,max}} / \text{nm}$</th>
<th>$\lambda_{\text{em,max}} / \text{nm}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>1 ± 0.02</td>
<td>1.63 ± 0.02</td>
<td>448 ± 1</td>
<td>613.7 ± 0.3</td>
</tr>
<tr>
<td>PS (0.1x dye)</td>
<td>1.02 ± 0.12</td>
<td>1.87 ± 0.01</td>
<td>439 ± 5</td>
<td>604.7 ± 1.2</td>
</tr>
<tr>
<td>PS (ETH\textsuperscript{T} 3001)</td>
<td>0.49 ± 0.02</td>
<td>1.53 ± 0.01</td>
<td>450 ± 1</td>
<td>611.4 ± 0.4</td>
</tr>
<tr>
<td>PS (Ru(dpp)$_3$)</td>
<td>0.94 ± 0.02</td>
<td>1.66 ± 0.01</td>
<td>447 ± 0</td>
<td>612.9 ± 0</td>
</tr>
<tr>
<td>PS/1x o-CPOE</td>
<td>0.83 ± 0.01</td>
<td>1.33 ± 0</td>
<td>446 ± 1</td>
<td>610.1 ± 0</td>
</tr>
<tr>
<td>PS/2x o-CPOE</td>
<td>0.71 ± 0.01</td>
<td>1.17 ± 0.01</td>
<td>447 ± 2</td>
<td>612.5 ± 0</td>
</tr>
<tr>
<td>PS/1x o-NPOE</td>
<td>0.79 ± 0.02</td>
<td>1.29 ± 0</td>
<td>448 ± 1</td>
<td>611.2 ± 0.2</td>
</tr>
<tr>
<td>PS/2x o-NPOE</td>
<td>0.79 ± 0.04</td>
<td>1.17 ± 0.01</td>
<td>447 ± 1</td>
<td>612.2 ± 0.9</td>
</tr>
</tbody>
</table>

Table 4.6 Summary for investigated plasticized PS membranes
Data has been determined by individual determinations from two membranes. $\Phi (\text{PS}) = 0.19 ± 0.05$ in nitrogen atmosphere.

The data for the plasticized PS membranes needs more careful evaluation because experimental difficulties prevented the determination of the refractive index of the spin-coated membranes and hence the refractive index corrections given in 3.2.3 Refractive index corrections on p. 41.

If the refractive index of a PS membrane with 16\% o-NPOE is linearly interpolated between PS ($n_D^{20} = 1.5894$ [40]) and o-NPOE ($n_D^{20} = 1.511$ [41]) a value of 1.577 is obtained. This corresponds to an emission intensity of 0.98 relative to PS according to equation (3.11). o-CPOE is likely to have a very similar refractive index as o-NPOE even though it has not been experimentally determined. We can conclude that the quantum yields of ETH\textsuperscript{T} 3003 for o-NPOE/o-CPOE plasticized PS membranes mirror the actual trends, but deviations from the value in PS are slightly smaller.

The decrease in quantum yield seems to be linearly correlated with the decrease in $K_{SV}$, but not linear with respect to plasticizer content. For 8\% o-CPOE there is a decrease of -17\%, but for additional 8.2\% o-CPOE only a further decrease of -12\% in quantum yield is observed. Please note that the effect of plasticizers on polymers also must not depend linearly on the plasticizer content. It is difficult to tell whether the effect is more marked for any of the two plasticizers.

[Ru(bpy)$_3$]$^{2+}$ undergoes efficient oxidative quenching with nitroaromates in solution due to their relatively high redox potential [42]. o-CPOE, on the other hand, has been considered as non-quenching. Papkovsky et al. have investigated the luminescence quenching of coproporphyrin-ketone tetraethyl ester (CPK-TEE) and the
platinum(II) complex of etioporphyrin (PtEP) in ethanol with either plasticizer and found quenching with $o$-NPOE to be at least three orders of magnitude higher than with $o$-CPOE [37].

The quenching data provided by the authors for plasticized PVC, however, is not very convincing. They provide relative emission intensities which have not been referenced to the respective absorbance, furthermore, their membranes were in contact with a buffer solution which very likely changes the mobility of the species in the membrane. Even if their data was correct, they do not prove that $o$-CPOE does not quench. The solution behavior can obviously not be directly extrapolated to polymers.

The linear decrease in both $K_{SV}$ and $\Phi_F$ must be interpreted as that both plasticizer and oxygen compete for the same excited state.

Fig. 4.8 shows the emission and absorption maxima wavelengths for the investigated membranes.

![Absorption/emission wavelength shifts for plasticized PS membranes](image)

Little information can be retrieved from the absorption maximum wavelength because they scatter in the range of the depressed absorption peak with two maxima which “blur” in the membrane. Emission maximum wavelengths in PS are approximately 1 nm off the values in ethanol, i.e. the changes are very small. Addition of the plasticizers seems to decrease the emission maximum wavelength, though the dependence on plasticizer content is not consistent. Values for the 0.1x ETH 3003 membrane are largely off which is not astonishing considering its small absorbance and emission intensity.
More attention is given to ETH\textsuperscript{T} 3001 because it obviously behaves in polymeric membranes not as expected, at least in terms of quantum yields. 2D excitation/emission plots give valuable informations on the luminescence of the species contained therein. An example is given in Fig. 4.9.

![Emission/excitation plot of ETH\textsuperscript{T} 3001 in a o-CPOE/PS membrane](image)

**Fig. 4.9** Emission/excitation plot of ETH\textsuperscript{T} 3001 in a o-CPOE/PS membrane

1.5\% ETH\textsuperscript{T} 3001 in 16.1\% o-CPOE/82.4\% PS. Other luminescent species - if there were any - would create additional maxima in the $\lambda_{\text{em}}/\lambda_{\text{exc}}$-plane.

The thickness of this membrane together with a second identically prepared one were measured with a profilometer as 8.25 and 8.5 µm respectively; the corresponding absorbances were 0.270 and 0.259. We can assume that the membrane density is close to the one of 1.050 kg/l for PS [40] because of the comparably small 16.1\% o-CPOE content, provided that o-CPOE has a similar density as o-NPOE ($\rho = 1.041 \text{ kg/l, [41]}$).

Based on this data we can determine the molar decadic absorption $\varepsilon$ coefficient as $3.04 \pm 0.15 \cdot 10^4 \text{ Mcm}^{-1}$. This is -9\% of what has been determined for ETH\textsuperscript{T} 3001 in ethanol. One has to keep in mind that it cannot be guaranteed that the membrane thickness is measured at exactly the same spot as both luminescence and absorbance.

For the ruthenium(II) diimine complexes a difference spectrum of spectra recorded in nitrogen and oxygen atmosphere will only reveal the oxygen-dependent luminescence which is presumably exclusively the ruthenium(II) diimine complex luminescence. Such a difference 2D-spectrum of a membrane spin-coated was identical to a 2D-luminescence spectrum of its ca. 1:20 diluted cocktail of 1.1\% ETH\textsuperscript{T} 3001 in 59.6\%PS/39.3\% o-NPOE in chloroform.

These two experiments show that the dye ETH\textsuperscript{T} 3001, with the exception of the
quantum yield, obviously has almost identical absorption and luminescence properties both in solution and the plasticized PS membrane. Remember that the lifetimes for ETH$^T$ 3001 and ETH$^T$ 3003 were virtually identical. The cause of the decrease in quantum yield is very likely an impurity in ETH$^T$ 3001, compare the synthesis in 2.2.8 on p. 14. Because the lifetimes are not affected we must conclude that there are very small amounts of this impurity which strongly quench or which precipitate a part of the ETH$^T$ 3001.

Plasticized PS membranes which had been used for water measurements had gotten harder than plasticized membranes used exclusively in gas measurements. This suggests that plasticizer has been washed out.

It is noteworthy that plasticizers are not needed for a good mechanical stability of PS. Obviously they decrease both quantum yield and $K_{SV}$, i.e. clearly deteriorate oxygen sensor performance. This is not necessary the case for other polymers.

Humidity was found to have a negligible effect, as was confirmed by measurements with humidified N$_2$/O$_2$-mixtures.

### 4.4.2 Thermoplastic polymer membranes

All the membranes with thermoplastic polymers were prepared from ETH$^T$ 3003 and the respective polymer only. The compositions of the membranes are given in Table 4.7.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$w$(dye) / %</th>
<th>Relative absorbance</th>
<th>$n_\text{D}^a$</th>
<th>$\rho$ / kg·l$^{-1}^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>1.45</td>
<td>1</td>
<td>1.5894</td>
<td>1.050</td>
</tr>
<tr>
<td>PAMS</td>
<td>1.82</td>
<td>0.53</td>
<td>1.61</td>
<td>1.075</td>
</tr>
<tr>
<td>PtBuS</td>
<td>1.29</td>
<td>0.84</td>
<td>n.a.</td>
<td>0.950</td>
</tr>
<tr>
<td>PMOS</td>
<td>1.68</td>
<td>0.33</td>
<td>1.5967</td>
<td>n.a.</td>
</tr>
<tr>
<td>PtBrS</td>
<td>1.55</td>
<td>0.23</td>
<td>n.a.</td>
<td>2.1</td>
</tr>
<tr>
<td>PC</td>
<td>1.64</td>
<td>0.64</td>
<td>1.586</td>
<td>n.a.</td>
</tr>
<tr>
<td>PDPO</td>
<td>1.54</td>
<td>0.40</td>
<td>1.575</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

**Table 4.7 Composition of thermoplastic polymer membranes with ETH$^T$ 3003**

The relative absorbance is the ratio of the measured maximum absorbance of the main band divided by the concentration in weight percent, normalized to ETH$^T$ 3003 in polystyrene.

---

a. Values of pure polymers are from Scientific Polymer Products [40]
b. ditto

The ca. 150 mg of PAMS, PtBuS, PMOS dissolved immediately in 1.5 ml chloroform, whereas PDPO and PtBrS (both as powder) took 3 hours of continuous agita-
tion to dissolve completely. Three identical membranes were prepared by spin-coating of 0.3 ml from each cocktail, except for the polystyrene cocktail where only two membranes were prepared. The cocktails gave membranes of different thicknesses depending on their viscosity. The ratio of the measured maximum absorbance of the main absorbance band divided by the ETH\textsuperscript{T} 3003 concentration in weight percent, normalized to ETH\textsuperscript{T} 3003 in polystyrene, is given in Table 4.7. This relative absorbance of ETH\textsuperscript{T} 3003 in the membranes reflects variations in the membrane thickness and probably to a lesser extent changes in its absorption coefficients.

The membrane surface of PtBrS was not completely smooth compared to the others, but slightly waved. PtBrS is the only polymer among the selection which has a perceivable, yellowish color.

![Graph](image)

**Fig. 4.10** Relative quantum yield *versus* Stern-Volmer constant of ETH\textsuperscript{T} 3003 in different polymers

Error bars indicate the standard deviation of the values determined for each membrane. Quantum yields are relative to ETH\textsuperscript{T} 3003 in PS and have not been corrected for refractive index changes.

Fig. 4.11 shows Stern-Volmer plots of sample membranes which have been recorded first in gas, then in water. In the first case, the membrane is directly exposed to an nitrogen/oxygen atmosphere, in the latter case to water which was in equilibrium with a corresponding nitrogen/oxygen mixture with the given oxygen concentration, experimental details are given in 8.5.2 *Measurements in oxygenated water* on p. 250.

A summary of the key data of 7 different polymer membranes is given in Fig. 4.10.
4.4 Evaluation of suitable polymers

The displayed $K_{SV}$ and relative quantum yield values are mean values for three (or in the case of polystyrene membranes two) different membranes with the respective standard deviation error bars. $K_{SV}$ has been determined with the one-site model in (4.7), the relative quantum yield is calculated as the maximum emission intensity divided by the maximum absorbance of the main band.

PtBrS has an especially large uncertainty in the relative quantum yield. This is likely due to the combination of the smallest overall absorbance of 0.027 with a very curved background which gives large determination uncertainties. The large error bar for PAMS can be tracked to an outlier caused by a distorted absorption spectra. If this measurement is ignored the relative quantum yield for PAMS rises to 0.98.

The most interesting observation perhaps is that high quantum yield and high sensitivity to oxygen (i.e. $K_{SV}$-value) seem to virtually exclude one another. Authors usually never document their relative quantum yield or data which would make their calculation possible. Therefore, no literature data is available for verification.

Please note that these quantum yields have not been corrected for refractive index differences. For those polymers where the refractive index is known corrections are given in Table 3.1 on p. 45. With the corrections ETH$^T$ 3003 would have a virtually identical quantum yield in PAMS, PS and PC (+2%, 0 and 1%), PDPO would have a slightly lower (-3%) and PMOS a slightly (+3%) higher quantum yield.

For the investigated polymers a high sensitivity not only comes with an unwanted low quantum yield, but also the undesirable non-linearity as can be seen in Fig. 4.11 for PDPO and PtBuS.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$\Phi_{rel}$</th>
<th>$K_{SV}$/ bar$^{-1}$</th>
<th>$\lambda_{abs,max}$/ nm</th>
<th>$\lambda_{em,max}$/ nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>1 ± 0.03</td>
<td>1.63 ± 0.02</td>
<td>448 ± 1</td>
<td>613.7 ± 0.3</td>
</tr>
<tr>
<td>PS/<a href="ClO$_4$">Ru(dpp)$_3$</a>$_2$</td>
<td>0.94 ± 0.02</td>
<td>1.66 ± 0.01</td>
<td>447 ± 0</td>
<td>612.9 ± 0.0</td>
</tr>
<tr>
<td>PAMS</td>
<td>0.96 ± 0.07</td>
<td>1.49 ± 0.01</td>
<td>454 ± 10</td>
<td>617.7 ± 0.3</td>
</tr>
<tr>
<td>PtBuS</td>
<td>0.83 ± 0.01</td>
<td>3.99 ± 0.09</td>
<td>450 ± 0</td>
<td>620.9 ± 0.4</td>
</tr>
<tr>
<td>PMOS</td>
<td>1.36 ± 0.03</td>
<td>1.26 ± 0.01</td>
<td>469 ± 3</td>
<td>614.8 ± 0.3</td>
</tr>
<tr>
<td>PtBrS</td>
<td>1.22 ± 0.10</td>
<td>0.94 ± 0.01</td>
<td>456 ± 7</td>
<td>617.9 ± 0.6</td>
</tr>
<tr>
<td>PC</td>
<td>1.02 ± 0.03</td>
<td>1.24 ± 0.01</td>
<td>452 ± 2</td>
<td>618.3 ± 0.3</td>
</tr>
<tr>
<td>PDPO</td>
<td>0.89 ± 0.00</td>
<td>4.42 ± 0.09</td>
<td>449 ± 5</td>
<td>619.7 ± 0.7</td>
</tr>
</tbody>
</table>

Table 4.8 Summary for investigated thermoplastic polymers

Data has been determined from individual determinations from three membranes (except PS membranes with two individual determinations). $\Phi_{(PS)} = 0.19 ± 0.05$ in nitrogen atmosphere.
A standard analysis with the two-site model (according to (4.8)) is summarized in Fig. 4.12.

Measurements with several membranes with identical composition as in this case make it possible to do statistics on the obtained Stern-Volmer constants. Each determination, i.e. a fit of a two-site model to a Stern-Volmer plot, yields a standard devi-
4.4 Evaluation of suitable polymers

Evaluation of suitable polymers for each of the three Stern-Volmer constants $K_{SV,1}$, $K_{SV,2}$ and $f_1$. With several determinations one can calculate the average standard deviation of individual determinations and also a standard deviation of an average value calculated from all determinations.

It is expected that the standard deviation of average values should be smaller than the standard deviation of individual values. This, however, is not the case for all the membranes except PAMS and the two PS membranes which are less relevant because they are only based on two determinations. It is no surprise that the two-site model fit yields large standard deviations of individual values for data which is essentially linear, such as PtBrS. In that particular case, we have to consider that it is only the $K_{SV,1}$ component with $f_1 \approx 0$ which is very undetermined. We, however, do not expect such a behavior for membranes like PDPO and PtBuS, which are adequately fitted only by the two-site model.

We must conclude that the two-site model is not robust enough to describe the quenching behavior of a membrane. We have already learnt that the obtained Stern-Volmer parameters have no physical meaning, but they cannot even be used for purely descriptive means. Different membranes like PtBuS and PDPO or the cluster PC/PS/PMOS cannot be distinguished based on their two-site model constants. On the other hand, arguably identical membranes like PS/[Ru(II)(dpp)$_3$](ClO$_4$)$_2$ and PS/ETH$^T$ 3003 are almost separated in the $K_{SV,1}$-$K_{SV,2}$ plane.

Fig. 4.12 Stern-Volmer constants calculated with the two-site model
The size of the circles indicates $f_1$, the filled circles (very small) their respective standard deviation.
Fig. 4.13 gives an overview of emission/absorption maxima wavelengths of ETH\textsuperscript{T} 3003 in different polymers, the respective values are found in Table 4.8.

![Absorption/ emission wavelength shifts of ETH\textsuperscript{T} 3003 in different thermoplastic polymers](image)

Error bars indicate the standard deviation of the values determined for each membrane, circles give the absolute value of the absorption.

A bit of statistics on these measurements with three identical membranes show that $\lambda_{\text{em}}^{\text{max}}$ can be determined within $0.4 \pm 0.2$ nm accuracy for a given membrane set. This holds true provided the true peak form is not structured, because in that case the determination of the polynomial would introduce an error of a similar order of magnitude. Therefore, we can assume that changes in $\lambda_{\text{em}}^{\text{max}}$ of more than $\pm0.9$ nm are significant for a 95% probability. One should keep in mind that the specified wavelength reproducibility of the PE LS-50B is $\pm0.5$ nm and the wavelength accuracy is $\pm1$ nm. The emission wavelengths in 0.1 nm precision are intended for comparison within this work only.

A comparison of the emission wavelength shifts with respect to the emission maximum of ETH\textsuperscript{T} 3003 in ethanol ($\lambda_{\text{em}}^{\text{max}}$ 613.4 nm) we obtain the following order:

PS (0.3 nm) $\approx$ PMOS (1.4 nm) $\ll$ PAMS (4.3 nm) $\approx$ PtBrS (4.5 nm) $\approx$ PC (4.9 nm) $< $ PDPO (6.3 nm) $< $ PtBuS (7.5 nm)

The absorption maxima wavelengths are much more difficult to assess. The main absorption band has two maxima with almost identical absorption coefficients (only 3\% difference for ETH\textsuperscript{T} 3003). In polymers, such as PAMS or PtBuS, the two
emission maxima of ETH\textsuperscript{T} 3003 are even more flattened than usual which causes the maxima to scatter over the “plateau”.

Table 4.9 gives the Stern-Volmer constants and response times for the investigated polymers in oxygenated water.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$K_{SV}(H_2O),\text{bar}^{-1}$</th>
<th>$t_{90%}$ / min</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS</td>
<td>1.16</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>PS/\text{[Ru(II)(dpp)$_3$(ClO$_4$)$_2$]}</td>
<td>1.27</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>PAMS</td>
<td>1.25</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>PtBuS</td>
<td>3.09</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>PMOS</td>
<td>0.74</td>
<td>&lt; 0.5</td>
</tr>
<tr>
<td>PtBrS</td>
<td>0.91</td>
<td>4</td>
</tr>
<tr>
<td>PC</td>
<td>0.99</td>
<td>1</td>
</tr>
<tr>
<td>PDPO</td>
<td>1.95</td>
<td>&lt; 0.5</td>
</tr>
</tbody>
</table>

Table 4.9 Stern-Volmer constants in water
The response time $t_{90\%}$ (time for a 90% of the total signal change) has been determined for the change from 40% to 21% oxygen in the supernatant.

PtBuS and PMOS showed a poor adhesion and detached at some moment during the experiment, indicated by an exclamation mark; PtBrS had detached when the membrane was removed from the cell. This possibly distorts the Stern-Volmer plots because the zero oxygen concentration which corresponds to $I_0$ was recorded at the very end of the experiment. Plasticized membranes of ETH\textsuperscript{T} 3001 in o-NPOE/polystyrene had detached from the glass support after two months of continuous storage in water, which suggests that the other investigated polymers are very probably also incompatible with untreated glass on a long timescale.

Silanization of the glass plates very likely might have solved this problem, but because water measurements were not a major concern the experiments were not repeated.

As a general trend one can say that the degree of curvature of the Stern-Volmer plot in water follows the one in air, only that it is very much enhanced.

Response times are very fast, though there are always uncertainties in their determination as what exactly the final state is. Going to higher oxygen concentrations was always at least as double as fast as \textit{vice versa}. The experimental procedure can introduce various errors which can greatly slow down apparent response; they are discussed in 8.5.2 \textit{Measurements in oxygenated water} on p. 250.
The $K_{SV}$-values were consistently smaller than for a gas atmosphere as expected. If the polymer is very hydrophilic we expect no difference in the $K_{SV}$-values because the water acts as an oxygen carrier between the gas atmosphere and the membrane. On the other hand, if the polymer absorbs a considerable fraction of water the diffusion of oxygen and may be also the solubility is hampered, hence we expect that the $K_{SV}$-constant decreases.

The ratio $K_{SV}(\text{water})/K_{SV}(\text{air})$ could therefore be used as a coarse measure for the water uptake. A value of one means no structural change of the polymer upon water contact, decreasing values mean increasing structural change.

$\text{PtBrS} \ (1.03) \gg \text{PAMS} \ (0.83) \ > \text{PC} \ (0.78) \approx \text{PS} \ (0.76) \ = \text{PtBuS} \ (0.75) \ > \text{PS/} \ [\text{Ru(II)(dpp)}_3]\text{ClO}_4 \ (0.71) \ > \text{PMOS} \ (0.59) \ > \text{PDPO} \ (0.42)\n
This contrasts to polymers where water has a plasticizing effect. The correlation of the polymer series with decreasing hydrophobicity is obvious.

Possible indicators for water in polymers are solvatochromic dyes, such as Reichardt’s Dye [43]. Unfortunately, the correlation between water content and absorption maxima shifts is a priori unknown.

### 4.5 Sterilization

#### 4.5.1 Dye leaching

In order to investigate dye leaching into a solution the solution’s oxygen content has to be accurately known. This is easily done by using a disodium sulfite solution, where the sulfite reduces any oxygen contained therein and the solution can therefore considered to be oxygen-free.

A membrane containing 1.5% ETH$^T$ 3003 in polystyrene was taken to investigate dye leaching over 33 days. 0.5 l of a 5 g/l solution of disodium sulfite thermostated at 25°C was continuously cycled through the flow-through cell with a flow rate of 1 ml/min. The headspace of the sulfite solution was being purged with nitrogen to avoid its oxidation by air. The disodium sulfite solution was exchanged after two weeks of continuous use, with no signal change.

Great care was taken to avoid bleaching during the test and hence to reduce the total light exposure of the membrane. Only every 7000 s there was a 200 s-period where the xenon flash lamp was on and data points were sampled. The excitation slit was set to 2.5 nm, the smallest available calibrated slit width, giving the smallest light exposure.

Initially there was a relatively steep increase of 2%/d during the first day on first exposure to the sulfite solution, but then luminescence increased slowly but steadily
with an average of 1.3‰/d over the next ten days. When the experiment was resumed 2 months later for another 23 days, luminescence kept increasing 1.0‰/d.

A continued experiment with the slit width set to 15 nm and the xenon flash lamp continuously on (see 5.3.1 Bleaching in the luminescence spectrophotometer on p. 126) gave an approx. ten-fold light power increase combined and a 35times longer exposure, which results in an light exposure increase of a factor of 330. Despite the dramatically larger light exposure, there was even a larger increase in luminescence of ca. a 6‰/d over three days, rather than the expected decrease.

A membrane of 1.3% ETH T 3001 with 20.0% o-CPOE in PS changed its $K_{SV}$ from 1.84 to 1.93 bar$^{-1}$ when going from soaked to dry. $K_{SV}$ in the dry state before and after soaking were identical, though the membrane had not been soaked in water for the first time which precludes the conclusion that water has really no effect.

**4.5.2 Phosphate buffer sterilization of polystyrene membranes**

In a feasibility study with *Metroglas AG* (Affoltern am Albis, Switzerland) sterilizable membranes for oxygen sensors based on luminescence quenching of ruthenium dyes were to be developed. Sterilization was specified to take place in a phosphate buffer (8.0 g NaCl, 0.2 g KCl, 1.0 g Na$_2$HPO$_4$, 0.2 g KH$_2$PO$_4$ in 1 l distilled water, pH 7.3 ± 0.1) at 130°C for 30 min. The membranes should retain their oxygen measuring capability, i.e. transparency of the membranes should be maintained and dye losses minimized.

Membranes for these experiments had been prepared mainly with ETH T 3001 because the more lipophilic octyl-homologue ETH T 3003 had been available only in small amounts.

Initial experiments were carried out with a set of three different plasticized polystyrene membranes (~1% dye, ~20% plasticizer), namely with ETH T 3001 with/without o-CPOE and ETH T 3003 with o-CPOE.

All the membranes were submitted to one sterilization cycle. Due to a handling error at the *Metroglas AG* during the sterilizations the membranes could not be identified. Some of the membranes had “melted”, some had detached from the support while others remained fully operational though with a slight fogging. The membranes had turned harder and brittle, which suggests that the plasticizer had essentially been removed. Obviously the membrane formulation was unsuitable for such a harsh sterilization method.

Part of the adverse effects observed could be attributed to heavy mechanical stress in the boiling solution during sterilization. This was remedied for later sterilization experiments by constructing a suitable glass wafer holder.
If we assume that the intact membrane was unplasticized PS we would have had a loss in $K_{SV}$ of more than a third and a general absorbance increase due to turbidity of $\sim0.05$.

### 4.5.3 Phosphate buffer sterilization of polysiloxane membranes

According to earlier experiments in our group by Taiping Lü with polysiloxanes, including a Siloprene K 1000/Crosslinking Agent K-11 (from Fluka, reacts at room temperature) membrane for oxygen sensors, we focused on the favorable properties of polysiloxanes. Membranes have been prepared with RTV-2K silicone and LSR 2030 as well as Siloprene K1000 polysiloxanes, see Table 4.3 on page 84.

The usual method of membrane preparation is not applicable with polysiloxanes because they are virtually insoluble in organic solvents. All of the polysiloxanes precursor components were dissolved in a solvent (THF or dichloromethane) prior to membrane fabrication and then applied to our glass wafers with either spin-coating or knife-coating, if necessary with a time delay. The knife-coating technique proved to be useful to overcome the non-wetting property of the polysiloxane precursor solution without using chemical glass modifications.

After these preliminary coating optimization processes membranes with ETHT 3001 of all three investigated polysiloxanes were obtained. Unfortunately, the complex did not show any detectable luminescence which of course is a prerequisite for the oxygen sensor. Low emission intensities had already been encountered by Taiping Lü, together with a 10 to 15 nm red-shift of the emission maximum. In the LSR 2030 membrane the absorption maximum of the dissolved dye was shifted to 477 nm, an indication for dye precipitation.

Sterilization experiments proved the very good adhesion to glass, maintenance of transparency with no change in visual aspect after sterilization. There was very little fogging, mostly due to phosphate buffer deposits on the surface which is unavoidable without intermediate washing steps.

Reasons for the non-detectable luminescence are not evident. Causes may be dye aggregation/crystallization due to poor solubility, the curing treatment or a heavily quenching environment. Other research groups had used lipophilic counterions, such as laurylsulfate, to incorporate $\text{[Ru(dpp)$_3$]}^{2+}$ successfully into polysiloxanes [36].

If luminescence quenching is governed by vibrational relaxation to the media as outlined in 2.4.2 Luminescence quantum yields on p. 22, it is only natural that the quenching behaviors of polymers are different, depending on their major vibrational modes. Polysiloxanes for instance feature two strong bands of asymmetric Si-O-Si stretch vibrations (from 1100 to 1070 cm$^{-1}$ and 1060 to 1010 cm$^{-1}$) and a strong
symmetric Si-CH$_3$ vibration (1280-1250 cm$^{-1}$). These bands are very well in the range of the 1300 cm$^{-1}$ mentioned above and might be partly responsible for the deactivation of the Ru(II) diimine complexes in polysiloxanes.
4.5.4 Steam sterilization of thermoplastic polymers

Fig. 4.14 Stern-Volmer Plots of the membranes for the sterilization investigation

More systematic experiments were made with the thermoplastic polymers investigated in 4.4.2 Thermoplastic polymer membranes on p. 92. A membrane of each series had been tempered for 2h 20’ in a Nabertherm L3 heating chamber (Nabertherm, Lilienthal/Bremen, Germany) at 135°C. There was no perceivable change in surface morphology nor color.
Sterilizations with these membranes were carried out in a Napco Model 8000 Autoclave (Napco, Winchester, VA, USA) at 1 bar, 121°C during 20 min. The membranes were secured in a Teflon holder which was wrapped in aluminium foil to protect them from extra mechanical stress.

The Stern-Volmer plots of the investigated membranes after preparation, after the tempering at 135°C and the first as well as the second sterilization are given in Fig. 4.14.

The qualitative effects of tempering and the subsequent sterilizations on the Stern-Volmer plot don’t follow a general pattern, but are very individual for every polymer.

PAMS loses in sensitivity after tempering, but shows little variation afterwards (-3.3% in $K_{SV}$). PtBuS shows the same initial sensitivity loss after the tempering, but recovers to a slightly higher level than originally where it remains after the first sterilization (-1.3% in $K_{SV}$). PMOS follows the pattern of PtBuS, though with a smaller initial change and less stability after sterilization (+3.8% in $K_{SV}$). PtBrS has a higher initial loss in sensitivity with rather small subsequent decreases in $K_{SV}$ (-5.5% after the second sterilization to $K_{SV} = 0.63$ bar$^{-1}$).

PC behaves very differently. It is the only membrane which is barely affected by tempering apart from getting slightly curved. After each sterilization it turns to more pronounced non-linear behavior with a tremendous sensitivity loss ($K_{SV}$ from 1.20 to 0.77 to 0.15 bar$^{-1}$). PDPO repeats the pattern of PtBuS, though with a larger sensitivity decrease after tempering (-34%) and +6.1% in $K_{SV}$ after the second sterilization.
A more detailed analysis with the one-site and the two-site model is given in Fig. 4.15 and Fig. 4.17. The plots clearly show that except for the reasonably linear data (for PtBrS and PAMS as well as some of PC) trends are hard to define. For the non-linear data the parameters for the two-site model show only for PtBuS an understandable development, whereas the fit parameters for PMOS and PDPO go up and down without apparent reason. This illustrates the physical uselessness of two-site model data, compare 4.2 Theory of Stern-Volmer kinetics on p. 71.

Images of the membranes after their second sterilization are shown in Fig. 4.16. The horizontal incident lightening of the membranes to highlight their surface features overemphasizes e.g. dust particles. PAMS for instance can be considered unaltered by the successive sterilizations, but nevertheless gives the impressions of being speckled. PtBuS seems unchanged at first sight, but got a thin wrinkle network which shines polychromically at certain lightning angles. On its outskirts where the membrane had rested on the Teflon support membrane aggregates have been formed. PMOS stayed intact, but looks like it...
was covered with one large salt spot which gets more compact to the center of the glass plate. In PtBrS there are small wrinkles, at the edge a small part of the polymer had fused and the membrane seems to be spotted with small, ETH² 3003-colored dots. PC remained physically intact, but showed increasing turbidity to the center. With PDPO the membrane had detached on its very rim and showed some long, very thin cracks covering the surface.

The PC membrane was still opaque/turbid after 71 min at 135°C, which clearly suggests that a structural alteration of the polymer had taken place, and that it was not incorporated water which was responsible for the turbidity.

The optical assessment of the membrane state is reflected in the absorption spectra changes of the individual membranes, see Fig. 4.18. All the membranes grew less transparent and dirtier. The PAMS, PtBuS, PtBrS and PDPO membranes all show an almost identical offset of 0.1 in absorbance at 460 nm though the PDPO membrane has a larger absorbance change below 400 nm. The PMOS and especially the PC membrane have an absorbance increase of 0.20 and 0.78 respectively at 460 nm.
The steady increase towards smaller wavelengths shows that light is not absorbed, but scattered.

It must be stressed that the shape and intensity of ETH$^\text{T}$ 3003’s main absorption band at 460 nm did not change, i.e. no dye leaching took place. This is what was expected from the findings in 4.5.1 Dye leaching on p. 99.

Very interesting is the absolute emission change after drying and the two subsequent sterilizations. The peak emission intensity in nitrogen atmosphere of the indi-
individual membranes normalized by their initial peak emission intensity is plotted in Fig. 4.19.

Tempering at 135°C gave an emission intensity increase of +14 to +19% with a significantly larger increase for PtBrS (+26% and PDPO (+27%). After the first sterilization all the emission intensities return more or less to their initial state except for PC which further increases to +42% compared to the initial state. Following the second sterilization all the emission intensities decreased in a linear-like fashion with respect to the second data points (after tempering). The decrease with respect to the second point (to the third for PC) is in the order of PMOS (-9.3%) > PAMS (-17%) ≈ PDPO (-19%) ≈ PtBrS (-19%) ≈ PtBuS (-22%) > PC (-44%). Subsequent sterilizations are very likely to further reduce the total emission and hence the S/N-ratio to an extent that the sensor membrane has to be discarded.

A look at the emission maxima wavelengths in Fig. 4.20 conveys also clear trends. After a more or less pronounced decrease (+0.3 to -3.9 nm) the values change little during the first sterilization (+1.4 to -1.0 nm) to increase again after the second sterilization (+0.7 to +3.8 nm). A clear trendbreaker is PC whose emission wavelength decreases by -8.0 nm after the second sterilization. This is not surprising seen the complete loss in oxygen sensitivity, decrease in absolute emission intensity or the great increase in turbidity. PDPO, on the other hand, has the largest increase in the emission maximum wavelength and the emergence of an absorbance starting below 400 nm. This suggests to a chemical modification within the polymer. Note that emission intensities are influenced by different scattering behavior as visible in the absorbance spectra or the membrane images.
4.6 Discussion

Two-site model data ($K_{SV,1}, f_1, K_{SV,2}$ and $f_2$) gives very little idea of the sensitivity of the oxygen sensor at first sight. Therefore, two-site model data provided in the literature has been refitted as a one-model fit ($K_{SV}$) by the author. Such data is marked with “(calc.)”. Stern-Volmer constants have been recalculated to [bar$^{-1}$].

Hartmann et al. had investigated [Ru(dpp)$_3$](ClO$_4$)$_2$ in PS with lifetime and intensity measurements [4]. They found $K_{SV} = 1.48$ bar$^{-1}$ (calc.) based on the lifetime data. He had used PS with a $M_w = 240'000$ (280’000 for our PS) from the same supplier. This is less than reported here (1.63 bar$^{-1}$, see Table 4.6 on p. 89).

Oxygen sensitivities in the investigated polymer membranes ($K_{SV} < 4.4$ bar$^{-1}$) are very different from those in ethanol ($K_{SV} \approx 100$ bar$^{-1}$). Obviously there must be more potential for oxygen sensitivity.

Silicones have a two orders of magnitude larger permeation than PS [20]. Demas et al. achieved a $K_{SV} = 22.1$ bar$^{-1}$ (calc.) with [Ru(dpp)$_3$]$^{2+}$ in RTV 118 silicone rubber [10]. With pyrene in poly(dimethylsiloxane) $K_{SV} = 118$ bar$^{-1}$ was achieved [44].

From the experiments it is obvious that neither o-CPOE nor o-NPOE plasticization of PS improves sensor characteristics. For many other polymers, such as cellulose acetate, cellulose acetate butyrate or PVC, oxygen sensor response was improved with increasing plasticizer content [6, 36]. It should be noted that PVC and few other polymers are unique in showing a beneficial response to plasticization [28]. Furthermore, the use of plasticizers precludes real long-term measurements or sterilizations.

Some of the highest sensitivities reported for luminescence-based oxygen sensors are those obtained with Pd(II) octaethylporphyrin ketone (PdOEPK) and Pt(II) octaethylporphyrin ketone (PtOEPK) in PS and PVC [45]. $K_{SV}$ was 1020 bar$^{-1}$ (calc.) for the PdOEPK/PS combination! For PdOEPK/PS there was still a respectable $K_{SV} = 14.9$ bar$^{-1}$. Sensitivities in PVC were less impressive for both dyes with 8.4 and 1.4 bar$^{-1}$, respectively. Such high sensitivities can only obtained by very long lifetimes which were ca. 450 and 60 µs in both polymers. The dyes’ alleged moderate photostability has not been investigated up to date.

They few reported luminescence quantum yields of Ru(II) diimine dyes in polymers are usually higher than those in solution.

Thomson et al. had determined the quantum yield of 8 tris(4,7-diaryl-1,10-phenanthroline) ruthenium(II) complexes in unplasticized PVC [46]. They reported quantum yields which were equal or up to a factor of 2.3 higher than in EtOH/MeOH (4:1). There is no obvious correlation, but sturdier ligands gave generally lower quantum yields. Their dye concentrations in PVC were ca. 0.03 mM, i.e. two orders of magnitude smaller than ours. The emission maxima were 17 to 28 nm blue-
shifted, whereas for the polymers investigated here, they were usually red-shifted by up to +10 nm. Considering the obvious differences in dye behavior PVC, we may well expect a different behavior for the luminescence quantum yields too.

_Hartmann et al._ had measured the quantum yield of [Ru(dpp)₃](ClO₄)₂ in PS as 0.6 ± 0.1 in both the 1 mM and the 10 mM sample. Even though the quality of this measurement cannot be easily assessed, we can assume that the 1 mM and 10 mM sample have approximately equal quantum yields [4].

For _Kopelman et al._ a two-fold decrease in luminescence quantum yield when Ru(II) diimine dyes are entrapped in an organic polymer media is a common observation [7]. This discrepancy is the reason why luminescence quantum yield determinations of dyes in polymers are of vital interest. As long as they are not considered many effects will pass unnoticed.

Interesting is a comparison of the sterilization results in 4.5.4 on p. 103 with the characterization experiments in water in 4.4.2 on p. 92 done with identical membranes. PtBuS and PtBrS showed poor adhered to glass when exposed to water, but fared better in the sterilization experiments. This confirms that these polymers have a bad adhesion to glass, but seem to be _a priori_ water-compatible. PMOS has undergone some physical transformation, but apparently with little effect on the dissolved ETH⁰ 3003 as emission maximum wavelength and absolute emission intensity show.

PC, however, which behaved close to ideally in water failed badly in the sterilizations, but not in the tempering. Presumably the combination of water and heat hydrolyzes PC. PDPO’s degradation is most probably only temperature-related. PC remains interesting for low temperature applications, PAMS is the most suitable material for sterilizations, though far from perfect. There is no apparent correlation with any of the observed effects with the glass transition temperature.

We believe that with subsequent sterilizations ETH⁰ 3003 molecules increasingly precipitate or - essentially the same - aggregate. The observed shifts in the emission maxima wavelengths, the decrease in absolute emission intensity and oxygen sensitivity with an approximately constant absorbance of ETH⁰ 3003 are consistent with this. It is not clear whether this is due to a decrease in the free volume of the polymers or induced by water.

It is known that an increasing particle size shifts the color of yellow, orange or brown particles to the red [31]. The absorption spectrum of _solid_ [Ru(bpy)₃]Cl₂·6H₂O for instance is shifted 20-30 nm to the red in comparison to its aqueous solution [42]. There has been no evidence for an absorption maxima wavelength shift in the sterilized membranes.

_Hartmann et al._ deduced from lifetime data for their oxygen sensor using 1 mM [Ru(dpp)₃](ClO₄)₂ in polystyrene that some aggregation of the dye had taken place after that the membranes were stored several days at T = 120°C (i.e. > Tₐ) [4].
Spectral and overall quenching parameters were not significantly affected. They found no evidence for precipitation prior to tempering by means of x-ray diffraction.

When they repeated the experiment for a membrane with 10 mM [Ru(dpp)$_3$]$^{2+}$ in PS, they observed a red shift of the emission maximum from 612 nm to 630 nm (neat crystals having $\lambda_{\text{max}} = 665$ nm) which greatly affected all quenching parameters. Emission intensity decreased by a factor of 3 with a decrease in $K_{SV}$ from 1.56 to 0.56 bar$^{-1}$ (calc.). This suggests that at lower ETH$^T$ 3003 concentrations precipitation might have been less marked or inexistent.

A method to investigate the dye distribution in a membrane is Scanning Confocal Laser Microscopy, which would make dye profiles possible with a resolution down to 1 µm, provided the luminescence intensity can be measured accurately enough (reabsorption in the polymer!). An established method to investigate dye migration is fluorescence correlation spectroscopy of molecules, such as Rhodamine B [47].

There is very little reported in the literature which allows to assess the sterilization experiments. Li et al. have carried out a single steam sterilization at 125°C for 30 min with [Ru(dpp)$_3$](ClO$_4$)$_2$ in RTV 732 silicon rubber [48]. They found a change in $K_{SV}$ of a few percent. Klimant et al. carried out steam sterilizations at 130°C for 1 h of [Ru(dpp)$_3$](trimethylsilylpropanesulfonate)$_2$ in ormosil glasses [9]. After 10 cycles they found a -8% change in $K_{SV}$ and -3% change in $\tau_0$.

Voraberger et al. from Joanneum Research, Graz, have presented sterilizable oxygen optodes based on polysulfone (PSU) and polyetherimide (PEI) at Europt(r)ode 2000 [49]. They found that thermal pre-treatment considerably improved sensor stability. Subsequent sterilizations at 135°C for 60 minutes gave no detectable changes. There was no indication to what exactly the ruthenium(II) diimine dye was or whether there was an emission intensity decrease with subsequent sterilizations. Presumably most of the research in this field will not be reported.

The ETH$^T$ 300x series may have a higher lipophilicity as other ruthenium diimine dyes, but are still essentially ionic species. Lipophilization strategies which worked well with a large host of neutral compounds may reach a limit. Exchanging the counterion helps to some extent [36]. We believe that a covalent linkage with the polymer is necessary to prevent dye aggregation. Few examples have been reported in the literature [50, 51], which, however, do not address issues, such as dye distribution or luminescence quantum yields, in the bound state.

Extruding is a method of dye incorporation which to the author’s knowledge has never been attempted. This would significantly widen the scope of potential polymer matrices. Now that e.g. [Ru(dpp)$_3$](ClO$_4$)$_2$ has become commercially available economical considerations matter less. Heating experiments in the oven have shown that the ETH$^T$ 300x dyes fade between 400 and 500°C, so very probably the complex withstands processing temperatures for most commercial polymers.
4.7 Summary and Perspectives

The new lipophilic ruthenium(II) diimine dye, ETH$^\text{T}$ 3003 (tris(4,7-bis(4-octylphenyl)-1,10-phenanthroline)-ruthenium(II) perchlorate monohydrate), was investigated in different polymers: polystyrene (PS), polystyrene plasticized with $o$-nitrophenyl octyl ether ($o$-NPOE) and $o$-cyanophenyl octyl ether ($o$-CPOE), poly($\alpha$-methylstyrene) (PAMS), poly($p$-tert-butylstyrene) (PtBuS), poly(4-methoxystyrene) (PMOS), poly(2,4,6-tribromostyrene) (PtBrS), poly(2,6-dimethyl-$p$-phenylene oxide) (PDPO) and poly(bisphenol-A-carbonate) (PC). Dye concentrations were usually $\approx$ 8 mM.

Membranes were routinely characterized by their relative quantum yields, their oxygen sensitivity in both air and oxygenated water in terms of Stern-Volmer constants, and their absorption and emission maxima wavelengths. These quantities were usually determined from two or even three identically prepared membranes. The standard deviations for these quantities are given in the full text. A set of membranes was subdue to tempering at 135°C with two subsequent steam sterilizations at 125°C.

The luminescence quantum yield of ETH$^\text{T}$ 3003 in PS ($M_w = 280'000$) was determined as 0.19 ±0.05 in a nitrogen atmosphere. This is lower than e.g. in ethanol ($\Phi_L = 0.52 \pm 0.05$). $\Phi_L$ was identical at $\approx$ 0.8 mM dye concentration. We believe that quantum yields in polymers are generally overestimated [4]. [Ru(dpp)$_3$(ClO$_4$)$_2$ had a $\Phi_{rel}$ (i.e. relative to ETH$^\text{T}$ 3003 in PS) of 0.94 which agrees very well with the quantum yields in ethanol. $K_{SV}$ constants for ETH$^\text{T}$ 3003 and [Ru(dpp)$_3$(ClO$_4$)$_2$ were virtually identical, namely 1.63 and 1.61 bar$^{-1}$, respectively. Emission and absorption maxima wavelengths were the same as in ethanol. Note that all $K_{SV}$ constants are given for the one-site model even for non-linear curves.

The plasticization of PS with $o$-CPOE or $o$-NPOE resulted in a similar decrease in both $K_{SV}$ and $\Phi_{rel}$. For $w = 0.08$ plasticizer, $\Phi_{rel}$ was $\approx$ 0.8 and $K_{SV} \approx 1.3$, for $w = 0.16$, $\Phi_{rel} \approx 0.75$ and $K_{SV} \approx 1.2$ bar$^{-1}$. This shows that $o$-CPOE and $o$-NPOE are equally quenching in polymers and that the quenching behavior in solution should not be extrapolated to polymers [37]. The luminescence lifetime in PS with $w = 0.19$ $o$-CPOE was 4.90 µs in nitrogen and 2.77 µs in oxygen.

$\Phi_{rel}$ was high in oxygen optodes with PMOS (1.36) and PtBrS (1.22), but $K_{SV}$-values were low (1.26 and 0.94 bar$^{-1}$, respectively). PDPO and PtBuS had high, pronounced non-linear oxygen responses with $K_{SV} = 4.42$ and 3.99 bar$^{-1}$, but low $\Phi_{rel}$ with 0.89 and 0.83, respectively. PAMS and PC behaved very similarly to PS. In all the polymers the absorption and emission maxima wavelengths were shifted to higher wavelengths with respect to PS. PMOS had a pronounced red-shift in absorption with $\lambda_{max}^{abs} = 469$ nm (448 nm in PS). Emission in the other polymers
was red-shifted by 4 to 8 nm. Measurements in water gave response times $t_{90}$ of < 0.5 min for all the polymers, apart from PtBrS (~4 min) and PC (~1 min). Oxygen responses in water in terms of $K_{SV}$ with respect to the equivalent oxygen concentration were usually about two thirds of those in gas.

No leaching of ETH T 3003 from PS was observed when cycling with sodium sulfite solutions over a period of 33 days. Sodium sulfite solutions are discouraged for characterization since they distort the oxygen response. Tempering of the polymers at 135°C produced either no change or a decrease of up to -33% in $K_{SV}$ (PDPO). Emission maxima wavelengths generally shifted to lower wavelengths by some nanometers, while absolute emission intensities increased in all membranes by up to +20%. This demonstrates the importance of tempering to separate temperature from sterilization effects.

Emission wavelength maxima shifted to higher wavelengths with subsequent sterilizations and with shifts up to +4 nm per sterilization. After two subsequent sterilizations, absolute emission intensities had decreased by typically -20% per sterilization, with the smallest change in $K_{SV}$ of -1.3% and the largest change of +6.1% with respect to the state before the second sterilization. Absorbance spectra showed no loss of ETH T 3003 but a general increase in turbidity of +0.1. This data was interpreted as meaning that ETH T 3003 increasingly precipitates in the polymers, which precludes their prolonged use for sterilization. It is not clear whether this could be due to the high dye concentration of $\approx 8$ mM.

PC that had probably undergone a partial hydrolysis showed the largest deterioration of all the parameters; but there were also signs of a slight chemical modification in PDPO. PAMS is the best material among those investigated. The increase in lipophilicity of ETH T 3003 is not sufficient to dissolve it directly into the three polysiloxanes investigated.

The investigations with three identically prepared membranes for each composition revealed some statistical trends. Two-site model Stern-Volmer constants differed more within the same composition than between different compositions. Absorption maxima wavelengths and above all relative luminescence quantum yields must be determined for several membranes, considering that standard deviations may be as large as 10%.

Changes in oxygen sensitivity during sterilizations were modelled with the usual one- and two-site models, as well as with $K_{SV}$-distributions following a normal, log-normal, Maxwell-Boltzmann, exponential or a Gamma distribution. Normal, Gamma and Maxwell-Boltzmann distributions did not fit the data as well as the two-site model, but can also be sensibly interpreted.
4.8 References


5 Photobleaching of ruthenium(II) diimine dyes

5.1 Introduction

What determines the lifetime of a luminescence-based oxygen sensor? If there are not especially harsh conditions, such as sterilizations, aggressive chemicals or high temperature, there is still a dye’s most common enemy: light. Every dye fades one day [1] and this applies to ruthenium(II) diimine dyes as well. The issue is of special importance for the ruthenium(II) dyes because they must be flooded with light in order to give a maximal luminescence signal. The question is how fast bleaching occurs and how this affects sensor response.

The term (photo)bleaching used throughout this work refers to any photochemical transformation of dye molecules which precludes their primary function, in this case their luminescence used for oxygen sensing. Other terms frequently used are photochemistry, photodegradation or -decomposition, but these terms do not suggest an observable change in absorption in the same way as the term bleaching does. The products created upon photobleaching will be termed photoproducts.

Until rather recently it was widely accepted that the problem of photobleaching was one of intensity-based sensors, rather than of lifetime-based ones (see p. 67 for the measurement principles). This would hold true if a bleached dye simply lost its luminescence, but was otherwise photochemically inert. Put in other words, it was assumed until recently that an average dye’s quenching microenvironment would not change. In the following I will show that this is not true because photoproducts act as quenchers. Even if the initial assumption were true, we would still expect a reduction in concentration quenching upon considerable bleaching.

Most photodecomposition studies found in the literature have only been carried out with hydrophilic ruthenium(II) complexes in water or aqueous solutions. Peter Belser et al. have observed the photosubstitution of biq (2,2’-biquinoline) with water in Ru(bpy)$_2$(biq)$_2^{2+}$ when the complex was irradiated in acetone/water at pH 1.5. They argue that the same excited $^3$MC state is responsible for photosubstitution with water as well as for photoracemization since they have similar activation energies [2, 3] (for the photophysics and explanation of the states see section 2.1 on p. 7).

Watts et al. [4] have carried out a bleaching experiment with 436 nm-irradiation on a $10^{-4}$ M Ru(bpy)$_3^{2+}$ solution in 0.1 M HCl at both 25° and 95°C. They found no detectable changes in spectra at 25°C within 5 hours, but observed spectral changes
for Ru(bpy)$_3^{2+}$ at 95°C. A shoulder in the absorption band at 500 nm emerged and then disappeared while a new band at 365 nm appeared. After photolysis Watts et al. used fluorescence spectroscopy and found uncomplexed 2,2’-bipyridine in the solution.

Concluding from their spectra at different time intervals bleaching was accelerated with progressing bleaching. Watts et al. claim from the uncomplexed 2,2’-bipyridine and the appearance of the band at 500 nm that a ligand substitution has taken place.

Vaidyalingam et al. recently carried out an elegant study on photodecomposition of Ru(bpy)$_3^{2+}$ [5]. Based on retention times and detector absorption spectra in HPLC they showed that Ru(bpy)$_3^{2+}$ loses bpy upon irradiation in aqueous solution. Rodgers et al. investigated the energy and electron transfer from Ru(bpy)$_3^{2+}$ to molecular oxygen in D$_2$SO$_4$ / water solutions [6]. They observed a “chemical” bleaching, which they attributed to Ru(bpy)$_3^{3+}$. The solution reverted within 10 hours to Ru(bpy)$_3^{2+}$, i.e. the reaction was reversible. They found that bleaching increased with both increased proton and oxygen concentrations.

Demas et al. were the first to investigate the photochemical decomposition of ruthenium(II) diimine complexes in silicone rubber [7], i.e. in a luminescence-based oxygen sensor. They believed that singlet oxygen was not the primary cause of sensor deactivation. They based this assumption, however, only on emission decay measurements, and conducted no further investigations. Semi-empirical investigations have been made with [Ru(dpp)$_3$](trimethylsilylpropanesulfonate)$_2$ in ormosil glasses [8].

Hartmann has just published a paper on ruthenium complex photodecomposition [9] to which the author of this work contributed to the discussion. It is the only literature source which reports the photochemistry of similar ruthenium(II) diimine dyes under comparable circumstances, and is therefore worth discussing at length. Hartmann carried out photobleaching experiments with a blue LED on Ru(bpy)$_3^{2+}$, Ru(phen)$_3^{2+}$ and Ru(dpp)$_3^{2+}$ in PVC, PS as well as on silica gel. He has mainly published absorption difference spectra, weighted decay times and relative phase shift changes.

He clearly showed for Ru(dpp)$_3^{2+}$ in PS that the presence of oxygen during photobleaching increased the relative phase shift change measured in the absence of oxygen. After 16 h of irradiation with 16 mW, he found a phase shift of -0.9% for photobleaching at 0 bar O$_2$, which increased quickly with [O$_2$] and leveled off to -5.3% at 1 bar O$_2$. He also demonstrated that the photobleaching effect depends on the concentration of Ru(dpp)$_3^{2+}$ in PS. The relative phase shift when irradiated with light in an oxygen or air atmosphere was a single order of magnitude larger for the 10 mM than for the 0.1 mM dye concentration.
Another important experiment was photobleaching Ru(dpp)$_3^{2+}$ in PVC plasticized with dioctyladiapate (DOA) in the presence and absence of oxygen. It was only in the presence of oxygen that the long-wavelength bands around 600 nm formed, whereas in the absence of oxygen there was only a decay of the main absorption band around 450 nm. In general he found larger photobleaching effects in PVC than in PS.

In an earlier paper he and coworkers had already shown that singlet oxygen scavengers slow down the photoinduced decrease of the decay time, but did not affect the rate of decrease in the overall luminescence intensity [10].

I decided to investigate photobleaching in more depth to try to identify a mechanism for the process. Empirical studies have their benefits, but cannot provide the basis for a focussed strategy on how to prevent photobleaching in the first place. All the studies reported here were carried out with ETH$^\text{T}$ 3001 (tris(4,7-bis(4-propylphenyl)-1,10-phenanthroline)-ruthenium(II) perchlorate), but the conclusions are likely to hold for most other ruthenium(II) diimine complexes as well, especially the popular [Ru(dpp)$_3$]$^{2+}$.

The first experiment investigated whether any significant bleaching can occur under the standard characterization conditions given in section 5.3.1 on p. 126, and found that none took place. A second experiment, however, showed that bleaching with a blue LED under the conditions prevailing in commercial instruments causes significant bleaching, see section 5.3.2 on p. 126.

In order to speed up the timescale of photobleaching, I resorted to using a well-characterized mercury lamp (5.2.1) for the next experiments. A representative plasticized polystyrene membrane was extensively bleached. Bleaching progress was monitored by both full luminescence and absorption spectra. The results are discussed in section 5.4. Special attention was paid to self-absorption and the actual number of photons absorbed. An innovative application of Matrix-Assisted Laser Desorption/Ionization (MALDI) mass spectrometry allowed the photoproducts in the bleached membrane to be directly investigated, see 5.7.1.

A similar bleaching experiment was also carried out, but monitored by Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) spectroscopy. This allowed the photodegradation of the polymer to be assessed and possible acceleration of the bleaching to be determined. The results are presented in 5.6 and discussed in 5.8.1 Influence of the polymer matrix on p. 145. Additionally, ETH$^\text{T}$ 3001 bleaching was monitored by ATR FT-IR spectroscopy (see 5.6).

Carefully designed solution experiments at different dye concentrations allowed the kinetics of photobleaching in solution to be assessed as well as the production of photoproducts in larger quantities. The solution experiments are discussed in 5.5. The photoproducts produced in these experiments were investigated using ATR FT-
IR spectroscopy (see 5.6) and Electrospray Ionization (ESI) mass spectrometry, which has a very high information content (5.7.2).

Finally, a comprehensive mechanism for the photobleaching based on the experimental evidence and literature is elucidated in 5.8.2.

All the experimental details are given in 5.2, except for the LED set-up which is described in the Appendix (section 8.3.3 on p. 239).

5.2 Methods and materials

5.2.1 Bleaching light source

A Sylvania Par 38 Mercury Lamp, type H44GS-100, 100 Watt (GTE Products Co., Winchester, KY, USA) was used to bleach the membranes. The mercury lamp takes approximately 10 minutes to reach its full, stable intensity with most of its intensity focused on a small spot on the lamp’s symmetry axis. An emission spectrum of the lamp was recorded on the PE LS-50B luminescence spectrophotometer and corrected to represent energy intensities, see Fig. 5.1. The UV light was of very small intensity since most of the UV light is absorbed by the outer glass bulb.

![Fig. 5.1 Emission spectrum of the Sylvania Par 38 mercury lamp](image)

Tags for emission lines indicate estimated power density [W/m²] at the spot center 20 cm from the lamp, assuming that all the light intensity is incorporated in the tagged lines. Most of the UV intensity is absorbed by the outer light bulb.

Its absolute intensity was measured with the Newport Digital Power Meter (Newport, Fountain Valley, CA, USA), using a neutral density filter with an optical density of 3.0. A weighted calibration factor derived from the energy spectrum of the
lamp and the photodiode’s surface were used to calculate a light energy density of approximately 1.5 kW/m² at 20 cm distance. This value is not very accurate since several uncertainties intervene. The emission correction of the spectrum is not very accurate, especially below 400 nm. No UV range calibration factors are given for the Newport Digital Power Meter, those used are linearly extrapolated below 400 nm. There is no reasonable standard in chemical actinometry [11] which can handle the broad spectral range of the mercury lamp.

5.2.2 Membrane bleaching set-up

The membrane was placed at 10 cm distance at the center of the lamp’s light spot (intensity 6 kW/m²), using a set-up depicted in Fig. 5.2. The glass plate with the membrane was horizontally placed with the membrane side up on a white holder. The holder itself was placed on a rail-guided sled and could be reproducibly positioned under the mercury lamp in a light-protection box. This allowed to run the lamp continuously during the experiments in a defined state whilst ensuring UV protection and minimize heating. Continuous irradiation over 10 min lead to a noticeable heating of the membranes, hence the maximum interval size was limited to 10 minutes. At the beginning of the experiment and after each exposure emission and absorption spectra were recorded. The glass plates had been marked to make accurate repositioning in the spectrometers possible. Emission spectra were recorded on the PE LS-50B luminescence spectrophotometer in ambient atmo-
sphere with the membrane side facing the spectrometer entrance, which was done to average out as much as possible the remaining positioning uncertainty (compare 3.2.4 Methodology of luminescence and absorbance measurements on p. 46). The slit width combination 2.5/10 nm was used to increase the S/N-ratio.

5.2.3 Solution bleaching set-up

The long irradiation times for bleaching in solution necessitated a more sophisticated set-up than in Fig. 5.2 which prevented heating of the sample solution. The mercury lamp was again placed at 10 cm distance from the sample, resulting in 6 kW/m² irradiated light. Experience showed that lamp exposure > 1 h is likely to give a temperature rise of 10-20°C, which will accelerate chemical reactions in solution, force solvent evaporation and also lower oxygen solubility. These problems were effectively solved by placing a heat-absorbing quartz glass plate in front of the cuvette which was cooled by a fan, see Fig. 5.3. Solution levels were marked by pen and evaporation losses replaced with solvent prior to absorbance measurements. 10 x 10 mm or 1 x 10 mm Suprasil™ cuvettes with stopcocks were used. Great care was taken that lamp and cuvette were aligned because the lamp intensity drops off laterally quickly.

The quartz cuvette wall can reflect or absorb light additionally, hence reducing the total light intensity. Absorption of a quartz cuvette filled with ethanol amounts to a constant 7-8% down to 250 nm, giving only 3-4% less light intensity for one cuvette wall. This small decrease of the total light intensity has been neglected for the calculations.
5.2.4 Bleaching monitored with ATR FT-IR

All spectra have been recorded with the diamond crystal of the Golden Gate ATR unit ($n_D = 2.47$, single reflection) as waveguide for a Bruker Vector 33 FT-IR spectrophotometer (Bruker, Billerica, MA, USA). Resolution was 1/4 cm$^{-1}$ for all measurements.

A similar bleaching set-up as in Fig. 5.3 had been used with the same light power and distance to the sample, see Fig. 5.4. ETH$^T$ 3001, PS and the 1.3% ETH$^T$ 3001 with 20.0% o-CPOE/78.7% polystyrene membrane had been deposited as a chloroform solution onto the diamond crystal.

![fig5_4](image)

**Fig. 5.4 Set-up for in-situ bleaching and IR-monitoring with the Vector 33**

5.2.5 Mass spectrometry

MALDI mass spectra were recorded by Edda Lehmann in the group of Prof. Renato Zenobi at ETH Zürich on their linear MALDI-TOF mass spectrometer [12]. The sample for the unbleached membrane was the original membrane cocktail in chloroform used to prepare the membrane. Some microliters of this solution were deposited on a MALDI tip and air-dried. The same procedure was done for the bleached membrane which had been redissolved in chloroform. Spectra were recorded in positive ion mode, the work was carried out in one session to avoid instrument artifacts, mass accuracy was +/- 2 Da.

ESI-MS spectra have been recorded on a Finnigan TSQ 7000 (San Jose, USA) by Oswald Greter of the MS service of the Laboratory of Organic Chemistry. Samples were redissolved in methanol for spectra recording. Displayed ESI-MS spectra have been smoothed and normalized to the base peak.
5.3 Bleaching under measuring conditions

5.3.1 Bleaching in the luminescence spectrophotometer

A vital question for anyone concerned with dye bleaching is whether standard measurements in spectrometers result in a sufficient light exposition to cause bleaching. In this work a prudent approach was pursued which tries to minimize photochemistry. In order to estimate the effect of light bleaching an experiment was performed with a membrane exposed to air with maximized light irradiation. The xenon flash light lamp was continuously on with the excitation slit width set to the largest possible value of 15 nm. Remember that the excitation slit width used throughout this work was 2.5 nm. The total excitation light power at 460 nm increased from 0.750 µW (2.5 nm slit width) to 13.5 µW (15 nm slit width), accompanied by a larger spot size of 2.5 x 9 mm. Power density hence rose from 0.063 W/m² to 0.6 W/m².

During 5 days the membrane was exposed alternatingly to 3500 s nitrogen and 100 s oxygen at a reduced flow of 40 ml/min to save gas. The following 5 days the pattern was inversed, with 3500 s oxygen and 100 s nitrogen. Alternating the two regimes allows to constantly assess sensitivity, whilst still maintaining an “oxygen” or “nitrogen” atmosphere. As with the previous experiment, the xenon flash light lamp was continuously on with the excitation slit width set to 15 nm. During the nitrogen regime the nitrogen signal increased +2.6‰/d whereas the oxygen signal increased even +6.0‰/d. This was astonishing since a constant or decreasing signal had been expected. In the oxygen regime the increase leveled off with -0.6‰/d for the nitrogen and +1.6‰/d for the oxygen signal, i.e. still no conclusive trend. Interesting is the appearance of a pronounced shoulder in the excitation spectrum below 300 nm with almost double the intensity, though this is in an unreliable wavelength range of the PE LS-50B.

Even a maximized light exposure in the PE LS-50B luminescence spectrophotometer over 5 days gave no clear evidence of bleaching of a ruthenium(II) diimine dye in a polystyrene membrane. Therefore, we can conclude that bleaching occurs during the standard membrane characterization processes reported here.

5.3.2 Bleaching with a blue LED

Most professional and semi-professional measurement set-ups with ruthenium(II) diimine dyes which claim some practical usability rely on a blue LED as an excitation source, be it for intensity or life-time based measurements. Since involved light intensities are considerably larger than in a spectrometer the bleaching effect in a LED-based set-up has been investigated.
For the measurement a membrane of 1.2% ETH T 3001 in 80.3% PS/ 18.3% o-CPOE had been used in the set-up as described in 8.3.3 Self-referenced two-photodiode set-up on p. 239. The experiment ran over holidays when the laboratory was not in use which minimized light and vibration interferences. The result is shown in Fig. 5.5.

The signal from the reference photodiode is fairly stable which indicates that the excitation light level from the LED had been more or less constant. The signal from the luminescence photodiode, however, decreases in a linear fashion which demonstrates that bleaching has occurred. The actual luminescence signal is only a fraction of the whole signal, the rest being unwanted excitation light, luminescence from membrane parts which are not exposed to the sample or an offset of the amplifier, as could expected from the kind of prototype set-up used.

The dynamic range of the signal \((S(N_2)-S(O_2))/S(N_2)\) covered originally 8.89% of the total signal and fell down to 7.75% after 8 days (relative change 12.45%) whilst \(S(N_2)\) fell 9.53%. We can calculate the background contribution of the luminescence photodiode signal if we assume that the dynamic range relative to the total luminescence remained constant. In other words, the oxygen quenching characteris-
tics as expressed by $K_{SV}$ are assumed constant. We obtain that 76.6% of the measured signal are actually luminescence which corrects the initial bleaching rate of -1.096% to -1.43% / d.

It is important to note that the membrane had been used for different previous experiments with a total light exposition of a week, though in nitrogen atmosphere.

A good estimate of the light intensity on the membrane can be derived from the photodiode signal and the geometry of the set-up, compare Fig. 8.6 on p. 242. Given a reference photodiode current of 7.5 µA (corresponds to a signal of 1.65 V with an amplification gain of 2.2·10^5) and a calculated spectral sensitivity of 0.61 A/W at 650 nm we must have had 21 µW light at the reference photodiode. With its surface of 7 mm^2 and an irradiation angle of 45° (detected light 0.71 times the irradiated light density) we obtain 2.5 W/m^2 light power density. This translates into 1.3 W/m^2 at the membrane based on light intensity being proportional to $1/r^2$ (distances $a = 25$ mm and $b = 35$ mm in Fig. 8.6).

We must conclude that considerable bleaching can occur with a LED which will rule out any real long-term application.

5.4 Bleaching of ETH$^\text{T}$ 3001 in the membrane

Given the long irradiation times needed with a blue LED to cause noticeable bleaching a more powerful irradiation source was needed to reduce the time scale of bleaching. A mercury lamp (see 5.2.1 Bleaching light source on p. 122) was used which, however, is no longer a monochromatic light source. An accelerated bleaching experiment was performed with a membrane of 1.2% ETH$^\text{T}$ 3001 in 80.2% polystyrene with 18.5% o-CPOE cast onto a quartz glass plate.

The temporal evolution of the absorbance/luminescence intensity can be seen in Fig. 5.7, the corresponding absorption spectra are shown in Fig. 5.6.

Both luminescence intensity and absorbance of the membrane decrease rapidly upon accelerated bleaching. In the absorption spectra two isosbestic points reveal that bleaching seems to be a stochiometric reaction with one molecule of ETH$^\text{T}$ 3001 yielding one molecule of a photoproduct. This does not preclude the existence of other photoproducts from the same or subsequent reactions which do not absorb or only absorb slightly.

Exponential fits to the curves yield $32.9 + 68.1 \times \exp(-0.0176 \times t)$ for the absorbance and $2.6 + 95.9 \times \exp(-0.080 \times t)$ for the luminescence. The luminescence intensity is falling approximately four times faster than the absorbance. It is important to note that the luminescence data are values at $[O_2] = 21\%$, i.e. they only accurately reflect the temporal evolution of the unquenched luminescence in the case that relative oxygen sensitivity stays the same. Presuming that the exponential fit is
valid and that there is no mechanism change during photobleaching, the final absorbance would be 33% of the initial absorbance. Based on this, the half-life for the dye transformation would be 39 min. This compares to a 5.5 min half-life of the azo dye ETH\textsuperscript{7} 4012 [13] in polysiloxane, which fades away completely under identical experimental conditions.
The number of absorbed photons per molecule $N_{\text{abs}}$ can be calculated based on the known light power distribution of the mercury lamp (see Fig. 5.1 on p. 122), the absorption spectra of ETH$^T$ 3001 and its absorption coefficient.

The total absorbed light intensity $I_{\text{abs}}$ in quanta·m$^{-2}·s^{-1}$ can be calculated from the known light power $I(\nu_i)$ compromised in the $n$ individual mercury lamp lines at the frequencies $\nu_i$ and the fraction of light which is absorbed by ETH$^T$ 3001 with its absorbance $A(\nu_i)$ at the respective frequencies:

$$I_{\text{abs}} = \frac{1}{N_A} \cdot \sum_{i=1}^{n} \frac{I(\nu_i)}{h \nu_i} \cdot (1 - 10^{-A(\nu_i)}).$$

Equation (5.1)

$I_{\text{abs}}$ amounts to $2.7 \cdot 10^5$ mol photons·m$^{-2}·s^{-1}$ at the beginning of photobleaching. For practical reasons the number of photons is given in moles, hence the division by Avogadro’s number $N_A$.

The surface concentration $\Gamma$ of $2.85 \cdot 10^{-5}$ mol·m$^{-2}$ ETH$^T$ 3001 molecules can be calculated according to Beer’s law as

$$\Gamma = \frac{A(\lambda_r)}{\varepsilon'(\lambda_r)}$$

from the absorbance $A(\lambda_r)$ and the absorption coefficient $\varepsilon'$ of $3.317 \cdot 10^3$ m$^2$/mol given in the unusual dimension [mol·m$^{-2}$]. By simple division

$$\frac{dN_{\gamma}}{dt} = \frac{I_{\text{abs}}}{\Gamma}$$

Equation (5.3)

the number of absorbed photons of $9.7 \cdot 10^9$ molecule$^{-1}·s^{-1}$ is obtained even though the actual number of molecules is unknown. Based on the initial signal decrease an average ETH$^T$ 3001 molecule has a quantum efficiency of $1.4 \cdot 10^{-13}$ for photobleaching with respect to luminescence (including quenching) or $3.0 \cdot 10^{-14}$ with respect to absorbance under the chosen experimental conditions.

A second two-point determination with a membrane with identical composition which had been exposed 15 min continuously had shown a $20\%$ faster photobleaching rate. This could be a temperature effect because the membrane had turned luke-warm.

The wavelength of the emission maximum of ETH$^T$ 3001 of initially 610.0 nm shifts linearly $+(0.18 \pm 0.01)$ nm/($\%$ loss in absorbance). Furthermore, the peak width grows at half maximum $+0.8$ nm/($\%$ loss in absorbance) to the red side. There are two possible explanations for this observation: either the peak really red-shifts or the blue-side of the luminescence peak gets increasingly self-absorbed within the
membrane due to the increasing red shoulder in the absorption spectra. The effect of self-absorption can be easily estimated based on the absorption spectra series. Luminescence which is emitted from the flow-through cell side of the membrane will have to cross the whole membrane until its detection by the luminescence spectrometer, with the membrane acting as filter. On the other hand, light which is emitted from the glass support side of the membrane will be detected undistorted. It follows that for an average emitting position half the membrane’s absorbance will act as a filter. This has to be corrected for the slightly longer absorption path because the emitted light is not detected at the normal to the membrane, but at a 60° angle. For a PS membrane this yields a 33° angle of the light path to the normal within the membrane due to refraction, resulting in an 1.2 times longer absorption path which is, considering the small variations in the refractive index of polymers, a typical value. This corrects the factor to 0.6 of the absorption spectra which acts as filter.

The absorption spectra need to be corrected for the background introduced by the glass support, which is an offset of 0.049 for the emission wavelength range. The original emission spectra $I_{corr}$ is filtered by multiplication of the recorded emission spectra $I$ by the membrane filter transmission spectra $T_f$. We, however, have the filtered emission spectra and want to reconstruct the original emission spectra which is calculated as

$$I_{corr} = \frac{I}{T_f} = \frac{I}{10^{-0.6 \cdot (A - \text{offset})}}.$$

(5.4)

The self-absorption effects for the membrane with an ca. absorbance of 0.1 are minuscule. The initial self-absorption loss is 3‰ and increases to 8‰ during photobleaching.

Another very similar optical characterization is NIR-VIS spectroscopy which might yield further informations. Unfortunately, usual sensor membranes cannot be investigated, because the glass wafers cause oscillations in the spectra and overall absorptions are too small. Measurements in transflection (e.g. on a white Teflon strip) work could detect changes in the bulk compounds like polymer and plasticizer.

5.5 Bleaching of ETHT 3001 in solution

Even though the bleaching experiment in the polystyrene membrane in 5.4 on p. 128 reflects a true life situation, results are limited because only very small amounts of ca. 30 nmol ETHT 3001 are bleached which is too little to investigate with most standard methods, such as IR or NMR. In addition most standard methods need solutions, which requires a difficult extraction step of the dye from the
plasticized polymer. Thus, for further investigations on the nature of the photoproduct bleaching experiments were carried out in ethanol. Trial experiments showed that bleaching in solution is much slower than bleaching in the polystyrene membrane. Solutions in two cuvettes sealed with septum caps, one being purged regularly with nitrogen, the other open to admit air, were bleached simultaneously at 10 cm distance with the mercury lamp. In either case bleaching of an approximately 10^{-5} M solution of ETH^T 3001 in ethanol was orders of magnitude slower than bleaching in the membrane. Bleaching was definitely faster with oxygen being present, but the small absorption changes, lacking long-term gas tightness of the septum and concentrating effects with nitrogen purging did not allow a proper quantification.

Carefully designed experiments were made with the improved set-up in 5.2.3 Solution bleaching set-up on p. 124. A 0.013 mM solution of ETH^T 3001 in ethanol in a 10 x 10 mm Suprasil™ and a 0.257 mM solution in a 1 x 10 mm Suprasil™ cuvette with the supernatant air were exposed to the same amounts of light from the mercury lamp. The similar absorbance of the solutions (ca. a factor of 2 difference) guaranteed that both solutions absorbed an approximately equal number of photons. Fig. 5.8 shows the absorption spectra and the temporal evolution of the absorbance at three selected wavelengths.

Unfortunately, because of the unknown nature of photoproduct(s) no clear measure for the reaction progress can be extracted from the absorption spectra. The main difference in the mechanism for the two solutions is best visible at the temporal evolution of the absorbance at 460 nm and 394 nm, which represent the absorption band which is responsible for the luminescence and the isosbestic point found for the photoproduct earlier respectively, see Fig. 5.6 on p. 129.

The curve for the absorbance at 460 nm is upward-curved for the higher concentration and downward-curved for the lower concentration. The reaction for the lower concentration seems at first glance to be either catalyzed by the photoproduct or inhibited by the intact dye which is unlikely as this is obviously not the case for the higher concentrated solution. The downward-curving for the concentrated solution shows a quick initial decay, but the decrease levels off.

The absorbance evolution of the concentrated solution would most probably proceed as the diluted solution after half time if the experiment had been continued. The absorption band at 280 nm and the shoulder at 320 nm, however, are still substantial in the concentrated solution whereas in the diluted solution they have almost completely gone at that point, which suggests that different photoproducts have been formed.

The initial number of absorbed photons was 3.2 \cdot 10^2 and 5.0 \cdot 10^2 mol/s with a total of 3.5 \cdot 10^{-7} mol and 6.9 \cdot 10^{-7} mol ETH^T 3001, respectively. Please note that the number of absorbed photons does not depend linearly on the absorbance or dye concentra-
Photobleaching of ruthenium(II) diimine dyes

Taken per molecule this gives the very large numbers of $7.2 \times 10^9$ and $9.1 \times 10^9$ photons·molecule·s$^{-1}$ absorbed. Based on the lifetimes of 4.13 µs in an aerated solution of ETH$^T$ 3001 in ethanol (see Table 2.4 on p. 24) the molecules would be constantly excited.

A major difference between the two experiments is the absolute number of dis-

Fig. 5.8 Bleaching of ETH$^T$ 3001 in ethanol at different dye concentrations

The wavelength of 394 nm represents the isosbestic point found for the photoproduct earlier (see Fig. 5.6 on p. 129), 460 nm the band responsible for the luminescence and 550 nm the band of the appearing photoproduct. The absorbance values of the 0.26 mM solution have slight discontinuities due to uneven replacement of evaporated ethanol in the 1 x 10 mm cuvette. All absorbance timetrack values have been normalized to $t = 0$. 

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A major difference between the two experiments is the absolute number of dis-
solved oxygen molecules if an equal saturation of the different cuvette volumes is assumed (2.1 mM at 25°C [14, 15]); the diluted solution has ca. 20 times more oxygen molecules per dye molecule than the concentrated solution.

A plot of the normalized absorbance against the number of photons absorbed gives a clearer picture of the kinetics, see Fig. 5.9.

The most obvious finding is the very quick decay in the diluted solution which is terminated after $1.8 \times 10^8$ mol photons have been absorbed, i.e. an average quantum efficiency for disappearance of $1.2 \times 10^{-15}$ per original molecule of ETH\textsuperscript{T} 3001. The absorbance decrease of the concentrated solution at 460 nm is not proportional to the quantity of absorbed photons as expected, but is getting slower instead, which suggests that photoproduct(s) stabilize(s) the remaining ETH\textsuperscript{T} 3001. A part of this could be explained by the fact that the fraction of energy-rich photons has been halved since the beginning of the experiment even though the average energy of absorbed photons has remained stable within 5% during the whole experiment.

For further experiments a sample with ~0.27 mM solution of ETH\textsuperscript{T} 3001 in ethanol in a 1 x 10 mm-cuvette with the supernatant air was irradiated for 48 h as the 0.257 mM solution above; its absorbance at 620 nm had decreased to ~67%. The resulting product has been used for investigations in 5.6 Investigation of bleaching by ATR FT-IR spectroscopy on p. 135 and 5.7.2 Electrospray Ionization Mass Spectrometry on p. 142.

An attempt to produce the photoproduct in large quantities in CDCl\textsubscript{3} (Dr. Glaser AG, Basel) for structural identification in an NMR study failed because the system
obviously behaved differently. NMR spectroscopy would have allowed to clearly identify the structure of the photoproduct. The solution in the sealed quartz cuvette was much more concentrated (6.5 mg in 0.7 ml, i.e. 6 mM) compared to the ethanol experiments, the supernatant being air. Within 4 hours of bleaching the completely opaque solution had turned transparent, the previously undissolved dye had dissolved due to the formation of a non-absorbing photoproduct. However, slight changes in the corresponding spectra (taken from diluted samples of the concentrated solution) were observed, indicating that a small fraction of the same species as in the membrane might have been created. Since this small amount does not account for the considerable dye dissolution during photobleaching, we deduced that bleaching in CDCl3 seems to be faster than in ethanol, but must be based on a different bleaching process which leads directly or via short-lived intermediates to a non-absorbing photoproduct. The crucial difference is very likely the high “concentration” of the oversaturated solution rather than the nature of the solvent.

5.6 Investigation of bleaching by ATR FT-IR spectroscopy

IR spectroscopy has been routinely used to investigate the photochemistry of polymers. Techniques, such as the Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) spectroscopy, are especially useful because they make in situ observation of photochemical processes possible. The principle of Attenuated Total Reflection relies on light which is trapped by total reflection inside a waveguide of a high refractive index. Absorption takes place only in a very thin layer outside the waveguide due to the so-called evanescent field. This eliminates the need to prepare polymer samples with a given thickness or surface topology, furthermore, in most spectrometers the sample becomes freely accessible from above. Thereby it is possible to record IR spectra during simultaneous irradiation.

The penetration depth $d_p$ of the evanescent wave into the polymer can be calculated according to

$$d_p = \frac{\lambda/\hat{n}_1}{2\pi(\sin\theta_1^2 - (\hat{n}_2/\hat{n}_1)^2)^{1/2}}. \quad (5.5)$$

$\lambda$ is the IR wavelength, $\hat{n}_1$ and $\hat{n}_2$ are the complex refractive indices of the ATR crystal and the sample, respectively. $\theta_1$ is the angle with which light is coupled into the ATR crystal [16]. For a PS membrane with light coupled into the crystal under a 45° angle, we obtain a penetration depth of 0.22·$\lambda$ if we ignore the small complex contribution. At 1500 cm$^{-1}$ the penetration depth is 1.5 µm, and at 3000 cm$^{-1}$ 0.7 µm.
This implies that the low-energy vibrations are overemphasized in contrast to a normal transmission measurement.

Fig. 5.10 shows IR spectra of the membrane consisting of 1.3% ETH\textsuperscript{T} 3001 with 20.0% $\alpha$-CPOE/78.7% polystyrene recorded prior to the bleaching experiment as well as spectra of its individual components. Residual chloroform (peaks at 754 and 668 cm\textsuperscript{-1}) which had been used to dissolve the membrane cocktail completely disappeared from the spectra within 5 minutes. No peak from ETH\textsuperscript{T} 3001 is discern-
ible in the composite membrane spectrum, obviously due to its low concentration of 1.3%. It is important to note that the band of ETH\textsuperscript{T} 3001 with the highest absorbance stems from perchlorate which is \textit{a priori} exchangeable and gives little information on structural changes of ETH\textsuperscript{T} 3001, compare 2.3.4 \textit{IR spectra} on p. 18.

The spectrum seem to be roughly a superposition of the pure polystyrene and the \textit{o-CPOE} spectra despite the latter’s 4-fold lower concentration. Noteworthy is the complete absence of the polystyrene band at 668 and 1217 cm\textsuperscript{-1} as well as the unexpectedly small \textit{o-CPOE} band at 1164 cm\textsuperscript{-1}. One could speculate whether the \textit{o-CPOE}’s large contribution is due to enrichment at the interface to the diamond crystal. A complete assignment of PS peaks can be found in [17].

A sample of solid ETH\textsuperscript{T} 3001 had been bleached in situ on the ATR crystal. The lower part of Fig. 5.11 shows five spectra out of 21 which had been recorded during the experiment’s 20 hour-duration; the upper part contains IR spectra of a ETH\textsuperscript{T} 3001 sample which had been bleached as a 0.27 mM solution in ethanol before and after bleaching, compare 5.5 \textit{Bleaching of ETH\textsuperscript{T} 3001 in solution} on p. 131. The diamond crystal’s low transmission is responsible for the poor spectra quality in the 2400 to 1800 cm\textsuperscript{-1} range.

The spectra of the in-situ bleaching needed extensive correction for water (4000 to 3400 cm\textsuperscript{-1} and 2100 to 1400 cm\textsuperscript{-1} range) as well as for carbon dioxide peaks (2400 to 2150 cm\textsuperscript{-1} and 750 to 600 cm\textsuperscript{-1} range). Even though the instrument had been allowed to warm up for a day and was constantly purged with nitrogen, water peaks gave up to ±7% change in transmission which would have completely distorted low-resolution spectra. This was corrected by subtracting a previously recorded high resolution spectrum of water and carbon dioxide such that the corresponding water/carbon dioxide peaks disappeared. The increased noise is very apparent in comparison with the sample bleached in solution whose IR spectra has less such artifacts. No satisfactory spectra correction could be achieved in the range above 3500 cm\textsuperscript{-1}, possibly because water was present in a form different than water vapor.

The ex- and in-situ bleaching experiments yield essentially the same result, though with slight quantitative differences. Bands at 1092, 622, 743, 838, 1415 and 1507 cm\textsuperscript{-1} turned weaker whereas bands around 1679, 1729, 1282, 3357 and 3483 cm\textsuperscript{-1} grew stronger (bands are listed in order of absolute transmission change).

The change of the bands around 1679, 1729 and 1282 cm\textsuperscript{-1} is much stronger for the in-situ bleaching whereas the change is bigger in the ex-situ bleaching for all the other peaks listed. The bands at 2920 and 2850 cm\textsuperscript{-1} which grow considerably in intensity (∼6% in transmission) in the ex-situ bleaching spectrum, however, are not present at all in the in-situ bleaching spectrum. There is a general transmission decrease between 1750 to 900 which appears in either spectra and is therefore not due to any long-term instability of the instrument.

The decrease in intensity of the 1092 and the 622 cm\textsuperscript{-1} bands must be due to a deg-
After 14 h of in-situ bleaching most bands reach a stable state, including the most important ones at 1679 and 1729 cm\(^{-1}\), but not the one at 1282 cm\(^{-1}\) whose transmission is still decreasing at almost the initial speed. It is tempting to conclude that the band at 1282 cm\(^{-1}\) should be attributed to a follow-up or competitive reaction rather than the initial degradation which obviously yields a product with bands at 1679 and 1729 cm\(^{-1}\).
Attribution [18] of the newly formed bands of the photoproduct is not possible without further data. If we assume that it must be an oxygen-containing functional group we would expect a peroxide, ketone, hydroxyl group, carboxylic acid, N-oxide or a ruthenium oxide.

A deprotonated carboxylic acid can be ruled out because there is no strong band between 1610 and 1550 cm\(^{-1}\). A protonated carboxylic acid would have broad COO-H\(_{\text{st}}\) associated band between 3550-2500 cm\(^{-1}\), a strong C=O\(_{\text{st}}\) from 1700 to 1680 cm\(^{-1}\) for ar-COOH (1725-1700 cm\(^{-1}\) for al-COOH, 1715-1690 cm\(^{-1}\) for C=C-COOH) as well as OC-OH\(_{\text{st}}\) and OC-OH\(_{\delta}\) bands between 1440 and 1210 cm\(^{-1}\) which is all consistent with the spectra.

A ketone group is very likely with an aliphatic C=O\(_{\text{st}}\) at around 1715 cm\(^{-1}\) whereas a conjugated would be in the range from 1700 to 1660 cm\(^{-1}\). This attribution is only consistent if we assume that there are two different sites where the oxygen has attacked and that the band above 3100 cm\(^{-1}\) is due to water adsorption.

Lactones and ester can be dismissed because their ketone group would be situated above 1710 cm\(^{-1}\) and the strong C-O\(_{\text{st}}\) band between 1330 and 1050 cm\(^{-1}\) is lacking. Peroxides have no strong band around 1700 cm\(^{-1}\) and can therefore be ruled out, the same applies for a hydroxyl group.

N-oxide IR data is not easily available. There is IR data of 1,10-phenanthroline N-oxide (phenO) [19] and of its transition metal complexes [20]. Free phenO has strong N-O\(_{\text{st}}\) vibrations at 1269 and 1249 cm\(^{-1}\), strong N-O\(_{\delta}\) deformation vibrations at 808 with a shoulder at 811 cm\(^{-1}\). Furthermore, there are medium-intensity bands at 1550 with shoulders at 1546 and 1558 cm\(^{-1}\) and at 1590 cm\(^{-1}\) as well as a weak band at 1612 cm\(^{-1}\) which have been attributed to C=C\(_{\text{st}}\) and C=N\(_{\text{st}}\) ring vibrations [19]. CH\(_{\gamma}\) are without practical use since there also present in all compounds.

Upon complexation to transition metals the N-O\(_{\text{st}}\) vibrations shift 10-20 cm\(^{-1}\) to lower energy and loss a bit of energy. The N-O\(_{\delta}\) band shifts only slightly and may loose in energy. With a strong ligand field the ring vibrations may shift up to 1628 cm\(^{-1}\) and gain in intensity. There are M-N\(_{\text{st}}\) and M-O\(_{\text{st}}\) vibrations which are, unfortunately, all below 500 cm\(^{-1}\). Almost all phenO transition metal complexes form outer-sphere hydrates with a strong, broad O-H\(_{\text{st}}\) absorption in the range of 3400-3350 cm\(^{-1}\).

An attribution of the observed bands in Fig. 5.11 to phenO is consistent in that all bands are present, however, only in small quantities. Other species are necessary to explain especially the strong bands at 1679 and 1729 cm\(^{-1}\).

An oxidation directly at the ruthenium can be ruled out because sharp intense Ru=O\(_{\text{st}}\) bands in the range 785-850 cm\(^{-1}\) are missing [21].

An 18 h-experiment identical to the in-situ bleaching of ETH\(^{3}\) 3001 has been carried out with the membrane used for the membrane spectra in Fig. 5.10. The spectra (not shown) which have been corrected for water and carbon dioxide bands had a
much neater baseline compared to the spectrum of the bleached dye. Bands at 1288 and 1260 (+11% transmission), 754 (+16.6%), 1598 (+6.4%), several bands at 1500 to 1450 (up to +5.5%), 2228 (cyano group, +5.3%), 2955 and 2856 (+4%) and at 1165 cm\(^{-1}\) (+3.3%) all lost in intensity whereas new broad bands between 1800 and 1600 cm\(^{-1}\) (maximum at 1720 cm\(^{-1}\) with -3% transmission) and above 3100 cm\(^{-1}\) have formed. All the peaks which lost in absorbance can be attributed to \(\text{o-CPOE}\).

A detailed analysis of the corresponding absorption spectra shows that all major \(\text{o-CPOE}\) bands decrease correlated in an exponential fashion with approximately -4.7%/h. The two new bands are not attributable to neither polystyrene nor \(\text{o-CPOE}\), but probably belong to the latter which very likely could have a ketone group in its degradation products. The apparent inertness of polystyrene is most likely only due to its much smaller absorbance at the wavelengths of the mercury lamp. From this data we do not expect any significant matrix bleaching effect for the 15 minutes exposure used for the investigation of the bleaching effects on absorbance and luminescence.

### 5.7 Analysis of ETH\(^T\) 3001 photoproducts

#### 5.7.1 Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry

One of the few analytical techniques which can directly investigate compounds in polymers is Matrix-Assisted Laser Desorption/Ionization (MALDI) mass spectrometry. The method relies on embedding the analyte in an excess of a strongly light-absorbing matrix. Upon laser irradiation the matrix is explosively desorbed. The embedded analyte is “softly” desorbed with the matrix and ionized in the resulting plume. Ionization usually takes place by protonation or cationization (\(\text{Na}^+, \text{K}^+\)). In a MALDI spectrum mostly molecular ions are observed. Preliminary tests by Frédéric Dubois had shown that ruthenium diimine complexes in polystyrene can be desorbed directly from the polymer film without redissolving or adding MALDI matrices. In this case, the polystyrene acts as MALDI matrix. The addition of the plasticizer \(\text{o-cyanophenyl octyl ether (o-CPOE)}\), which closely resembles the known MALDI matrix \(\text{o-NPOE}\) [22], additionally helps to desorb the complex. Due to its aromatic ring system the ruthenium complex significantly absorbs the nitrogen laser’s UV photons, which induces fragmentation of the complex, a known MALDI artifact from investigations with transition metal bipyridyl complexes [12, 23]. For the low masses (< 2000 Da) only singly-charged ions are expected in the spectra.

In order to obtain more chemical information on the photoproduct mass spectra of the membrane before and after bleaching had been recorded, see Fig. 5.12. The
ETH\textsuperscript{T} 3001 sample had been bleached in ethanol, see p. 134. The resulting product corresponds to the last absorption spectrum in the series of the lower graph in Fig. 5.8. The lower mass range (< 200 m/z) presumably presents fragments from polystyrene and \(o\)-CPOE (\(M_r 231\)).

Clearly detectable in both spectra are \(\text{RuL}_3\) at 1351 m/z (\(L = \text{ligand 3-dpp, } M_r 416.6\)), \(\text{RuL}_2\) at 935 m/z and \(\text{RuL}\) at 519 m/z. Very interesting are the signals in the spectrum of bleached ETH\textsuperscript{T} 3001 which correspond to the addition of 16 and 32 to the masses of this series. Considering the dependence of the photobleaching on oxygen this has been interpreted as the original complex plus one or two oxygen atoms. The adduct with one oxygen atom is clearly a species which had evolved during bleaching. The adduct with mass plus two oxygen atoms, on the other hand, coincides with a product impurity \([M + 14]\) (see ESI-MS spectra in Fig. 5.14) and its contribution cannot be easily estimated.

Fig. 5.13 shows the peak integrals of \(\text{RuL}_x\) series normalized to the \(\text{RuL}_x\) species without oxygen. The peaks of the \(\text{RuL}_2\) series show that the ratio of \(\text{RuL}_2\) to \([\text{RuL}_2 + 2\text{O}]\) remained constant which suggests that the impurity underneath the \([\text{RuL}_2 +
2O] signal observed in the MALDI spectra was indeed present prior to bleaching or that only little [RuL₂ + 2O] has been formed during bleaching. The intensity of the [RuL₂ + O] peak, however, cannot be directly related to its abundance because its precursor (the photoproduct) has very likely a different desorption/ionization efficiency than the original RuL₃ complex.

Explanation of the RuL₃ series is more difficult because the [RuL₃ + O] as well as the [RuL₃ + 2O] signals are increased after bleaching, which was not the case for the RuL₂ series. The ratio 4:1 of [RuL₃ + O] to RuL₃ compared to the ratio of 1:5 for the corresponding RuL₂ species makes only sense if the alleged photoproduct [RuL₃ + O] fragments less. This is the case if it is either more stable or absorbs less energy from the nitrogen laser.

5.7.2 Electrospray Ionization Mass Spectrometry

Electrospray Ionization Mass Spectrometry (ESI-MS) is a very soft ionization technique which usually does not introduce any fragmentation of the analyte. It generates multiply charged ions, but will leave ions at low molecular masses at their intrinsic charge state. The combination of electrospray ionization with a high reso-
Photobleaching of ruthenium(II) diimine dyes

The Fourier Transform Ion Cyclotron Resonance (FT-ICR) Analyzer allows to accurately measure the charge state.

Fig. 5.14 shows the positive ion ES mass spectra of ETH\textsuperscript{T} 3001 in methanol before and after bleaching in ethanol, see p. 134. The product corresponds to the last absorption spectrum in the series of the lower graph in Fig. 5.8.

The base peak at m/z 675.5 in the unbleached ETH\textsuperscript{T} 3001 corresponds to the doubly charged RuL\textsubscript{3} ion (denoted as M with $M_r 1351$). A high resolution spectrum of the peak at m/z 675.5 unambiguously supported the attribution to RuL\textsubscript{3} based on the mass, mass distribution and its native charge state of 2, with no protons added. The second most intense signal at m/z 1450 is attributed to the singly charged adduct of perchlorate to RuL\textsubscript{3}, also backed by a high resolution spectrum. There is a faint signal at m/z 1350.5 which corresponds to singly charged RuL\textsubscript{3}.

The origin of the relatively intense (5% of base peak) signal at m/z 762.5 and at 661.3 (3%) present in both spectra which correspond to doubly charged species with the masses 1525 and 1323 respectively is unknown. The same applies to the small peaks at m/z 1001.1 and 1177.6. These, however, are no longer present in the spectrum of the bleached sample and are probably a contamination.

The spectrum of the bleached ETH\textsuperscript{T} 3001 show a whole series of peaks in the vicinity of RuL\textsubscript{3} which correspond to RuL\textsubscript{3} plus 16, 32, 48 and 64, equalling the mass of plus one, 2, 3, or 4 oxygen atoms. Their intensity is decreasing exponentially with respect to [RuL\textsubscript{3} + O]\textsuperscript{2+} with [RuL\textsubscript{3} + (n+1)-O]\textsuperscript{2+} having 50% intensity of [RuL\textsubscript{3} + n-O]\textsuperscript{2+}. This suggests - assuming an identical ionization efficiency - that the oxidation is statistically governed with the addition of one oxygen atom at once with no preference for even partially bleached complexes. With respect to the base peak, the whole series of oxygen adducts of RuL\textsubscript{3} has a ten-fold lower intensity than what would be expected from their extrapolation to the RuL\textsubscript{3} intensity. The question is whether this is due to different ionization efficiencies of ETH\textsuperscript{T} 3001 and its oxidation products because otherwise it must grossly underestimate the amount of bleaching as seen in absorption spectra.

The [RuL\textsubscript{3} + O]\textsuperscript{+} peak has double the intensity of the unbleached [RuL\textsubscript{3}]\textsuperscript{+}, which comes much closer to the expectation of its abundance. It is not clear whether these singly-charged species are created in the ion source or are actually present in the sample. The very intense signal of the singly-charged adduct [RuL\textsubscript{3} + ClO\textsubscript{4}]\textsuperscript{+} shows that there is no systematic discrimination of singly-charged ions in the ion source. Therefore, we can conclude that in either case the actual contribution of [RuL\textsubscript{3} + O]\textsuperscript{+} to the bleaching products is small.

There are no peaks which correspond to triply-charged species, to ruthenium complexes with one or several ligands lost or to the single ligand ($M_r 416.6$) or phenO
This of course does not preclude the existence of any short-lived intermediates during bleaching.

(M_r 432.6). This of course does not preclude the existence of any short-lived intermediates during bleaching.

Fig. 5.14 ESI-MS spectra of ETH^T 3001 before and after bleaching
M denotes RuL_3. Spectra are normalized to the base peak at 675.5 (M^{2+}) = 100%. Note that most intensive peak in the ETH^T 3001 spectra cover up identical peaks behind.
A very good study on the photobleaching of (ethylenediamine)bis(2,2′-bipyridine)ruthenium(II) complexes by on-line ESI-MS has recently been published by Arakawa et al. [24]. They found a direct oxidative dehydration of their ethylenediamine ligand to form a nitroso species with a mass plus 14, corresponding to plus oxygen minus 2 hydrogen which they confirmed by using $^{18}$O$_2$. The suggested that the source of the oxygen atom would be from dissolved oxygen rather than from water. Their spectra showed only species in their native doubly-charged state or with one perchlorate ion added.

In a similar experiment with [Ru(bpy)$_2$(N,N,N’,N’-tetramethyl-ethylenediamine)](ClO$_4$)$_2$ the ligand was substituted by the solvent acetonitrile, but no oxygenated product was observed. No such mechanism seems possible with ETH$^T$ 3001 because it has neither amino hydrogens nor exchanges ligands.

### 5.8 Bleaching mechanism

#### 5.8.1 Influence of the polymer matrix

A very important question is the influence of the matrix on photobleaching, in this case polystyrene/o-CPOE. Polystyrene and/or o-CPOE could either accelerate or slow down the photodecomposition of ETH$^T$ 3001. It has been noted in the literature that luminophors and dyes exert a light-stabilizing effect on polystyrene [25, 26] although the contrary also holds if dyes act as sensitizers [27].

IR data in 5.6 Investigation of bleaching by ATR FT-IR spectroscopy on p. 135 shows that very little happens with PS in the space of an hour, during which time all of ETH$^T$ 3001 becomes bleached. o-CPOE, on the other hand, lost 4.7% in IR absorbance, with a reaction involving its cyano group ($\tilde{\nu} = 2228$ cm$^{-1}$). This can be rationalized by the absorption spectra of the two components given in Fig. 5.15.

The apparent preponderance of the o-CPOE absorbance ($\epsilon_{\text{max}} = 17.2$ g$^{-1}$·l·cm$^{-1}$ at 296 nm) has to be seen in relation with the 4-fold concentration of polystyrene ($\epsilon_{\text{max}} = 6.2$ g$^{-1}$·l·cm$^{-1}$ at 262 nm) in the bleached membrane, resulting in approximately equal levels of light absorption for the two components in the membrane. PS starts absorbing below wavelengths of 280 nm, whereas o-CPOE even absorbs below 320 nm, i.e. much closer to the visible spectral range. This is very important for the bleaching experiment because the Sylvania mercury lamp had no measurable intensity below 300 nm. Since PS does not absorb the bleaching light, we expect that only o-CPOE will show any photodecomposition, if it takes place.

o-NPOE, another popular plasticizer (investigated in 4.4.1 Plasticized polystyrene membranes on p. 85), was added for comparison. It starts absorbing below 400 nm and its absorption is overall larger, because it is broader ($\epsilon_{\text{max}} = 11.5$ g$^{-1}$·l·cm$^{-1}$ at 330 nm, $\epsilon_{\text{max}} = 14.8$ g$^{-1}$·l·cm$^{-1}$ at 260 nm).
5.8 Bleaching mechanism

In absolute terms, the -4.7% of \(\text{o-CPOE}\) loss, given a molar excess of \(\text{o-CPOE}\) over \(\text{ETH T 3001}\) of 93 in the membrane, equals a roughly equimolar decay of both species. It is not at present possible to say whether the two processes interfere much with one each other. Decomposition of \(\text{o-CPOE}\) could be caused by a photoinduced reaction or by singlet oxygen, in which case the reaction would be sensitized by \(\text{ETH T 3001}\). When considering the absorption weighted with the mercury lamp intensity, it is \(\text{ETH T 3001}\) which in absolute terms absorbs much more (by several orders of magnitudes) incident light than \(\text{o-CPOE}\).

Based on this, we believe that it is \(\text{ETH T 3001}\) that sensitizes - via singlet oxygen - the decomposition of \(\text{o-CPOE}\) rather than \text{vice versa}.

Pure PS is not very lightfast and its photochemistry has been well investigated [28]. The lightfastness can be improved by copolymerization of PS with acrylnitrile (SAN copolymers) [17] or additives [29, 30]. From the oxidation of PS, we would expect mainly peroxides, alkoxy, carboxylic, per-carboxylic, \(\alpha,\beta\)-unsaturated carbonyls and carbonyl groups as a result of chain scissions and phenyl ring-opening photo-reactions [27]. The changes in IR bands for the membrane bleaching experiments monitored by ATR-IR spectroscopy on p. 139 were, except for changes in \(\text{o-CPOE}\) bands, too small to be attributable to any of those functional groups.

5.8.2 How does photobleaching work?

Which mechanism is consistent with all the data presented in sections 5.3 to 5.7? We do not need to start from the scratch. Hartmann has shown that the presence of oxygen greatly accelerates the effects of photobleaching and that different products are obtained when oxygen is present than when it is absent [9, 10]. We also know

![Absorption spectra PS and the plasticizers \(\text{o-NPOE}\) and \(\text{o-CPOE}\)](image-url)
that ruthenium(II) diimine complexes produce the very reactive singlet oxygen $^{1}\text{O}_2$ in the luminescence quenching process with oxygen with a yield of close to unity [6]. Therefore, we will assume an oxidation by singlet oxygen as the dominant mechanism of one of the first degradation steps.

For the reader’s convenience I will outline the suggested mechanism first and then present all the evidence which supports it. In the following I will discuss why we can dismiss existing theories, such as ruthenium oxidation [6], ligand loss [2, 4, 5] or oxidation at the 5,6-position of dpp [9]. Finally, some obvious questions, such as how photobleaching behaves differently in solution from in the membrane, and the different kinetics will be addressed.

The suggested mechanism for the photodecomposition of highly luminescent ruthenium(II) diimine dyes in the presence of oxygen is given in Fig. 5.16.

Please note that the wording in the previous sentence has been carefully chosen. It must be a *highly luminescent* ruthenium(II) diimine complex because otherwise the singlet oxygen yield will be too low and other mechanisms will prevail. The same holds true for the presence of *oxygen*; without oxygen, ligand loss may take over.

The first step in the photodecomposition process is a reversible, partial ligand detachment to a five-coordinate complex. In the second step, singlet oxygen $^{1}\text{O}_2$ oxidizes the accessible imine nitrogen to the N-oxide, which recoordinates to the ruthenium(II). The resulting complex, $\text{[RuLO}_2]^2+$ (LO = N-oxide of L), is less stable than the original $\text{RuL}_3^{2+}$ complex and is non-luminescent. In a polymer, the reaction virtually stops here. In solution, the labile photoproduct is oxidized preferentially to non-absorbing, low-molecular weight compounds.

The first step is probably not very controversial. The fact that pure *cis* or *trans*-ruthenium(II) diimine complexes racemize upon irradiation strongly suggests that a five-coordinate intermediate exists. Racemization of $\text{[Ru(bpy)}_3]^{2+}$ has approximately a 100 times higher quantum efficiency than substitution [2]. In general, the $^3\text{MC}$ state with larger Ru-N distances is thought to be responsible for the photo-
chemistry of ruthenium(II) diimine complexes, such as photoracemization or -substitution [2, 31]. The \(^3\)MC state can be reached from the \(^3\)MLCT-state by thermal activation. For a general discussion of the photophysics of ruthenium(II) diimine complexes, see 2.1 on p. 7.

Since no acids or potential ligands were present in the solution or membrane experiments in sections 5.4 and 5.5, photosubstitution was effectively curbed. Nothing, however, is known about the lifetime of the five-coordinate complex, especially about its lifetime in polymers.

Support for the bleaching hypothesis that a partial ligand detachment must be the first decomposition step is provided by the \([\text{Ru(cage)}_3]^{2+}\) compound synthesized in the groups of Alex von Zelewsky and Peter Belser [3]. Cage is a ligand consisting of three completely rigidified bpy-ligands, see Fig. 5.17. Even after weeks of continued irradiation in dichloromethane this compound did not show any detectable sign of photodecomposition; the observed increase in stability against photodecomposition compared to \([\text{Ru(bpy)}_3]^{2+}\) was higher than \(10^4\!\) !

Note that \([\text{Ru(cage)}_3]^{2+}\) must be synthesized based on the template effect of Ru(II); a later incorporation of Ru(II) is not possible. \([\text{Ru(cage)}_3]^{2+}\) was found to have double the quantum yield of \([\text{Ru(bpy)}_3]^{2+}\). If we assume that a temporary, partial ligand detachment prior to an oxidation of bpy by singlet oxygen to the N-oxide is necessary for photodecomposition, this is sterically not feasible with \([\text{Ru(cage)}_3]^{2+}\). All the other possible oxidation sites, however, are as accessible as in \([\text{Ru(bpy)}_3]^{2+}\), but seem not to be targeted by singlet oxygen. This is in very good agreement with our bleaching hypothesis.

Where my bleaching hypothesis is “revolutionary” is in proposing a N-oxide formation step. Even though no isolation with a subsequent structural analysis of the photoproducts was possible, there is ample evidence for the formation of a N-oxide.

![Fig. 5.17 \([\text{Ru(cage)}^{2+}]\) according to [3]](image-url)
The strongest evidence which excludes a wide variety of potential photoproducts comes from mass spectrometry. Both ESI-MS (Fig. 5.12 on p. 141) and MALDI-MS (Fig. 5.14 on p. 144) spectra clearly show that the dominant species appearing upon bleaching corresponds to \([M + 16]^{2+}\), where \(M\) is the molecular ion. In the ESI-MS spectrum of ETH\(^T\) 3001 bleached in ethanol, there are additional adducts \([M + 32]^{2+}\), \([M + 48]^{2+}\) and \([M + 64]^{2+}\) with exponentially decreasing intensities. These signals are the only ones which appear upon bleaching. The multiples of 16 can only be sensibly explained by the mass of oxygen. There are only two structures which succeed in explaining this mass. Based on the \([\text{Ru(cage)}]^{2+}\) evidence presented above, a hydroxyl group is not very likely. This leaves the N-oxide. IR spectra (see p. 139) support the N-oxide hypothesis.

1,10-phenanthroline-N-oxide (phenO) is synthesized by oxidation of phen with acidic hydrogen peroxide solutions at 70-80°C [32]. For the synthetic route, we expect the imine nitrogen atom to be the preferred oxidation site [33] if it becomes accessible on partial ligand detachment. The exponentially decreasing intensities in the \([M + n\cdot O]^{2+}\)-series in the ESI mass spectrum suggest that the bleaching process in ethanol is the kind of random process we expected.

There is no data in the literature on the postulated ruthenium(II) diimine complexes with N-oxide ligands. There is data, however, on transition metal ions which form high-spin complexes with phenO [20]. The complexes have the general formula \(M(\text{phenO})_{1-2}\cdot\text{Cl}_{2-3}\cdot n\text{ H}_2\text{O}\), in which phenO functions as a bidentate chelating agent. The compounds are generally stable in air, have charge-transfer absorption bands in the visible range and are, with the exception of the Fe(II/III) and Cr(III) compounds, poorly soluble in almost any solvent. Transition metal complexes with N,N'-dioxides of bpy (bpyO\(_2\)) exist as low-spin complexes which decompose slowly in aqueous solutions [34].

This data leads me to assume that a ruthenium diimine complex of the type \([\text{RuL}_2\text{LO}]^{2+}\) (LO = N-oxide of the diimine ligand L) is stable. It is also very likely to be a relatively strongly absorbing high-spin complex with a broader absorption band than RuL\(_3\) due to its distorted octahedral structure. Since the N-oxide considerably affects the Ru(II) diimine chromophore, we expect the luminescence also to be affected.

Oxidation of ETH\(^T\) 3001 to the corresponding Ru(III) species can be ruled out. Spectroelectrochemical experiments (compare Fig. 6.16 on p. 183) clearly show that the absorbance of Ru(III) species of ETH\(^T\) 3001 decreases around 550 nm, whereas that of the photoproducts increases (Fig. 5.6 on p. 129). The ESI-MS spectrum revealed no triply-charged species or fragments which might have indicated a triply-charged mother compound. IR spectra preclude the formation of Ru(IV)\(=\)O species (no intense band at \(\tilde{\nu} = 785-850 \text{ cm}^{-1}\)).
There is no evidence for significant ligand substitution prior to the formation of the N-oxide. Neither ESI nor MALDI mass spectra show fragments corresponding to a ligand loss/substitution or to free ligands or to LO. Thin-layer chromatograms of bleached solutions of ETH\textsuperscript{T} 3001 in ethanol running with ethyl acetate on reversed phase or with KNO\textsubscript{3}/H\textsubscript{2}O/AcN/EtOH (1:10:40:10) on silica gel showed no uncomplexed ligand under UV or with Fe\textsuperscript{2+}-staining.

The usual oxidation products of ruthenium(II) phenanthroline complexes with chemical oxidizing agents all give rise to other masses than [M + 16]\textsuperscript{2+} (see Fig. 5.18). Note that these are reactions which take place in the “dark”, with the imine nitrogen atoms protected by complexation.

![Chemical oxidation of ruthenium(II) diimine complexes](image)

The bleached ETH\textsuperscript{T} 3001 is insoluble in a saturated NaHCO\textsubscript{3} solution, but prefers chloroform instead. It is not possible to say whether this indicates the absence of acidic groups or just a general insolubility of the different photoproducts.

Hartmann’s theories that a dialdehyde forms in the 5,6-position on the dpp-phenanthroline moiety [9] or that there is a ligand loss [10] can be easily disproved. The structural data for these theories came from MALDI mass spectra where inherent ruthenium complex fragmentation and generally-formed adducts artifacts might
have been misinterpreted [36].

Hartmann’s other argument emphasizes the apparent photobleaching stability in the order \( \text{Ru(bpy)}_3^{2+} \gg \text{Ru(phen)}_3^{2+} > \text{Ru(dpp)}_3^{2+} \), attributing it to the fact that \( \text{Ru(bpy)}_3^{2+} \) lacks the 5,6-bridge which precludes the production of his proposed photoproduct. Demas et al. had a similar idea back in 1991, and refuted it on the grounds that the photolysis quantum yields corrected for the absorption coefficient were almost equal [7].

One must consider that IR spectra show ample IR bands which cannot be explained by N-oxide formation. We can assume, however, that the photoproduct’s subsequent degradation to volatile products must be very fast given the enormous light stability of \( \text{ETH}^\dagger \ 3001 \) with quantum efficiencies \( < 10^{-13} \). It seems that much of the original \( \text{ETH}^\dagger \ 3001 \) must have disappeared according to absorption spectra, otherwise the relatively small contributions of photoproducts in the mass spectra make no sense. There are only two mass spectrometry peak series which probably represent the true proportions. One is the RuL\(_3\)-series in Fig. 5.13 on p. 142 with a ratio of RuL\(_3\) to RuL\(_2\)LO of 1:4. The preferred fragmentation of RuL\(_3\)LO could lead to the much lower ratios for the RuL\(_{1,2}\)-series and to the appearance of RuL\(^+\). The other peak series is M\(^+\) to [M + O]\(^+\) in Fig. 5.14 on p. 144 with a 1:2 ratio.

The major differences between bleaching in solution and in polymers concern isosbestic points and the intensities of the long-wavelength absorption band. The neat isosbestic points observed in the plasticized PS suggest a stochiometric formation of [RuL\(_2\)LO]\(^{2+}\).

Hartmann also found neatly respected isosbestic points in the absorption spectra of bleaching experiments with [Ru(dpp)]\(^{2+}\) in polymers, even though they passed unnoticed. For [Ru(dpp)]\(^{2+}\) adsorbed on silica gel, there were two isosbestic points at around ~390 and 480 nm [9]. For PVC, there was one around 500 and one at 680 nm (very likely there is another below 400 nm where the spectra end) [10].

Note that MALDI mass spectra (Fig. 5.13 on p. 142) show a small fraction of [M + 32]\(^{2+}\). Determining whether this is rather [RuL\(_2\)LO\(_2\)]\(^{2+}\) or [RuL(LO)]\(^{2+}\) (LO\(_2\) = N,N’-dioxide of L) is not possible.

For a stochiometric reaction as given by the isosbestic points, we would expect an equal decrease in luminescence and absorbance. Luminescence, however, we found decreased four times faster than absorbance. This can only be explained if the photoproduct [RuL\(_2\)LO]\(^{2+}\) heavily quenches the luminescence. Reabsorption effects cannot account for this (see p. 131). It appears that a direct energy transfer must take place, although we have not yet been able to conduct any experiments to check this.

For the bleaching in ethanol (compare Fig. 5.8 on p. 133) the isosbestic points were only initially respected. In the concentrated solution, there are more photoproducts
with the longer wavelengths absorbed and in the diluted solution, there is only very little.

How can these findings be explained? Approx. 20 times more oxygen was dissolved per molecule of ETH T 3001 in the diluted solution than in the concentrated solution. Therefore, based on the similar absorbance of the two solutions, it is likely that more singlet oxygen is generated in the diluted solution. For the concentrated solution, partial ligand detachment is probably initially accelerated because the $^3$MC state is likelier to be populated by energy transfer from other dye molecules. In the diluted solution, the labile photoproducts degrade quickly due to the high concentrations of singlet oxygen. In the concentrated solution, quenching photoproducts build up after a fast initial degradation phase. The singlet oxygen yield goes down since the luminescence is increasingly quenched by the photoproducts rather than by oxygen. This mechanism is much more important in the concentrated solution because the dye molecules are much closer to each other (19 nm in the concentrated versus 50 nm in the diluted solution).

The slightly accelerated decomposition in the diluted solution towards the end can be explained by the decreasing probability of quenching by other ETH T 3001 dye molecules or photoproducts, as well as by the reduced inner filter effect of the initially strongly absorbing solution. For the concentrated solution, the reason for the decrease in the decomposition rate is simple: large quantities of photoproducts effectively curb singlet oxygen production.

Spectral changes were very similar to those observed with [Ru(bpy)$_3$]$^{2+}$ in solution [4, 5].

Why bleaching is much slower in ethanol than in plasticized polystyrene remains a difficult question. We have obtained quantum efficiencies for photobleaching (absorbance) of $1.2 \cdot 10^{-15}$ in solution (see p. 134) and $3 \cdot 10^{-14}$ in plasticized PS (see p. 130), i.e. 25 times faster than bleaching in the polymer.

One of the main differences is the different lifetimes of the singlet oxygen. Whereas the lifetime in (pure) PS is $19 \pm 2 \mu$s [37], that in ethanol is only $5.6 \mu$s [27]. The diffusion constant, $D$, of oxygen in ethanol is $2.64 \cdot 10^{-5}$ cm$^2$·s$^{-1}$ (at 29.6°C [38]) and must be above $1 \cdot 10^{-7}$ cm$^2$·s$^{-1}$ in plasticized PS [39]. The Einstein-Smoluchowski equation [40]

$$D = \frac{d^2}{2\tau}$$  \hspace{1cm} (5.6)

relates the diffusion constant $D$ to a stepwidth, $d$, in time, $\tau$. This allows the distance range which can be reached by singlet oxygen within its lifetime to be calculated. In ethanol, this is 172 nm and >19 nm in the polymer. The latter is in the range of the average dye distance of 6 nm for the approx. 8 mM plasticized PS membrane. Note that equation (5.6) only holds for isotropic media. It is possible that, in amorphous
domains of plasticized PS, \( ^1 \text{O}_2 \) does not migrate far from its origin and will preferentially oxidize the very complex that it created (no “cage escape”) [37]. Subsequently, singlet oxygen creation in that pore would be curbed, so that not much further photodegradation will take place. This could explain why we have neat isosbestic points in the absorption spectra even though a further photodegradation seems plausible.

\( ^1 \text{O}_2 \) in ethanol, on the other hand, can migrate well beyond the usual distances of a few nanometers with a Förster energy transfer mechanism for quenching. Thus, singlet oxygen production and reaction sites are effectively decoupled.

We can roughly estimate the singlet oxygen yield:

\[
\Phi_{^1 \text{O}_2} = \frac{\Phi_L(\text{N}_2) - \Phi_L(\text{air})}{\Phi_L(\text{N}_2)}
\]

(5.7)

if we assume that all luminescence quenching produces singlet oxygen. Data for ETH\(^T\) 3001 in ethanol can be extracted from Table 2.3 on p. 22 and data for the plasticized PS can be estimated from Table 4.6 on p. 89 if we assume a 4 times lower \( \Phi_L \) than in ethanol (see p. 62).

We obtain a ~17 times higher singlet oxygen yield in ethanol than in the plasticized PS. Both the singlet oxygen yield and the diffusion data suggest that the second bleaching step should be faster in ethanol. We must conclude that the first step is rate-determining.

Recent temperature experiments by Draxler have shown widely differing activation energies for the radiationless decay of \([\text{Ru(dpp)}_3]^{2+}\) in polystyrene below the glass transition temperature \( T_g \) and solutions or in solution-like media [41]. The activation energy barrier \( \Delta E \) derived from Arrhenius plots is supposed to be the energy difference between the \(^3\text{MLCT}\) and \(^3\text{MC}\) state. The other extracted parameter is the rate constant \( k \) in the case of a zero energy activation energy barrier.

In PS (\( M_w = 280'000 \) from Aldrich), a \( \Delta E = 583 \pm 40 \text{ cm}^{-1} \) with \( k = (2.9 \pm 0.5) \cdot 10^6 \text{ s}^{-1} \) was obtained below \( T_g \) (\( \approx 100^\circ \text{C} \)) and \( \Delta E = 4491 \pm 235 \text{ cm}^{-1} \) with \( k = (6.0 \pm 5.5) \cdot 10^{12} \text{ s}^{-1} \) above \( T_g \). In methanol the values were \( \Delta E = 1597 \pm 143 \text{ cm}^{-1} \) with \( k = (4.1 \pm 2.8) \cdot 10^8 \text{ s}^{-1} \). Similar values were obtained for toluene and styrene. In the case of the PS below \( T_g \), we would expect a two orders of magnitude faster reaction from the \(^3\text{MC}\) state as in methanol, based on the energy barriers. It is not clear whether the identical PS plasticized with 18.7% \( o\)-CPOE is still above \( T_g \), or whether methanol and ethanol behave similarly. In any case this provides a good clue that suggests that the partial ligand decoordination is by one or two orders of magnitude faster in the polymer as in solution.
Please note that most other polymers besides PS have longer singlet oxygen lifetimes [37] and other luminescence-quenching efficiencies which can result in considerably larger photobleaching effects.

For the set-up with the mercury lamp (see 5.4 on p. 128), the light intensity was approx. 6 kW/m² compared to 1.3 W/m² for the LED set-up (see 5.3.2 on p. 126), i.e. 4700 times stronger. The corresponding initial photobleaching rates (luminescence) were -8.0%/min and -9.9·10⁻⁴%/min, respectively, i.e. 8000 times more, which is in good agreement. We expect a higher photobleaching rate from the mercury lamp set-up due to the temperature increase and the larger fraction of energy-rich photons. Hartmann et al. cited an intensity of 13 W/m² for their bleaching experiment with a LED on a membrane with 5 mM [Ru(dpp)₃]²⁺ in PS [10]. From the figure 1 in [10], we can estimate their initial photobleaching rate (luminescence) as -0.010%/min. Taken per irradiated energy, this photobleaching rate is identical to ours. This confirms that our determined photobleaching rates hold under the usual measurement conditions as well.

If we presume that the singlet oxygen yield from the luminescence quenching process is close to unity, we will have photobleaching effects proportional to the quantum yield. A potentially higher sensitivity is at the cost of a faster degradation. This throws doubt, at first glance, on the prevailing credo of “the more the better” for the quantum yields of ruthenium(II) diimine complexes. Now, however, that it is clear how the main photodecomposition mechanism works, better complexes, such as [Ru(cage)₃]²⁺, can be designed to prevent the first degradation step, the partial ligand detachment, by sterical means.

Zelewsky et al. have shown that a rigid cap on one side can achieve the same photo-stability as with [Ru(cage)₃]²⁺, but the same does not apply with a more flexible, non-aromatic cap [3]. Unfortunately, synthesis yields were very low with 1-5%. Oxygen sensor design should, in the future, focus on elucidating these areas further. Recently other complexes which explicitly address photostability have been suggested for oxygen optodes. The copper(I) complex [Cu(dbp)(dmp)](PF₆) (dbp = 2,9-di-tert-butyl-1,10-phenanthroline; dmp = 2,9-dimethyl-1,10-phenanthroline) absorbs moderately (ε = 7000 M·cm⁻¹) at 440 nm and emits in CH₂Cl₂ at 646 nm with Φₐ = 0.01 and τ₀ = 0.73 µs [42]. In polystyrene it had a very moderate KₜV of 0.6 bar⁻¹. In a photobleaching experiment, it did not decompose measurably under conditions where approx. 20% of [Ru(bpy)₃]²⁺ had decomposed [43]. Miller et al. attributed this to the lack of d-d excited states (³MC state) in the d¹0 metal copper. However, the dye decomposes in e.g. acetonitrile or DMSO. Even though the complex may be a “landmark improvement in the photophysics of [Cu(NN)₂]⁺” and the strategy for improving photostability is promising, it cannot yet compete with ruthenium(II) dyes.
5.9 Summary and Perspectives

The photobleaching behavior of the ruthenium(II) diimine dye ETH\textsuperscript{T} 3001 (tris(4,7-bis(4-propylphenyl)-1,10-phenanthroline)-ruthenium(II) perchlorate) has been extensively investigated. The dye is almost identical to the widely used \([\text{Ru(dpp)}_3]^{2+}\) dye. To my best knowledge, however, no one has, until now, proposed a comprehensive mechanism for the photobleaching of highly luminescent ruthenium(II) diimine complexes in the presence of oxygen. Such a mechanism is described in this chapter.

According to this mechanism, the first degradation step is a partial ligand detachment to a five-coordinate complex upon absorption. Evidence that such a five-coordinate complex exists comes from photosubstitution and -racemization experiments in solution [2, 4]. Synthesized cage diimine ligands for ruthenium [3], where partial ligand detachment is sterically blocked, shows that this step must be crucial.

In a second step, singlet oxygen \(^1\text{O}_2\) oxidizes the now accessible imine nitrogen to N-oxide, which recoordinates to the ruthenium(II) metal centre. The singlet oxygen is produced in the luminescence quenching process [6]. The resulting photoproduct \([\text{RuL}_2\text{LO}]^{2+}\) (LO = N-oxide of diimine ligand L) complex is less stable than the original \([\text{RuL}_3]^{2+}\) complex and quenches its luminescence, presumably by energy transfer. In solution, these intermediate photoproducts are preferentially degraded to low-molecular weight products. In the polymer, the reaction stops at this point, probably because of a “cage” effect for the singlet oxygen [37].

Note that in low-oxygen environments or with ruthenium(II) diimine dyes with low quantum yields, other mechanisms which have not yet been investigated may dominate. Oxidation can be much more pronounced in other polymers with longer singlet oxygen lifetimes, with higher diffusion coefficients or where the quenching efficiency of the dye is larger.

Photobleaching experiments were made with a membrane of approx. 8 mM dye in 80.3\% polystyrene/18.3 \(o\)-cyanophenyl octyl ether (\(o\)-CPOE) in air. The bleaching progress was characterized by absorption and luminescence spectroscopy. Upon bleaching, the main absorption band at 460 nm decreased, whereas new bands around 350 and 545 nm emerged. Two isosbestic points at 400 and 513 nm were neatly respected. Quantum efficiencies for photobleaching in the plasticized polystyrene membrane were calculated as \(3\cdot10^{-14}\) in absorbance and \(1.4\cdot10^{-13}\) in luminescence.

A membrane bleaching experiment monitored on-line with Attenuated Total Reflection Fourier Transform Infrared (ATR FT-IR) spectroscopy allowed polymer degradation to be assessed. There was significant \(o\)-CPOE disappearance, but no visible effect on the PS bands within the time it took for total ETH\textsuperscript{T} 3001 bleaching.
The phenomenology of the absorption spectra, the appearance of isosbestic points and the photobleaching rate comply with those reported by other authors [9, 10].

Our studies of photobleaching with diluted and concentrated dye solutions in ethanol indicate that the kinetics are concentration-dependent. Degradation was governed by the inter-dye distance and the amount of oxygen taken per dye molecule. Small distances between the dye molecules and a slight excess of oxygen molecules per dye molecule led to a considerable build up of intermediate photoproducts. As a result, the degradation slowed down. Large inter-dye distances and a large excess of oxygen favoured a decoupling of the $^1\text{O}_2$ production and reaction sites, leading to a quick degradation. Bleaching was found to be about 25 times faster in plasticized polystyrene than in ethanol. Bleaching in ethanol, however, is likely to be faster, given the $^1\text{O}_2$ lifetimes, the diffusion coefficients and the estimated singlet oxygen yields. We presume that the partial ligand detachment step is facilitated by a lower $^3\text{MC}$ state in the polystyrene [41] and is rate-limiting.

Evidence for the N-oxide photoproduct is mostly based on Matrix-Assisted Laser Desorption/Ionization (MALDI) mass spectra, taken directly from the bleached membrane, and the Electrospray Ionization (ESI) mass spectra from bleached dye solutions in ethanol. Masses $[M + n\cdot16]^{2+}$ ($n = 1,2$ in membrane, $1-4$ in ethanol) were found with exponentially decreasing intensities, but these were the only spectral difference found upon bleaching. The IR spectra of bleached dye are consistent with N-oxide formation.

Stable complexes of transition metal ions with bidentate N-oxide diimine ligands exist, and their properties correspond with those proposed for $[\text{RuL}_2\text{LO}]^{2+}$ [20, 34].

It has been possible to disprove alternative theories for photobleaching, such as ligand loss [2, 4, 5], ruthenium metal oxidation [6] or oxidation at the 5,6-position of the ligand’s phenanthroline moiety [9].

Our research leads us to believe that sterical hindrance [2, 3] of the partial ligand detachment is the most efficient way of reducing photobleaching, whilst retaining such desirable beneficial properties as high quantum yield.

### 5.10 References


[42] M. T. Miller, P. K. Gantzel and T. Karpishin, *A Highly Emissive Heteroleptic Copper(I) Bis(phenanthroline) Complex: [Cu(dbp)(dmp)]+ (dbp = 2,9-Di-

6 Electrochemical investigation of ETH\textsuperscript{T} 3001

6.1 Introduction

The electrochemistry of ruthenium(II) diimine dyes is routinely determined for every new compound. Cyclic voltammograms of Ru(II) diimine compounds usually look very much alike with a reversible one-electron oxidation at around +1.2 V and three reversible ligand reductions in the range of -1.3 to -2.0 V (vs SCE, $E^\circ = 0.2412$ V) \[1, 2\]. Based on the reversibility of their redox reactions, Ru(II) diimine complexes are often used as mediators or catalysts.

The experiments reported here were originally motivated by the studies of light bleaching with ETH\textsuperscript{T} 3001 (tris(4,7-bis(4-propylphenyl)-1,10-phenanthroline)-ruthenium(II) perchlorate), see chapter 5 Photobleaching of ruthenium(II) diimine dyes on p. 119. Using electrochemistry it is possible to mimic one half of the light absorption process in the MLCT-band where either the ligand can be reduced or the ruthenium centre is oxidized. Light absorption, i.e. promoting an electron from the metal-centered LUMO to the ligand-centered HOMO, takes place when both processes occur at the same time \[2\]. Cycling between the two corresponding redox potentials comes close to this situation and gives rise to chemiluminescence \[3, 4\].

Electrogenerated chemiluminescence, where excited Ru(II) complexes react with a strong reducing agent, are exploited for analytical goals, for instance, the quantitative determination of oxalate \[5\].

The redox potential of $[\text{Ru(dpp)}_3]^{3+}/[\text{Ru(dpp)}_3]^{2+}$, which is the key parameter for this reaction, was determined as -0.90 V vs SCE \[1\].

The accessibility of excited states of Ru(II) diimine complexes using electrochemistry makes it possible to estimate the absorption energy with the redox potentials determined by cyclic voltammetry.

Cyclic voltammetry experiments with ETH\textsuperscript{T} 3001 in different solvents are described in 6.4. What turned out to be much more interesting and revealing, however, were the spectroelectrochemical experiments \[6\].

Spectroelectrochemical cells come in a wide variety of forms \[7\], but essentially all contain a thin layer section around the working electrode which can be fully electrolyzed in a few seconds. When the cells are used in conjunction with spectroscopic techniques, such as UV-VIS, IR or luminescence, much more information can be gained than from cyclic voltammetry alone.

The development and characterization of a versatile high-quality spectroelectro-
chemical cell for absorbance and luminescence measurements, as well as thin-layer phenomena are described in 6.3.

Ruthenium(II) diimine complexes have been frequently investigated by spectroelectrochemistry in absorbance because they are readily accessible due to their strong absorption bands and because their electrochemistry is interesting [8, 9]. The spectropotentiostatic experiments in absorbance with ETH\textsuperscript{T} 3001 in ethanol are presented in 6.5.3 and the cyclic voltammetry investigations coupled with UV-VIS spectra in 6.5.4. For the latter, though, only the results at positive potentials are given.

Ethanol was chosen as the preferred solvent because it is suitable for anodic electrochemistry [10] and has been used in measuring quantum yields as well as in photobleaching experiments, compare chapter 3 Determining luminescence quantum yields on p. 31 and 5.5 Bleaching of ETH\textsuperscript{T} 3001 in solution on p. 131.

Section 6.5.2 describes one of the rare applications of spectroelectrochemistry to luminescence.

Experimental details for all the experiments are given in 6.2 on p. 162. Please note that some experiments were carried out at the Changchun Institute of Applied Chemistry, Changchun, P. R. of China and some at CCS under widely differing experimental conditions. All the potentials, including those from literature sources, are given with respect to a Ag/AgCl/KCl (sat.) reference electrode \((E^\circ = +0.197 \text{ V})\) unless otherwise noted.

# 6.2 Methods and materials

## 6.2.1 Materials

For the experiments conducted at the Changchun Institute of Applied Chemistry, Changchun, P.R. of China, i.e. all those apart from the ones described in section 6.3 and 6.5.4, the following materials were used: tetrabutylammonium perchlorate (TBPA) was obtained from Fluka (purum), potassium chloride from the Beijing Reagent Factory (Beijingshi hongxing huagong chang, analytical grade), ethanol, acetonitrile, chloroform from the Beijing Reagent Factory (Beijingshi huagong chang, all analytical grade). Chloroform was stored over calciumhydride, acetonitrile over bariumhydride. TBPA had been dried before use. All the other reagents were used as received.

In the experiments described in section 6.3 and 6.5.4 tetrabutylammonium perchlorate (Fluka, puriss. electrochemical grade), sulfuric acid 95-75\% (Riedel-de-Haën, puriss.), potassium hexacyanoferrate(III) (Fluka, puriss.) as well as potassium nitrate and ethanol (both MERCK, pro analysis) were used.
6.2.2 Cyclic voltammetry

For the cyclic voltammetry experiments a BAS100B Electrochemical Analyzer (Bioanalytical Systems Inc., West Lafayette, IN, USA) with a conventional three electrode arrangement was used. Experiments were done in a 10 ml four-neck conical flask with the working electrode (WE), the auxiliary electrode (AE), the reference electrode (RE) and a gas bubbler, respectively, at the angled necks. The working electrode consisted of a platinum rod with 1 mm diameter which had been incorporated into a glass body. The electrode was polished successively for several minutes with W40 grinding paper, 1.0, 0.3 and 0.05 µm alumina powder, rinsed with distilled water between each polishing step and eventually sonicated in acetone and distilled water. The electrode was used immediately after half an hour of cleaning scans between 1.2 V and -0.2 V (against Ag/AgCl/KCl sat. electrode) in deoxygenated 0.5 M sulfuric acid. A 10 x 6.5 mm platinum foil served as auxiliary electrode.

For a few experiments with 0.1 mM ETH\textsuperscript{T} 3001 in 0.27 mM tetrabutylammonium perchlorate (TBPA) chloroform solution a glassy carbon electrode (GCE) with a diameter of 4 mm was used to check for adsorbed species [11].

A Ag/AgCl electrode with a saturated KCl solution was used as reference electrode. The reference electrode had a silver wire bridge rather than a conventional diaphragm which is more suitable in non-aqueous solvents. For the measurements in acetonitrile a silver wire was used as so-called pseudo reference electrode according to [3]. If not otherwise mentioned, cyclic voltammograms have been recorded with an initial potential of 0 V against the reference with a scan rate of 100 mV/s and show the last segments of successive scans. Voltammograms have not been corrected for \textit{iR} drop. Experiments were carried out at ambient temperature (14-18°C).

Degassing of all solutions was achieved with a slight nitrogen (5.0 quality) stream using a syringe needle introduced through the bubbler neck. After half an hour of nitrogen purging the flask was sealed with a tube connecting both ends of the bubbler. Solutions were not stirred.

Solutions used for the cyclic voltammetry consisted of 0.44 mM ETH\textsuperscript{T} 3001 in chloroform, acetonitrile and ethanol with 0.2 M tetrabutylammonium perchlorate (TBAP) as supporting electrolyte for chloroform or acetonitrile and saturated in potassium chloride (KCl) for ethanol (approximately 22 mM as calculated from literature values [12]). Blank solutions had the same composition, but without ETH\textsuperscript{T} 3001 in it. For the saturated potassium chloride solution care was taken that precipitate was present at all times.

All cyclic voltammograms shown in 6.4 Cyclic voltammetry experiments on p. 173 represent the steady state after several scans.
6.2.3 Spectroelectrochemical cell (Changchun)

The spectroelectrochemical cell in Changchun was built based on the publication of Lin et al. [13] (see Fig. 6.1), but with slight differences. Instead of the platinum gauze (g in Fig. 6.1) with a hole size of 140 by 140 µm used in the reference a 2.5 by 10 mm platinum minigrid with a hole size of approximately 400 by 400 µm was used. The platinum foil of 10 by 15 mm which served as an auxiliary electrode (j) was placed 20 mm below the working electrode rather than symmetrically around the working electrode in close proximity as the original. Total cell volume amounted to 11 ml. The reference electrode was introduced through the opening at the top instead of a sideward neck. The design with its well enclosed minigrid tries to minimize edge effects at the cost of a high ohmic polarization ($iR$ drop) and is therefore thought for bulk electrolysis rather than cyclic voltammetry [7].

A Ag/AgCl electrode with a saturated KCl solution was used as reference electrode (e). It had a silver wire bridge instead of a diaphragm to make the electrical connection between measuring and inner solution ($E^\circ = +197$ mV vs NHE, +44 mV vs SCE with saturated KCl solution), its average distance to the working electrode was 4 mm. According to Niu et al. [14], the optically transparent thin layer cell had a volume of 6.6 ± 0.2 µl with a thickness (d) of 0.23 ± 0.02 mm. Own measurements gave a thickness of the thin layer section of 0.35 mm.

![Fig. 6.1 Spectroelectrochemical cell from Lin et al. [13]](image)

The cell volume was deaerated through a thin Teflon tubing with a slight nitrogen stream. Great care was taken to ensure complete deaeration (lasting approximately 40 minutes) and to minimize edge effects by convection within the cell induced by the nitrogen stream. Even with very vigorous bubbling the thin-layer could not be
exchanged within 15 minutes as visual luminescence observation confirmed. A EG&G Princeton Applied Research Polarographic Analyzer was used in “dc mode” to apply potentials between -4 and +4 V to the working electrode. Spectra were taken after the potential had been applied for 2 min for a 0.1 V step, 4 min for steps up to 0.25 V and 6 min for any larger potential step. These times correspond to the 200 s needed for a full diffusion-controlled electrolysis of the cell’s thin-layer section [15], which should permit to reach equilibrium.

The transmission of the minigrid was approximately 50% at 750 nm and 20% at 350 nm, with a sudden decrease below 350 nm.

Experiments with potentials with up to plus/minus 4 Volts imposed considerable stress onto the cell, especially on the working and the reference electrode. The reference electrode’s potential had shifted to a value between -104 to -108 mV against a standard calomel electrode (-44 mV is the standard difference) after the first run to +3 V. The reference electrode was therefore not replaced, but its potential was monitored after each experiment. All indicated potentials have not been corrected for the iR drop.

All experiments with the spectroelectrochemical cell were carried out with 0.16-0.43 mM ETH\textsuperscript{T} 3001 solutions in potassium chloride saturated ethanol. Actual concentrations were determined by the solutions’ absorbance after the experiments. Unless otherwise noted solutions were not replaced, but the thin-layer section volume was exchanged with the bulk volume by agitation.

6.2.4 Spectroelectrochemistry in luminescence

The luminescence spectra were recorded with a Tracor Northern (Middelton, Wisconsin, USA) TN-6500 Rapid Scan Spectrometer using a TN-6051 Multipurpose Spectrometer Base, a TN-6048 Spectrograph and a TN-6100 linear 512-element silicon photodiode array detector. The total luminescence accessory proved to be useless, but another solution could be found with the existing components. The modified set-up for the luminescence experiments is shown in Fig. 6.2.

In order to have a detectable luminescence signal a special slit adapter for the TN-6048 Spectrograph was needed, which minimized the distance from the cell’s mini grid to the spectrometer entrance. To achieve a sufficient discrimination of the excitation light a blue LED (Sloan, \( I_{\text{max}} = 20 \) mA, \( \lambda_{\text{max}} = 470 \) nm) was used as bright excitation source, which would interfere as little as possible with the dye’s luminescence (\( \lambda_{\text{max}} = 620 \) nm). A trapezoidal adapter with a 1 x 5 mm slit was made from black cardboard, which fitted well into the groove of the spectroelectrochemical cell and respected the focal distance of the spectrometer entrance.

The light of the blue LED was focused onto the spectroelectrochemical cell’s mini grid working electrode to excite exclusively the part of the dye solution in the thin
layer which undergoes electrochemical reactions. The best LED light discrimination was achieved when the LED was positioned at a $45^\circ$ angle with respect to the spectrometer entrance. The LED was operated in series with a $1 \, \text{k}\Omega$ resistor at currents of typically $5 \, \text{mA}$ after it had been allowed to equilibrate for an hour. LED current was regularly monitored and never changed more than 1% during an experiment. Spectra were averaged over 10 spectra with exposure times of 1 s.

For the experiments with oxygen an even larger LED light discrimination was necessary, because the luminescence of ETHT 3001 in ethanol is very effectively quenched by oxygen to about a 20th of its original level. This was achieved by introducing an $40 \times 40 \times 3 \, \text{mm}$ OG 570 cut-off filter (Jenaer Glaswerk Schott & Gen., Mainz, Germany) between slit and spectrometer entrance (as shown in Fig. 6.2) and increasing exposure time to 10 s, averaging over three spectra.

The luminescence intensity was determined by taking the maximum of smoothed and background-corrected luminescence spectra. For the positive potential range the spectra at $+4 \, \text{V}$, where the luminescence totally disappears, were taken as background. For the negative potential range the signal symmetrical to the LED peak was used as spectra background.

### 6.2.5 Spectroelectrochemistry in absorbance

Absorption spectra of the thin-layer section of the spectroelectrochemical cell were recorded with a VARIAN DMS 90 UV Visible Spectrometer. A wavelength scan in range from 350 to 750 nm was recorded within 2'45'', with a 2’ delay until the next
spectrum could be acquired. The applied potential was changed immediately after the spectrum had been recorded. Due to instrument limitations, it was not possible to have any usual background correction. The spectra were corrected by fitting background absorption spectra to the spectra and subsequently subtracting them. If available solution spectra recorded after the spectroelectrochemical experiments or spectra recorded at very positive potential where the dye is virtually destroyed (thus no absorption) have been used to improve the quality of this background correction. The spectrometer software did not allow any chronoamperometric experiments in absorbance.

The cell was aligned afresh with the beam at 530 nm after each manipulation to ensure that the light beam probes the inner part of the minigrid. Transmission was readjusted to 100% at 750 nm to ensure that the full dynamic range of the instrument was exploited.

### 6.3 Designing a spectroelectrochemical cell

The spectroelectrochemical experiments performed in Changchun motivated the design of an own spectroelectrochemical cell at CCS. Based on the experience the following requirements were set:

- thin-layer section made from quartz to make UV experiments possible
- flat, reproducible absorbance baseline to facilitate spectra background correction
- removable working (WE), counter (CE) and reference (RE) electrode for cleaning
- usable in a 30°/60° excitation/emission geometry for luminescence experiments
- smaller volume to save sample

All these requirements could be met. The cell has been manufactured by Bruno Nussberger of the Glasbläserei Hönggerberg, ETH Zürich. The cell was made from a quartz glass tube and two separate quartz glass windows for the thin-layer section.

The working electrode, counter electrode and reference electrode are introduced separately in the said order, each having its own neck. A fourth neck allows additional operations, such as degassing. The cell volume is 3 to 3.5 ml up to complete coverage of the working electrode, 6.5 ml up to the onset of the necks. The cell can be stirred by a small Teflon stirrer if desired and is vacuum-proof.

The transmission properties of the cell are given in Fig. 6.4. The transmission of the cell without the working electrode was between 82% in the visible range to 64% in the UV range, with the working electrode in place between 36 to 28%, respectively. The spectra are very smooth and without discontinuities.
A platinum Unimesh 48 mesh with a wire thickness of \(\sim 80 \mu m\) and a mesh size of \(480 \mu m\) (Johnson Matthey Inc., Valley Forge, PA, USA), which was connected by a thin platinum wire, was used both for the counter and working electrode. The reference electrode was manufactured by anodic oxidation of a silver wire in 1 M HCl at
currents below 20 mA during 100 minutes. A saturated solution of potassium chloride (Fluka, Chemika) in distilled water was used as electrolyte, which was connected to the sample solution by a platinum wire (ø 1 mm) welded into the glass. All electrodes are fully removable.

Experimental experience in Changchun told that any holder for a spectroelectrochemical cell should meet the following requirements:

- hold the cell firmly in place
- easy adjustment to the light beam needed
- easy, reproducible placement

A cell holder for absorption has been designed for the Uvikon 942. It is optically superior to the Hitachi U-3210, which has some flaws with extra reflecting surfaces (sudden baseline shifts). The existing massive brass support for two optode cells had been adapted, where the cell is now sandwiched between two custom-cut Plexiglas plates attached to the conventional absorption optode cell, see Fig. 6.5. The penetrating light beam is approximately 10.5 x 1 mm in size. The cell’s position and orientation can be easily adjusted to suit the spectrometer’s light path. The thickness of the thin-layer section has been determined by Lambert-Beer’s law to be 450 µm. A cardboard cover tailored to the cell’s dimensions has been made to keep ambient light off and to protect the mounted cell from damage and dust. Due to the very good optical properties of the Uvikon 942 even full admission of ambient light barely has any influence (0.002 change in absorption) on the spectra. This, however, changes drastically as soon as the cell is in place, therefore the cardboard cover was used at all times.

The scan rate of the spectrometer has no distinguishable influence on the quality of absorption spectra. Broad-banded spectra of 10 mM K₃[Fe(CN)₆] solution in 1 M KNO₃ showed no difference in the wavelength range of 200 to 850 nm if recorded with a scan rate of 200, 500, 1000 or 2000 nm/min. This reduces the time to record a full spectrum from 200 to 900 nm to 21 s.

For the spectroelectrochemical experiments absorption spectra on the Uvikon 942 were recorded continuously and automatically by a self-written LabVIEW 5 program, which controlled the spectrometer via the RS-232 interface. The software set all the required parameter, triggered the spectrum recording and transferred it to an external laptop computer where it was saved. The program also measured simultaneously the potential of the E_out BNC connector of the Autolab module with the National Instruments DAQ Card-1200. This enabled to accurately time-stamp each spectrum with the currently applied potential in order to match the cyclic voltammogram with the absorbance data.

Acquiring an absorption spectrum between 200 and 800 nm at scan rates of 1000 nm/min with subsequent ASCII transfer took 86 seconds, which set the time
6.3 Designing a spectroelectrochemical cell

resolution in absorbance. Absorbance measurements at a constant wavelength allow
time resolutions in the s-range, though discarding the complete spectra information.

Cyclic voltammograms have been recorded with the Autolab PGSTAT 20 or the
PGSTAT 10 system. Please note that this procedure is very different from the spec-
troelectrochemical experiments in Changchun (see 6.2.3), where each spectrum
reflected an equilibrated state. Cyclic voltammograms have a well-defined time-
potential relation which facilitates data interpretation.

Prior to use, the cell was cleaned in degassed 0.5 M sulfuric acid by continuous
voltage sweeps between 1.2 V and -0.2 V with a scan rate of 100 mV/s, and then
rinsed with the solvent intended for the experiments. Degassing was done by purg-
ing with argon (4.8, AGA, Zürich), a reduced purging was maintained during exper-
iments.

Oxidation/reduction in the thin-layer section of 95% of a 10 mM K₃[Fe(CN)₆] solu-
tion in 1 M KNO₃ took 110 s, full oxidation/reduction ca. 250 s. Obviously a full
electrolysis is possible, even though the dimensions of the thin-layer and the work-
ing electrode grid are generous. A sample spectroelectrochemical experiment with
the same solution is shown in Fig. 6.6.

The absorbance curves have been extracted from individual spectra and were plot-
ted against the potential which was applied when the wavelength scan passed
460 nm. Fig. 6.7 shows selected absorption spectra. Clearly visible is the creation of
the strongly absorbing \([\text{Fe(CN)}_6]^{3^-}\) and its disappearance in the subsequent reduction.

A redox couple \(\text{O} + e^- = \text{R}\), in which both the oxidized species \(\text{O}\) and the reduced species \(\text{R}\) exchange rapidly electrons with the working electrode, is termed an electrochemically reversible couple. For its cyclic voltammogram [16] we expect the anodic and cathodic peak potentials \(E_{\text{pa}}\) and \(E_{\text{pc}}\) in \([V]\) to obey the relation

\[
\Delta E_p = E_{\text{pa}} - E_{\text{pc}} \approx \frac{0.059}{n}
\]

and for the respective peak currents \(i_{\text{pa}}\) and \(i_{\text{pc}}\) (after several cycles)

\[
i_{\text{pa}} / i_{\text{pc}} \approx 1.
\]
6.3 Designing a spectroelectrochemical cell

The formal electrode potential $E^\circ$ with respect to the reference electrode is the potential midway between $E_{pa}$ and $E_{pc}$.

Note that these familiar equations hold under quasi-infinite conditions with ideal electrodes only! For a thin-layer situation at slow scan rates (such as the 0.001 V/s used) we expect $\Delta E_p = 0$ V with symmetrical peaks around $E^\circ$ [16]. The peak potentials depend in this case linearly on the scan rate, rather than on its square-root as for quasi-infinite conditions. An $iR$-drop, however, will still cause a peak separation. With very slow heterogeneous rate constants peaks will lose their symmetry and peak potentials will separate.

The redox couple $[\text{Fe(CN)}_6]^{3-} + e^- = [\text{Fe(CN)}_6]^{4-}$ is such an electrochemically reversible couple. Its half-wave potential is +0.36 V vs NHE in acidic solution [17], i.e. +0.16 V vs Ag/AgCl/KCl (sat.). The obtained half-wave potential of 0.095 V is clearly off the expected value, which was due to an insufficient AgCl-coverage of the reference electrode. This was remedied for later experiments and stresses the importance of reference electrode calibration.

The measured ratio $i_{pa}/i_{pc}$ of 1.02 is in good agreement with a reversible redox reaction. $\Delta E_p = 0.042$ V is an indication for the expected $iR$-drop, which had not been compensated during recording. Note that the potential scan range was larger than the usual -0.1 to +0.5 V and started in the potential range of the reduced species, which can account for some of the irreversibility. The solution was not freshly prepared and might have been partially oxidized at the beginning of the experiment as suggested by the different absorbance values at -0.1 V.

**Fig. 6.7 Absorption spectra of potassium hexacyanoferrate(III) at different potentials**

Selected spectra from Fig. 6.6 are shown. Spectra have been offset-, but not background corrected.
Absorbance and current in Fig. 6.6 are correlated as expected, with the current following the first derivative of the absorbance.

The rather large mesh size of the working electrode seems not to have any adverse effects since the diffusion-controlled reaction of $K_3[Fe(CN)_6]$ proceeds as expected. The characterization experiment at large confirms that the new spectroelectrochemical cell is suitable for cyclic voltammetry experiments at the slow scan rates for the simultaneous absorbance measurements.

### 6.4 Cyclic voltammetry experiments

For the 0.1 mM ETH$^T$ 3001 in a 0.27 mM tetrabutylammonium perchlorate chloroform solution the electrochemical window with the GCE (data not shown) was approximately from -1.5 V to +2 V which showed very little activity. Oxygen intrusion led to a reduction wave starting at -0.7 V. There was an oxidation wave at +1.65 V in the aerated solution when cycling from 0 to +2 V and back with no corresponding reduction wave, i.e. an irreversible electrode reaction or a slow electrode reaction with a fast subsequent chemical reaction.

To check whether this reaction occurred only on a GCE, the experiment was redone with the platinum electrode working electrode. The same wave appeared at 1.4 V at the platinum electrode if the potential was scanned from 0.8 to 1.6 V, with no change if the upper potential was extended to +3 V. There was a square-root dependence of the peak current on the scan rate showing that this was not an adsorption peak (linear dependence on peak current), but a diffusion-controlled reaction [18].

Fig. 6.8 and Fig. 6.9 show the corresponding voltammograms for acetonitrile and ethanol. The respective blank solutions have identical compositions, but without ETH$^T$ 3001.

The cyclic voltammograms in general failed to give additional information on the electrochemistry of ETH$^T$ 3001. Except for the above-mentioned peak in chloroform at 1.4 V all peaks are due to reduction or oxidation of species which have been created in previous segments of the voltammogram at very high or very low potentials. The oxidation waves are typical for irreversible reactions with slow electrode kinetics and sometimes fast follow-up chemical reactions [18]. There are some doubts about the purity of the acetonitrile which had been used for the experiments, though acetonitrile is generally regarded as the most suitable solvent for cyclic voltammetry with a large potential window [10]. Another aspect, why the acetonitrile cyclic voltammograms should be carefully looked at, is the silver wire pseudo-reference electrode which proved to be unreliable.

The recorded cyclic voltammograms have little resemblance with those of ca. 1 mM of $[Ru(bpy)_3]^{2+}$ or other ruthenium(II) diimine complexes that can be found in the
literature [2-5]. Bard et al. found their almost identical cyclic voltammogram of
[RU(dpp)$_3$]$^{2+}$ initially very different from [RU(bpy)$_3$]$^{2+}$ and difficult to explain [3, 4],
which suggests that the increasing lipophilic character of the ligands indeed inhibits
fast electrode kinetics or complicates cyclic voltammetry in other ways.

This has been confirmed by a cyclic voltammogram of ETH$^T$ 3001 in acetonitrile
kindly recorded by Peter Belser. He found the reversible Ru(III)/Ru(II) oxidation
potential at +1.17 V (vs SCE) with a difference of 80 mV between anodic and
cathodic peak.

Reduction of the ligands were strongly irreversible. In the cathodic range there was
a small peak at -1.31 V and a slightly larger one at -1.37 V. In the anodic range there
was a large, sharp peak at -1.295 V which suggests a desorption event. This is in
good agreement with the cyclic voltammogram shown in Fig. 6.8. Another voltam-
mogram (not shown) of these series recorded under identical conditions as in [4]
had shown a small reversible peak pair at +1.23/1.16 V (vs Ag wire), though with
much other activity probably due to solvent impurities.
6 Electrochemical investigation of ETH\textsuperscript{T} 3001

6.5 Spectroelectrochemical experiments

6.5.1 Cyclic voltammetry

Fig. 6.10 shows a cyclic voltammogram which has been recorded with 0.4 mM ETH\textsuperscript{T} 3001 in KCl-saturated ethanol in the spectroelectrochemical cell. The voltammogram shows a similar activity as in Fig. 6.9.

6.5.2 Luminescence

Eventually the ethanol system was chosen for the spectroelectrochemical study, mainly because the earlier bleaching experiments had been done in ethanol. The same solution as for the cyclic voltammetry experiments was used. Fig. 6.11 shows sample luminescence spectra for the positive potential range run in deaerated solutions. The peaks at 460 and 790 nm are LED and scattered LED light, respectively. The luminescence spectra for the other runs looked similar, but without the LED peaks which were removed by the OG 570 filter. A summary of the results for the spectroelectrochemical experiments in luminescence is given in Fig. 6.12.
No change in spectra shape has been found at the different potentials. In deaerated solutions at positive potentials the luminescence starts to decrease at approximately 1.4 V and completely vanishes at +4 V, but recovers within some minutes when the potential is set back to +2 V. If oxygen is present, the luminescence starts to decrease at 1.2 V with a shoulder at 1.6 V and disappears at 2.4 V. There was no
signal recovery when going back from +4 V to 0 V within 15 minutes, showing that this reaction is irreversible.

At negative potentials in the deaerated solution the luminescence essentially sustains until -4 V. The slight increase in luminescence around -1 V is due to the residual oxygen in the degassed solution which is reduced and no longer able to quench the luminescence. If the LED was switched off at -1 V, there was no luminescence below 1‰ of the original luminescence level. No electrogenerated luminescence has been observed at other negative potentials between -1 and -2.5 V as expected. Electrogenerated chemiluminescence usually needs a Ru(I) and Ru(III) species generated by rapid potential switching [3].

A control experiment with larger potentials steps showed less of a decrease at negative potentials, indicating that the change in luminescence is probably due to an obscuring effect of species formed at very negative potentials rather than due to a chemical reaction. This has been confirmed by the absorbance measurements at the same potentials. The control experiment spent less time at negative potentials, which could be an alternative explanation for the originally smaller luminescence signals in the case of a very slow reaction.

In the presence of oxygen there was a sharp increase below -0.1 V reaching 24 times the original level at -0.9 V. Then it slowly decreased until there was a steep
decrease from -2.3 V to -2.9 V down to zero luminescence. Please note the logarithmic extension of the top part of the luminescence curve and the normalization to 1; the actual curve would have ca. 1/25th of the intensity. The luminescence recovered within 5 minutes when going back from -4 V to 0 V, i.e., the reaction was reversible. The initial increase is due to the removal of the quencher oxygen at -0.8 V by the reaction

\[ \text{O}_2 + e^- \rightarrow \text{O}_2^2^- \]  

(6.3)

with subsequent adsorption to platinum. The oxygen reduction potential in acetonitrile had been estimated as -0.78 V [19]. The 24-fold increase is in good agreement with the ratio \( S_{\text{aerated}}/S_{\text{dearated}} \) determined above in Table 3.3 on p. 56 and Fig. 3.13 on p. 52.

The decrease below -2.3 V is most probably caused by the creation of a new luminescence quencher species from oxygen and ethanol, because the absorption spectra at the respective potentials showed no change.

Ohmic polarization within the cell was obvious for the luminescence experiments at high potentials in the absence of oxygen when the dye is reversibly transformed into a non-luminescent species. The central part of the rectangular optical thin layer - which experiences the smallest applied potential in case of anodic currents - was the last spot to show luminescence and was also the first spot where luminescence reappeared once the potential was decreased to +2 V where the dye recovers. A linear sweep voltammetry experiment with a rate of 5 mV/s showed an almost linear, featureless current increase to 22 µA when sweeping from 0 to -2.4 V. Assuming that this current reflects the conductivity of the solution the latter can be calculated to be at most 50 kΩ/cm. The \( iR \) drop between reference and auxiliary electrode is compensated by the potentiostat, but no provisions were made for the compensation between working electrode and reference electrode [20]. The working electrode is positioned at almost the same distance from the auxiliary electrode as the reference electrode and has free access to the bulk solution at all sides. These provisions should minimize the \( iR \) drop, but do not affect the potential gradient within the mini-grid in the thin-layer section which may amount to 0.5 V at very high currents.

### 6.5.3 Absorbance

Spectroelectrochemical studies in absorbance are much more common than those in luminescence. The results of several individual absorbance experiments are summarized in Fig. 6.13.

Fig. 6.14 shows the absorption spectra of a solution of 0.219 mM ETH T 3001 in KCl-saturated ethanol at positive potentials in the presence of oxygen. At potentials between 1.0 V up to 2.3 V the absorption spectra show a progression with an isos-
Fig. 6.13 Spectroelectroabsorbance experiments with ETH$^T$ 3001 in KCl-saturated ethanol
Absorbances have been normalized to 1 at 0 V. The “normalized” absorbance values at 460 nm for the 0.426 mM solution are obtained when the corresponding absorbance values at 394 nm are taken to be the 394 nm absorbance values of the air-saturated 0.219 mM solution.

Fig. 6.14 Absorption spectra obtained between 0 and 3 V in the presence of oxygen
Initially one isosbestic point at 394 nm is observed. The ethanolic solution was approximately 0.219 mM in ETH$^T$ 3001, the same solution had been used for the cyclic voltammetry and the spectroelectrochemical experiments in luminescence.
bestic point at 394 nm and a decrease in the absorption maximum at 460 nm. At higher potentials, there was a general decrease in absorbance which was not reversible as the absorption spectrum did not recover when going back to 0 V. A previous run to +1.2 V and back to 0 V showed that the spectrum did not recover within 10 minutes, indicating that the irreversible reaction takes place already around +1.2 V.

The same experiment with a freshly prepared 0.426 mM solution and a previously cleaned cell did not yield the same result. If the absorbance at 460 nm in Fig. 6.13 was normalized to the one at 394 nm of the experiment with the 0.219 mM solution, an identical decrease was found. It seems as if the oxidation coincides with another, faster process, like the one observed for the bleaching experiment in the concentrated chloroform solution (compare p. 134).

An absorption spectrum of the 0.219 mM solution, which had been acquired after having completed the spectroelectrochemical experiments, revealed a considerable fraction of oxidation products as seen in Fig. 6.16 on p. 183. Obviously most of the bulk solution had been oxidized by that time. The concentration of the oxidation products at the time when the experiments with the 0.219 mM solution were made must have been much smaller since most of the experiments with long application times of high positive potentials had been carried out thereafter.

Further experiments with a freshly prepared 0.215 mM solution still did not show the same behavior as the 0.219 mM, but this may be because the cell had not been cleaned previously. This suggests an electrode passivation by the deposition of oxidized/reduced species.

Explanation of the absorption spectra at positive potentials in the absence of oxygen is very difficult, because the background subtraction could not be satisfactorily resolved. The data does not allow any further conclusions.

At negative potentials with or without oxygen there was no change in the absorption spectra, except a small overall incremental increase which was probably due to the deposition of the black matter which had been found previously after the luminescence experiments. The black substance could be removed by acetone or other solvents, though the grid remained black even after subsequent cleaning scans, indicating that the platinum surface as well had undergone reactions (platinated platinum). Bard et al. had also observed the precipitation of a dark brown or black unidentified substance on the working electrode from a solution of \([\text{Ru(bpy)}_3](\text{ClO}_4)_2\) when varying the potential between the first oxidation wave and the fourth reduction wave [4]. Visual observation confirmed that the sudden increase of absorbance at -3 V is probably due to a desorption of previously adsorbed dye from the grid.

Brateman et al. found in the spectra of the singly, doubly and triply reduced species
of mixed ligand ruthenium diimine complexes a new band at 370 nm and a red-shift of up to 100 nm of the band at 460 nm [9]. None of this was observed here.

The possible reaction $\text{Cl}_2 + 2 \text{e}^- = 2 \text{Cl}^-$ has a standard reduction potential of 1.36 V in water [21], though this is likely to be different in ethanol. There was no gas formation observed in the spectroelectrochemical cell at either plus or minus 3 Volt, which might indicate that the overpotentials to generate any gaseous species were too high.

Experiments to verify a concentration dependence were not conclusive because of the spectrometer’s insufficient resolution power at high absorbances imposed by the spectroelectrochemical cell. The dye concentration of 0.426 mM was probably critical, because dye aggregation/adsorption/desorption processes might easily exceed the maximum solubility of 0.48 mM in ethanol, giving rise to similar acceleration effects as observed in the photobleaching process of concentrated ETH$^T$ 3001 chloroform solutions, compare 5.5 Bleaching of ETH$^T$ 3001 in solution on p. 131.

### 6.5.4 Experiments in absorbance with the new cell

In view of the controversial results obtained with the spectroelectrochemical cell in Changchun (see 6.5.3 on p. 178), the experiments at positive potentials were repeated with a self-designed spectroelectrochemical cell, see 6.3 Designing a spectroelectrochemical cell on p. 167.

Cyclic voltammetry scans of 0.13 mM ETH$^T$ 3001 in 0.10 M TBPA in ethanol from 0 to +2.5 and back to 0 V had been recorded with or without oxygen present, the one without oxygen additionally at three different scan rates. The experimental procedure was identical to those outlined in 6.3 on p. 167, the voltammograms have been carried out in the sequence specified in the legend without solution replacement. The results are summarized in Fig. 6.15, a few selected spectra are given in Fig. 6.16.

Absorbance curves in Fig. 6.15 correspond to the isosbestic point, which was temporarily found at 406 nm, and ETH$^T$ 3001’s absorption maximum at 463 nm. The first run with continuous nitrogen-purging also during the experiment has been corrected for evaporation losses (-4%), which were assumed to be linear throughout the whole cyclic voltammetry scan.

Please note that higher scan rates mean a higher potential gap between absorbance curves at different wavelengths (e.g. 406 and 463 nm), because the spectra cannot be recorded at once. Furthermore, a smaller potential resolution results because less spectra can be recorded in period of one CV. For the fastest scan rate of 0.004 V·s$^{-1}$, the potential gap between the two absorbance curves amounts even to 14 mV and potential resolution drops to 0.15 V.
From Fig. 6.15 we can conclude that the oxidation of ETH$^T$ 3001 in absence of oxygen must be reversible, because an identical absorbance curve is observed for the first and the second segment of the voltammogram. The oxidation/reduction half-wave potential must be between +0.5 and +1.5 V. The exact potential depends on whether the absorbance at 463 or at 406 nm is taken as reference. There is no significant corresponding activity in the cyclic voltammogram as expected for the very low scan rates.

In the absence of oxygen the main feature is the disappearance of the shoulder around 550 nm. It is noteworthy that this shoulder is the main difference between ETH$^T$ 3001 and ETH$^T$ 3003, compare Fig. 2.8 on p. 20.
In the presence of oxygen the voltammograms resemble qualitatively those recorded in acetonitrile, compare Fig. 6.8. The spectrum at 1.86 V with oxygen present nicely fits those in the experiments carried out in Changchun, compare Fig. 6.14. As expected the spectral change is more marked for the slower scan rate of 0.001 V·s⁻¹, which allows a higher turnover than at 0.004 V·s⁻¹ because the ongoing electrochemical reaction is slow and obviously not purely diffusion-controlled. Apart from that, the data is difficult to explain. Interesting features are the sharp desorption peaks at 1.63 and 1.35 V for the 1 mV·s⁻¹ and 4 mV·s⁻¹ respectively with no corresponding activity in absorbance.

An additional experiment up to +3 V still gave no annihilation of the dye as observed in Fig. 6.14. Most probably this complete destruction of ETH₇ 3001 in the earlier experiment was caused by oxidative products of the electrolyte KCl, which had been replaced by TBPA in this experiment.

At positive potentials in the absence of oxygen there is no increase in the absorption spectra around 400 nm where Brateman et al. had observed a Ru(III) species in their spectrotroelectrochemical experiments with [Ru(bpy)₂(X)]²⁺ systems [9], though these changes are likely to be at different wavelengths for ETH₇ 3001.

### 6.5.5 Discussion

Nonaqueous electrochemistry [22] is much more demanding than aqueous electrochemistry. Largely unknown for all experiments remains the effect of the reactive species created by ethanol, potassium chloride and oxygen at the unusually high or low potentials in the experiments.
Ethanol is considered to be a suitable solvent for anodic electrochemistry [10], though acetonitrile with tetrabutylammonium hexafluorophosphate (TBAHFP) and tetrafluoroborate (TBA TF B) is the usual recommendation. For cathodic electrochemistry DMF and DMSO are highly recommended [10]. TBAP can be considered inert. On the other hand, the rather nucleophilic acetonitrile can readily react with electrophilic intermediates or substitute ligands in organometallic complexes. Ethanol itself can form a host of various species [23-25], but these have not been investigated in such a large potential range. Cyclic voltammetry of a ~1 mM solution of ethanol in sulfuric acid/lithium sulfate with $[SO_4^{2-}]_{tot} = 0.5$ M, pH 1.9 on platinated platinum electrodes by Martin gave mainly ethanol below 200 mV, little ethanal around 100 mV and acetic acid above 250 mV as the dominant species with a HgSO$_4$/sat. K$_2$SO$_4$ ($E^\circ = +656$ mV) reference electrode and optimal scan speed of 50 mV/s [25]. Prepolarization at -350 mV (adsorption of ethanol) gave the highest sensitivity for analytical determination. Oxygen adsorption starts at above 250 mV, the first platinum oxides are formed above 350 mV. Oxygen adsorption above 550 mV displaces all other adsorbed organic species. If going back to -350 mV, the surface is voided of all oxygen/oxides. The zero surface charge potential of platinum is -490 mV.

Reactions seem to differ greatly from acidic to alkaline media which implies that these processes might be very different in non-aqueous media. Platinum oxides, adsorbed carbon monoxide, ethanal, adsorbed dye and ethanol all further complicate the picture in order that nothing definitive may be said.

The absorbance-spectroelectrochemical experiments (Fig. 6.13 and Fig. 6.15) look similar up to say +1.5 V, if the rather small absorbance variations around the alleged isosbestic point are not considered. At higher potentials the decrease in absorbance in Fig. 6.15 at 463 nm is stopped or even reversed, possibly because a subsequent reaction occurs or the reaction becomes diffusion-limited. The total destruction of ETH$^T$ 3001 at higher potentials in the Changchun experiments must be attributed to the unsuitable electrolyte KCl.

The reversible oxidation around +1.2 V in the absence of oxygen must be attributed the oxidation of Ru(II) to Ru(II). $E_p$ for [Ru(phen)$_3$]$^{2+}$ is slightly higher, around 1.45 V in AcN/0.1 M TBATFB [4].

The identical onset of the absorbance decrease in the absence and presence at around +0.5 V (see Fig. 6.15) suggests that oxygen reacts with oxidized ETH$^T$ 3001 species rather than undergoing a direct electrochemical reaction with ETH$^T$ 3001. This is in good agreement with Bard et al.'s observation that the bright green [Ru(bpy)$_3$]$^{3+}$ was stable in acetonitrile for several days under vacuum, but unstable to air [4]. The Ru(III) species possibly also reacts with ethanol, hence slowing down kinetics. Such a reaction could be prevented by using acetonitrile.

Oxygen concentration is very unlikely to be reaction-limiting in aerated ethanol.
because the solubility of oxygen at 20°C is 2.1 mM, i.e. approximately five- to 15-fold the concentration of ETH^T 3001 investigated [26, 27]. Further, the diffusion of the small oxygen molecule must be orders of magnitude faster than the one of the large ETH^T 3001 molecule.

Which oxidation product of ETH^T 3001 is formed with oxygen is unknown. It could possibly be a Ru(IV)=O species. Meyer found that already oxidized [Ru(bpy)_2(py)OH]^{3+} undergoes a second one-electron oxidation by Ce(IV) to precipitate as perchlorate salt of [(bpy)_2(py)Ru=O]^{2+} in concentrated solutions, which absorbs only in the UV [28].

In the case of the CCS spectroelectrochemical cell, the large grid size of ~0.5 mm is too large for very fast reactions or fast scan rates. No evidence for diffusion from the bulk solution to the thin-layer section on the timescale of the experiments was found.

### 6.6 Summary and Perspectives

In contrast to most of its other properties, the electrochemistry of ETH^T 3001 cannot be extrapolated from other ruthenium(II) diimine complexes. As expected, reversible oxidation Ru(III)/Ru(II) was found to occur at +1.21 (vs Ag/AgCl/KCl sat.) in the experiments with cyclic voltammetry and potentiostatic spectroelectrochemistry in absorbance and luminescence. The kinetics, however, are unusually slow. Ligand reductions are largely irreversible with little activity except desorption.

The oxidized species of ETH^T 3001 shows only slight spectral changes. The absorption maximum shifts to 438 nm with a decrease around 520 nm and an increase around 350 nm.

Its oxidation at positive potentials in the presence of oxygen is irreversible. Spectroelectrochemical experiments in absorbance suggest that this is due to a reaction of ETH^T 3001 with oxygen, rather than to a direct electrochemical reaction.

The lipophilization of ruthenium(II) diimine complexes seems to effectively protect from ligand reduction in conventional electrochemical experiments.

The work described here is a rare example of a spectroelectrochemical experiment in luminescence. After some experimental effort, it was possible to resolve the luminescence spectra of ETH^T 3001 at selected potentials even under oxygen-quenched conditions.

Some of the experiments were performed using a solid, versatile spectroelectrochemical cell. Its thin-layer section of approx. 450 µm thickness is made entirely from quartz glass and allows both luminescence and absorbance measurements.
from 200 to 900 nm. All the electrodes can be easily accessed for cleaning or replacement. Experiments can be carried out with as little as 3 ml of solution.

6.7 References


7 Impedance measurements with NO$_2$-sensitive membranes

7.1 Introduction to NO$_2$-sensitive polymeric membranes

Recent work in our laboratory has shown that an optical NO$_2$-sensor with a detection limit in the ppb-range is feasible [1]. Sensors for NO$_2$ are very attractive for early fire detection or environmental monitoring.

Such a NO$_2$-sensitive membrane is based on the same coextraction mechanism [2] in solution as for the nitrite optode developed earlier in our group by Caspar Demuth [3, 4]. The chemical structures of its components are given in Fig. 7.2. The principle of the coextraction mechanism is sketched in Fig. 7.1.

![Fig. 7.1 Coextraction mechanism](image)

An anion, $X^-$ (nitrite), is co-extracted with a proton, $H^+$, into a lipophilic membrane containing a ligand $L$ (NI 1) and chromoionophore $C$ (ETH 5418). A lipophilic counterion $R^-$ (KTTFPB) is used to stabilize the chromoionophore, which is positively charged in its protonated state.

The anion (in this case nitrite) transfers to a lipophilic membrane phase where it is selectively bound by a ligand (nitrite ionophore 1, abbrev. NI 1). The requirement of electroneutrality means that the nitrite’s negative charge must be compensated for. This is done by coextracting a proton into the membrane phase. The proton is abstracted by the chromoionophore (a pH indicator dye), which subsequently changes its spectral properties.

The chromoionophore fulfills three specific functions. First, it transduces the complexation of $XL$ into an analytically useful signal, i.e. an absorbance change. Second, it must be selective for protons, otherwise other anions would be coextracted as well. And last, the basicity of the chromoionophore determines the measuring range for the anion. A very basic chromoionophore, however, promotes an undesirably unselective coextraction of a cation with a proton.

A lipophilic counterion (in this case KTTFTP) is used to stabilize the chromoionophore in its protonated form where it is prone to be washed out into the aqueous phase.
sample solution. Note that KTTFTB decomposes slowly in a proton-catalyzed reaction [2].

Given the stochiometric coextraction mechanism, an equimolarity of the ligand, chromoionophore and lipophilic counterion in the membrane must be respected.

The main disadvantage of the sensor design for nitrite is its dependence on the sample solution pH. Sample solutions must be buffered for measurements.

In theory it would be possible to monitor the reaction based on the spectral changes of NI 1. Upon binding of the nitrite, the absorption maximum shifts from 527 to 560 nm, although the very small absorption coefficient of ~6000 M⁻¹ cm⁻¹ [4] makes
it difficult to observe the reaction. Therefore, the absorbance change of the much more strongly absorbing, lipophilized Nile Blue derivative ETH 5418 is used. The deprotonated form of ETH 5418 in MeOH has an absorption maximum at 524 nm ($\varepsilon = 3.9 \cdot 10^4 \, \text{M}^{-1} \text{cm}^{-1}$), and the deprotonated form at 656 nm ($\varepsilon = 7.9 \cdot 10^4 \, \text{M}^{-1} \text{cm}^{-1}$). In a PVC/DOS (2:1) membrane, the absorption maximum of the deprotonated form is 522 nm, whereas that of the protonated form shifts to 664 nm. ETH 5418’s $pK_a$ in PVC/DOS has been estimated as 9 [5].

There had already been speculation about the use of optodes for gas sensing of neutral species [6] in the group led by the late Wilhelm Simon, which can be considered the father of many optical sensors. At the time, however, the emphasis was on other gases, such as CO$_2$ and NH$_3$.

Research by Tomas Nezel has shown that the composition of the original nitrite sensor can be used in developing a NO$_2$-sensitive polymeric membrane. He has been working in collaboration with Bosch Telecom (Ottobrunn, Germany) and Penta-pharm (Basel, Switzerland) on finding an optimal formulation which can be adapted to industrial requirements and elucidating how the sensor works (see his forthcoming Ph.D. thesis).

So far, he has tested a wide variety of polymers, plasticizers and chromoionophores. Despite this variation, only a slightly altered membrane composition has resulted. The best combination was PVC/DOS with ETH 5418 and a precursor of nitrite ionophore 1 (PEFA 10105). Membranes without plasticizers were found to be too insensitive. Hydrophobic polymers have a very low diffusion rate for salts and a very large activation energy for ion formation [7]. Other polymer or plasticizer combinations were found not to have the required mechanical stability at 65°C or were not as reversible. The plasticizer DOS has been commonly used in conjunction with PVC, mostly in ion-selective electrodes. It has a $\varepsilon$ of 3.9 and a log $P_{\text{TLC}}$ of 11 ± 1 [8].

With this slightly modified combination, no cross-sensitivity to NO, CO or CO$_2$ was found and only a minor interference with SO$_2$. The detection limit was found to be 50 ppb NO$_2$, with a response time $t_{90\%}$ for 100 ppb NO$_2$ in the range of 30-40 min. Full reversion of the response took 1-2 days [1].

Nezel et al. believe that gaseous NO$_2$, which predominantly exists as N$_2$O$_4$, dissociates inside the membrane and reacts with the water in the membrane to give nitrite and nitrate:

$$\text{N}_2\text{O}_4 + \text{H}_2\text{O} = \text{NO}_3^- + \text{NO}_2^- + 2\text{H}^+. \quad (7.1)$$

Note that at concentrations of 1 ppm nitrogen dioxide predominantly (99.998%) exists as NO$_2$, not as N$_2$O$_4$ [9]. On the stochiometry of the disproportionation reaction, however, this has no influence. The subsequent reactions are thought to be the
same as those with the nitrite sensor membrane. The nitrite binding to NI 1 liberates its coordinated water.

In a feasibility study with Martin Forster and Markus Löpfe at Siemens Building Technologies - Cerberus Division (Männedorf, Switzerland; in the following abbreviated as Siemens-Cerberus), opportunities for measuring impedance with NO₂-membranes were investigated. Of special interest was whether the NO₂-response could also be sensed by impedance changes rather than optically.

Contrary to the original recipe in Demuth et al., Nezel omitted potassium tetrakis-[3,5-bis-(trifluoromethyl)phenyl]borate (KTTFPB) because his previous work had suggested that it was not necessary to measure gaseous NO₂ and because KTTFPB has limited stability. As a result ETH 5418 cannot be fully protonated in the membrane, but only up to 80-90%. The response up to 50% protonation is, however, identical.

In a first attempt, the impedance of NO₂-sensitive polymeric membranes were investigated in sandwich electrodes, see 7.4. The need for visual confirmation of the ETH 5418 protonation led us to conduct combined absorbance and impedance measurements with interdigitated microelectrodes (described in 7.5.1). To our knowledge, no such combined measurements have been reported to date to investigate reactions in a plasticized polymer with reactive compounds exposed to the gas phase.

The evolution of the data acquisition and treatment made it possible to do impedance timedrive measurements (given in 7.5.2).

The following section summarizes the essentials of impedance spectroscopy needed to understand how processes in a NO₂-sensitive polymeric membrane may be modelled (7.2.3), which parameters describe an impedance spectrum (7.2.1) and how they can be derived from data (7.2.2).

### 7.2 Theory of impedance spectroscopy

#### 7.2.1 Introduction to impedance spectroscopy

Systems investigated by impedance spectroscopy are commonly described by an electrical equivalent circuit [10], i.e. an electrical circuit which behaves as the chemical system investigated. This allows to use the powerful formalisms of electrodynamics [11] for the modelling which will be treated in this section. A widely different issue is on how the elements of this electrical circuit are correlated with chemo-physical processes in the system investigated. This is discussed in 7.2.3 Model for the NO₂-sensitive polymeric membranes on p. 201.
Impedance spectroscopy determines the frequency response of a current $I$ to a perturbing alternating voltage $U$. The current will change both in amplitude and phase shift $\phi$ with respect to the perturbing voltage depending on the frequency $\omega$ of the alternating voltage applied. These two parameters are summarized in the complex quantity *impedance*

$$Z = \frac{U}{I},$$

(7.2)
defined in analogy to Ohm’s law, but with a complex voltage $U$ and current $I$. The *admittance*

$$Y = \frac{1}{Z}$$

(7.3)
is the complex counterpart of the conductance. In a serial arrangement of circuit elements the individual impedance values add up for the overall impedance

$$Z_{12} = Z_1 + Z_2,$$

(7.4)
whereas in a parallel arrangement the individual admittances add up for the overall admittance

$$Y_{12} = Y_1 + Y_2.$$

(7.5)

The impedance of common elements of an electronic circuit are summarized in Table 7.1. Capacitor and resistors are commonly known elements, inductive elements are missing, because they do not occur in chemical systems. The Constant Phase Element (CPE) is the generalization of these three elements with a *constant* phase $\alpha$. With $\alpha = 1$ the CPE becomes a capacitor, with $\alpha = 0$ a resistor. The Warburg impedance finally is an empirical element tailored specifically for chemical diffusion processes and is discussed below. Its main difference to a resistor is its frequency-dependent resistance.

<table>
<thead>
<tr>
<th>Component</th>
<th>Symbol</th>
<th>Impedance</th>
<th>Phase shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacitor</td>
<td>C</td>
<td>$Z = \frac{1}{i \omega C}$</td>
<td>$-\pi/2$</td>
</tr>
<tr>
<td>Resistor</td>
<td>R</td>
<td>$Z = R$</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 7.1 Common elements in equivalent circuits
Phase shifts are relative to the perturbing alternating voltage $U$. 
It cannot be overemphasized that the formalisms of complex mathematics have originally been devised for electrodynamics and are very powerful. Remember that complex elements are plotted as a vector

\[ \rho e^{i\phi} = \rho \cdot (\cos \phi + i \cdot \sin \phi) = \alpha + \beta \cdot i \]  

in a plane with real and imaginary axis. \( \rho \) corresponds to the modulus, \( \phi \) to the phase factor of the vector \( z \). \( \alpha \) is the real contribution (has nothing to do with the phase factor \( \alpha \) of the CPE), \( \beta \) the complex contribution.

All following considerations address the equivalent circuit in Fig. 7.3, which has been found to describe best the \( \text{NO}_2 \)-sensitive polymeric membranes. The formalism can be easily applied to any different equivalent circuit. Please note that the model in Fig. 7.3 is imposed by experimental data and is not at the author’s discretion.

**Table 7.1 Common elements in equivalent circuits**

<table>
<thead>
<tr>
<th>Component</th>
<th>Symbol</th>
<th>Impedance</th>
<th>Phase shift</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warburg</td>
<td>W</td>
<td>[ Z = \frac{1}{Y_0 \sqrt{i \omega}} ]</td>
<td>(-\pi/4)</td>
</tr>
<tr>
<td>Constant Phase Element (CPE)</td>
<td>Q</td>
<td>[ Z = \frac{1}{(i \omega)^\alpha \cdot Y_0} ]</td>
<td>(\alpha \cdot (-\pi/2))</td>
</tr>
</tbody>
</table>

Phase shifts are relative to the perturbing alternating voltage \( U \).

\[ Z_{\text{tot}} \text{ for the equivalent circuit in Fig. 7.3 can be geometrically constructed step by step based on the complex vector representation, see Fig. 7.4.} \]

Because \( R_1 \) and \( W \) are in series, their combined impedance \( Z_{R1W} \) is the complex addition of their impedances

\[ Z_{R1W} = Z_{R1} + Z_W, \]
Impedance measurements with NO$_2$-sensitive membranes

which corresponds geometrically to a vector addition, compare step $a$ in Fig. 7.4. $C_2$ is in parallel with $R_1$ and $W$, therefore their combined admittance $Y_{R1WC2}$ is the complex sum of their individual admittances (step $b$)

$$Y_{R1WC2} = Y_{R1W} + Y_{C2} = \frac{1}{Z_{R1W}} + \frac{1}{Z_{C2}}. \quad (7.8)$$

Please note that the geometrical counterpart for the algebraic inversion is a reflection at a circle with radius unity; the complex inversion furthermore needs a reflection along the real axis. An example for the geometrical construction of the inversion is shown in Fig. 7.4b.

Fig. 7.4 Geometrical construction of $Z_{tot}$

(a) and (c) show impedance vectors, (b) admittance vectors. Subscripts denote the elements compromised in a vector. Please note that capacitance and the Warburg impedance depend on the frequency, i.e. the $Z_{tot}$-impedance vector has to be recalculated for every frequency. $R_1$ and $C_2$ are shown as non-ideal, i.e. out of their ideal phase angle of 0° and -90°; $R_0$ is ideal.

A geometrical inversion operation is shown with the transformation $Z_{R1W}$ to $Y_{R1W}$ in (b). $A''$ is $A'$ reflected along the real axis and, subsequently, $A'$ is $A$ reflected at the circle with $r = \text{unity}$. Note the construction of the geometrical inversion with the tangent.

$Z_{tot}$

$Z_{R0}$

$Z_{R1W}$

$Z_{C2}$

$Z_{W}$

$Y_{R1W}$

$Y_{C2}$

$A'$

$A''$
The overall impedance $Z_{\text{tot}}$ of the circuit in Fig. 7.3 calculates as the complex addition of the impedances of $R_0$ and the whole parallel circuit

$$Z_{\text{tot}} = Z_{R0} + Z_{R1W}C_2 = Z_{R0} + \frac{1}{Y_{R1W}C_2} = Z_{R0} + \left(\frac{1}{Z_{R1}} + Z_W + \frac{1}{Z_{C2}}\right)^{-1} \quad (7.9)$$

(step c). The construction of the vector $Z_{\text{tot}}$ is completely independent of the nature of the individual circuit elements, but depends only on the way they are interconnected. The simplicity of this algorithm makes modelling of even very complicated equivalent circuits possible. One should, however, be aware that for more complicated circuits we will have different possible topologies with the same frequency response function. A network of resistors and capacitors for instance can always be represented as a sum of parallel RC members (a resistor parallel to a capacitor).

$R_0$ is modelled as a pure Ohmic resistance

$$Z_{R0} = R_0, \quad (7.10)$$

which is intended to reflect the combined resistance of solutions, connections etc. The resistor $R_1$ is modelled as a Constant Phase Element (CPE) with an impedance $R_1$ and a potentially variable exponent $\alpha_{R1}$.

$$Z_{R1} = \lim_{\alpha \to 0} \left(\frac{R_1}{(i\omega)^{\alpha_{R1}}}\right) = R_1. \quad (7.11)$$

This has important implications, because it can make the absolute value of $Z_{R1}$ frequency-dependent. As for all CPEs, the phase change is directly proportional to $\alpha$, i.e. the phase is $\alpha \cdot (-90^\circ)$. The capacitor $C_2$ is constructed as a CPE with an admittance $C_2$ and a potentially variable exponent $\alpha_{C2}$

$$Z_{C2} = \lim_{\alpha \to 1} \left(\frac{1}{(i\omega)^{\alpha_{C2}C_2}}\right) = \frac{1}{i\omega C_2}. \quad (7.12)$$

Note that $1/R_1$ and $C_2$, respectively, replace the general admittance term $Y_0$ in the CPE element in Table 7.1. The Warburg impedance $W$ with the modulus $\Omega$ (not to confuse with $[R] = [\Omega] = [\text{Ohm}]$; in ambiguous situation “Omega” will be used) and an exponent $\zeta = 0.5$, but a constant phase of $-45^\circ$. It is notable that usual Warburg impedances assume a fix $\zeta$.

$$Z_W = \frac{\Omega}{\sqrt{i \cdot \omega^\zeta}} \quad (7.13)$$
Knowing the nature of the individual components, we can easily see what happens at either very low or very high frequencies. At very high frequencies (ideal behavior assumed), $Z_{C2}$ is zero and $Z_w$ is very small. In the admittance plane this means that $Y_{R1WC2}$ is very large with no $Y_{R1}$-contribution. Transformed to the impedance this gives a $Z_{R1WC2}$-vector of practically zero, which means that $Z_{tot} = Z_{R0}$. For very low frequencies $Z_{R1WC2}$ will not be altered by the negligible $Y_{C2}$-contribution and gives a $Z_{tot} = Z_{R0} + Z_{R1} + Z_w$. This corresponds to a line with a -45° phase with an offset ($R_0 + R_1$).

Impedance spectra in Fig. 7.5 have been simulated with typical parameter values for NO$_2$-sensitive polymeric membranes in the frequency range of 1 mHz to (largely theoretical) 1 GHz.

![Nyquist plot](image)

**Nyquist plot**

- $R_1 = 5 \, \text{M}\Omega$, $C_2 = 100 \, \text{pF}$, $\Omega_\text{g} = 1 \, \text{M}\Omega$
- $1: 10 \, \text{mHz}$
- $2: 100 \, \text{Hz}$
- $3: 100 \, \text{Hz}$
- $1: 100 \, \text{Hz}$
- $3: 10 \, \text{mHz}$

**Bode plots**

- $R_1 = 2 \, \text{M}\Omega$
- $C_2 = 500 \, \text{pF}$
- $\Omega_\text{g} = 100 \, \text{k}\Omega$

**Fig. 7.5 Simulated impedance spectra**

Simulation are for typical values of the equivalent circuit shown in Fig. 7.3. Nyquist plots continue outside the graph. The size of $R_1$ can be directly read from both Nyquist and Bode plots.
Fig. 7.5 shows to common impedance data representations, a Nyquist plot \((-\text{Im}(Z) \text{ vs } \text{Re}(Z))\) and the two Bode plots \((\log|Z| \text{ and } \varphi \text{ vs } \log(f))\). Nyquist plots are very popular for impedance spectroscopy, probably because RC members form easily recognizable semi-circles and Warburg impedances a straight line at a 45° angle. Nevertheless, Bode plots convey more information, because they do not compress the data at very high frequencies due to the logarithmic representation of both \(|Z|\) and \(f\). The simulation variations 1-4 clearly show that these are better discernible in the Bode plots than in the Nyquist plots. Apart from the change in resistance \(R_1\) - which can be read directly from the semi-circle’s diameter - simulations 1-3 very much look alike in the Nyquist plot except for the break region at the end of the semi-circle; the information is hidden in the frequency-shifts along largely identical curves. Despite this considerations exclusively Nyquist plots are reported for “backward compatibility”.

The Kramers-Kronig integral equations, which have been originally developed for the field of optics, can be applied to any system described by complex quantities which obeys to linearity, causality and stability. Bode has extended this concept to the electrical impedance and proved that the imaginary part \(\text{Im}(Z(\omega))\) of the impedance spectra can be obtained from the real part \(\text{Re}(Z(\omega))\) with the Kramers-Kronig transformation and vice versa, provided that the high and low-frequency asymptotes (for the integration from 0 to infinity) are known. There is also such a relationship between the modulus \(|Z(\omega)|\) and the phase angle \(\varphi(\omega)\). The two quantities contain in theory the same information, though for the observed frequency range the phase Bode plot for instance definitely contains more information, compare simulations 0 and 3 in Fig. 7.5.

The number of break points in the Bode plot yields the number of time constants of the equivalent circuit; these correspond to the number of discernible arcs in the Nyquist plot. The model in Fig. 7.3 has two time constants, one for the RC member and one for the Warburg impedance and is the simplest model which can describe the data. Please note that \(R_0\) is not needed because it is set to 0, i.e. \(R_0 << R_1\).

It is obvious that the frequency range above 100 kHz contains no additional information and does not need to be recorded. On the other hand, more frequency information would be needed below 1 mHz to better determine low Warburg impedances. The frequency range for the semi-circle does not allow to determine the Warburg impedance, whereas in the straight line part of the Warburg impedance \(C_2\) can no longer be determined, but still \(R_1\). Thus, the high-frequency range can be described by the RC member only, whereas the low-frequency range can be approximated by a Warburg impedance in series with a resistor.

What are the implications of these findings? First of all there is nothing like a set frequency range for impedance measurements; frequencies must always be adapted to the investigated system. Second, low \(R_1\) and Warburg impedances as well as high
C2-values shift the frequency range to lower frequencies, thereby increasing the time needed to record a spectrum. And last, a moderate frequency range may further simplify the model with subsequent information loss of which the experimentator is not aware.

A deviation from ideal behavior, such as the depressed arcs in Nyquist plots, can be described by a symmetrical distribution of relaxation times of orientational polarization processes according to Cole-Cole. It is important to note that it is the distribution of the relaxation times which makes the arc depressed. In an equivalent circuit this behavior is associated with the empirical element CPE which has been used throughout this work.

**7.2.2 Fitting an equivalent circuit to impedance data**

For the very large amount of data generated during timedrive experiments, programs, such as Boukamp’s commonly used EQUIVCRT, proved to be too inflexible. Instead a custom-made procedure in the software package IgorPro was created which seamlessly integrated into the data acquisition. Since many authors still rely on semi-empirical or graphical methods for their impedance data fitting, the implementation of the fitting is given in great detail.

The software uses the Levenberg-Marquardt algorithm to search for the coefficient values that minimize chi-square (see equation (4.16)), which is a sort of a non-linear, least-squares fitting. Most importantly, IgorPro can calculate chi-square, the estimated error (standard deviation) of every coefficient and the covariance matrix.

The implementation of a fitting procedure in IgorPro is demonstrated on the example of the model shown in Fig. 7.3, which was discussed above. The core procedure as it is reported is based on one mentioned by Gilberto Weissmüller [12] and was kindly provided by Klaus Adlkofer.

Programmed in an IgorPro procedure this model may have the following code:

```plaintext
Function /D Fit_ImpedanceData(w,x)
  wave /D w
  variable /D x
  variable /D value, angf
  angf = abs(2*pi*x)
  variable /D/C Z_tot, Z_R0, Z_R1, Z_C2, Z_W

  Z_R0 = cmplx(w[0],0)
  Z_R1 = w[1]*exp(w[2]*ln(cmplx(0, -1/angf)))
```

\[ Z_{C2} = \frac{1}{w[3]} \exp(w[4] \ln(\text{cmplx}(0, -1/\text{angf}))) \]

\[ Z_W = \text{cmplx}(w[9] \cdot \text{angf}^{-w[10]}, -w[9] \cdot \text{angf}^{-w[10]}) \]

\[ Z_{tot} = Z_{R0} + \text{cpowi}(\frac{1}{(Z_{R1} + Z_W) + 1/Z_{C2}}, -1) \]

```plaintext
if (x>0)
    value= log(cabs(Z_tot))
else
    value= atan(imag(Z_tot)/real(Z_tot))
endif
return(value)
End
```

Much of this function is determined by IgorPro conventions. All the functions used for fitting in IgorPro, be it built-in or user-defined, must have the following form

```
Function F(w, x)
    wave w; variable x
    <body of function>
    <return statement>
End
```

where \( w \) denotes the coefficient wave (in this case the elements of the equivalent circuit) and \( x \) the X data (in this case the frequency \( f \)).

The formula of the type \( \exp(p \cdot \ln(z)) \) is an IgorPro workaround for \( z^p \), with the complex base \( z \) and a real exponent \( p \) according to

\[
z^p = (e^{\ln z})^p = e^{p \cdot \ln z}.
\] (7.14)

If \( p \) is an integer, the function \( \text{cpowi}(z, p) \) can be used. The /D flag sets a variable to double precision, the /C flag makes it complex. The function \( \text{cmplx}(\text{real part, imaginary part}) \) is used to assign complex numbers, \( \text{cabs()} \) returns the absolute value of complex numbers, \( \text{real()} \) the real part and \( \text{imag()} \) the imaginary part of a complex number.

Normally, a function can return only one value, but here it has to return two. This is done with the conditional statement at the end which returns either \( \log(Z) \) or \( \theta \), depending on whether the frequencies are positive or negative. This could also be done using a global variable or one of the coefficients. Note that the sign of the frequency has no influence on the calculations, because of the \( \text{abs()} \) statement when calculating \( \text{angf} \), the angular frequency \( \omega = 2\pi f \).
The fact that the function returns $\log(Z)$ is very important, because it implies that $\log(Z)$ is fitted and not $Z$. If this kind of logarithmic weighting was not applied, only the high frequency data points would be fitted.

The coefficients represent: $w[0] = R_0$, $w[1] = R_1$, $w[2] = \alpha_{R1}$, $w[3] = C_2$, $w[4] = \alpha_{C2}$, $w[9] = \Omega/(\sqrt{2})$, $w[10] = \zeta$. Most of this coefficients have a meaningful range, i.e. all phase factors $\alpha$ must be between 0 and 1. An implementation of such a constraint can be

```c
if( w[2] > 1 )
    w[2] = 1
    break
endif

if( w[2] < 0 )
    w[2] = 0
    break
endif
```

The usual reason for a poor fit is that the model is not adequate or that one particular element is not significant.

### 7.2.3 Model for the NO$_2$-sensitive polymeric membranes

Chemical sensors based on reactive compounds dissolved in a polymer matrix have been studied by impedance spectroscopy. To the author’s knowledge, however, no chemical sensor exchanging analytes in the gas phase has been investigated with impedance spectroscopy. Investigations in the gas phase largely concern inert matrices, such as gas sensors based on polymers only. The majority of impedance spectroscopy activities cover corrosion or coating problems or deal more generally with charge transfer in solution, combined with other electroanalytical techniques.

Most literature citations that can be found on plasticized PVC membranes deal with investigation of ion-selective membranes in solution. Xie has investigated in his thesis [13] among others plasticized Valinomycin-PVC membranes. He was mainly interested in the charge-transfer part of the impedance spectrum to obtain information on the apparent exchange current density of potassium versus other ions at the interface membrane/solution, which governs the selectivity of the membrane towards potassium.

Xie [13] uses in his thesis an impedance model for an ion-selective membrane. It applies Fick’s 2nd law within the membrane and an ion exchange at the interface
according to a Butler-Volmer kinetics. It assumes a constant activity coefficient within the membrane, initially no analyte in the membrane, with only a small concentration change at the interface. The resulting equation for the Warburg impedance part is:

\[
|Z_\omega| = \frac{RT}{n^2F^2} \cdot \frac{1}{c_{I(m)}} \cdot \frac{1}{\omega} \cdot D_{I(m)}^{\frac{1}{2}} \cdot \frac{1}{\omega^{\frac{1}{2}}}
\]

(7.15)

\(D_{I(m)}\) and \(c_{I(m)}\) denote the diffusion coefficient and the concentration of the ionic species with the formal charge \(n\) responsible for the conductivity in the membrane, respectively. The phase shift is \(-\pi/4\), independent of the frequency.

Impedance spectra of membranes (PVC/dibutylphthalate 1:2, 5\(\times10^{-3}\) M Valinomycin and 3\(\times10^{-3}\) M sodium tetraphenylborate) in 10\(^{-2}\) M NaCl using symmetrical arrangements showed a full half circle for the charge-transfer part only after 4 hours preconditioning in bidistilled water. The bulk resistance was reduced by the uptake of water, which influences the diffusion coefficient of the charged species in the membrane.

The double logarithmic relation of the charge-transfer resistance on the concentrations of the ion solution - similar to redox reactions on metal electrodes - was well respected. This allowed Xie to extrapolate the charge-transfer resistance to the standard concentration of 1 M, where they are usually too low to be directly measured. A pure PVC membrane showed only a high bulk resistance and a Warburg impedance for 10\(^{-3}\) to 10\(^{-1}\) M solutions of alkali ions, a PVC/dibutylsebacate (1:2) membrane had all three elements, whereas a PVC/dibutylsebacate (1:2) with 3\(\times10^{-3}\) M NaBPh\(_4\) did not have the Warburg impedance line.

The very pronounced reduction of the bulk resistance by addition of the plasticizer was interpreted that not only the membrane conductivity is increased, but that the plasticizer also assumes an ionophore function. Addition of NaBPh\(_4\) increased the apparent exchange current density of 0.1 M solutions by two orders of magnitudes. The presence of the lipophilic BPh\(_4^-\) anion furthermore largely blocked the uptake of other lipophilic anions, such as picrate, and speeded up kinetics.

Van der Weijde has investigated organic barrier coatings with impedance spectroscopy [14]. He had used the model of Brasher and Kingsbury to describe the water uptake of the investigated polymers. The model assumes that water is uniformly distributed within the membrane, which is described as a parallel plate capacitor. The dielectric constant of the water as well as the thickness of the membrane are assumed to be constant when water enters the polymer structure. Van der Weijde had circumvented the problem of the uniform water distribution by slicing up the ca. 100 µm thick membrane into thin layers for which he had calculated the water con-
Impedance measurements with NO2-sensitive membranes

To monitor atmospheric water uptake of the coatings he deposited gold grids on top of the coating and applied the potential between these and the metal substrate. Water did not permeate the 100 nm-thick gold grids. Approximately 100 µm thick two-component epoxy coatings were not saturated even after 8 days. He found that ion uptake speeded up water uptake by a single order of magnitude, as well as water accelerated further water uptake.

He used Henry’s law to model the uptake of atmospheric water into the membrane.

\[ p_a = x_a \cdot K_a \]  

(7.16)

\( p_a \) is the partial pressure of water in the atmosphere (proportional to the relative humidity), \( x_a \) the molar fraction of water in the membrane and \( K_a \) the corresponding Henry’s constant.

How can these informations be integrated into the empirically determined model, i.e. the equivalent circuit in Fig. 7.3? To what processes correspond \( R_0 \), \( R_1 \), \( C_2 \) and the Warburg impedance in the polymer layer on the interdigitated microelectrode?

\( R_0 \) had been introduced in analogy to processes in water, where \( R_0 \) represents the (limited) electrolyte resistance. In our model, \( R_0 \) accounts only for the resistance of connecting cables and can be neglected, at least with respect to \( R_1 \), which is several orders of magnitudes larger.

\( R_1 \) and \( C_2 \) reflect the polymer bulk resistance and capacitance, respectively, as for ion-selective electrodes in solution.

The conductivity of PVC stems from contaminations, such as residues of polymerization catalysts or ageing products, and embedded water which may contain ions, such as \( H_2O^+, Na^+, K^+ \) and \( OH^-, Cl^- \) or \( Br^- \). For the NO2-sensitive polymeric layers the neutral ETH 5418, NI 1 and its counterion perchlorate have been added which will further increase conductivity, and hence decrease the resistance.

The measured conductivity in PVC can be interpreted according to

\[ \sigma = \sum_i q_i \cdot c_i \cdot \mu_i \]  

(7.17)

where \( \sigma \) is the conductivity, \( q \) the charge, \( c \) the concentration and \( \mu \) the mobility of the individual ionic species. A dissociation reaction for instance is expected to create new ionic species, thereby increasing overall conductivity or vice versa for an association reaction.

Water uptake will increase the mobility of ionic species in the plasticized PVC and increase the bulk capacity as in the work of \textit{van der Weijde}. It is unlikely that reactions, such as protonation or complex formation, will considerably affect the capacity.
It is very important to note that the dielectric constant (or permittivity) is not a constant, but depends on the frequency at which it was measured, usually 1 kHz. At very high frequencies the relative dielectric constant approaches unity, because dipoles no longer can follow the oscillations of the electric field. Every dielectric media has a dielectric loss factor $\tan \delta$ which is frequency dependent and indicates the fraction of the ohmic contribution. $\delta$ is the deviation from the expected phase shift of $-\pi/2$, the analogy with the phase factor $\alpha$ is obvious. Polymers have loss factors of $10^{-4}$ to 0.2, which corresponds to phase shifts of 0.006 to 11.3° [15]. This is yet another indication that real systems should be fitted with CPEs.

7.3 Methods and materials

7.3.1 Materials

The membrane preparation was according to Demuth et al. [4]. Apart from ETH 5418 (Fig. 7.2) all chemicals are commercially available. ETH 5418 was synthesized in our group according to [16]. Bis(2-ethylhexyl)sebacate (DOS), poly(vinyl chloride) (PVC) high molecular weight, nitrite ionophore I, tetrahydrofuran (THF, stabilized with 0.025% butylated hydroxytoluene) were all Selectophore quality from Fluka Chemie AG (Buchs, Switzerland). Trifluoroacetic acid was from Fluka (purum). THF was freshly distilled prior to use, all other reagents were used as received.

7.3.2 Sandwich electrodes

A prototype developed by Siemens-Cerberus measured the membrane impedance by sandwiching it between two gold-coated surfaces, one a glass slide, the other a porous ceramic to allow gas diffusion. The frit was pressed against the membrane to assure a good contact. A picture of the set-up is shown in Fig. 7.6.

A series of thin membranes was spin-coated, and a series of thick membranes 0.3 ml of the cocktail was cast in a glass ring with a 23 mm inner bore. The thickness of the spin-coated membranes was approximately 5 µm and for the cast membranes around 0.1 mm.

7.3.3 Interdigitated microelectrodes

For the combined optical and impedance investigation Siemens-Cerberus had designed interdigitated gold microelectrodes on glass. Size and dimensions of the interdigitated gold microelectrodes are given in the image in Fig. 7.7. Versions with a 10 µm, 20 µm and 40 µm width of the electrodes have been photolithographically
produced with a 100 nm thickness of the digits. Close examination under a microscope of the specimen with 20 \( \mu \text{m} \)-digits shown in Fig. 7.7 revealed that probably a third or half of the digits are ruptured somewhere. In some areas the gold seems to fade out whereas in others gold seems to have detached. The interdigitated microelectrodes with even thinner structures (10 \( \mu \text{m} \)) were useless, because the fingers mainly consisted of individual gold islands along the finger, equalling a rupture probably every 20 \( \mu \text{m} \) or worse. The microelectrodes with thicker structures (40 \( \mu \text{m} \)), on the other hand, showed very neatly the intended design, but still featured occasional scratches of approximately 2 \( \mu \text{m} \) in size.

An impedance plot of an uncoated 20 \( \mu \text{m} \)-electrode is shown in Fig. 7.8. The two contact areas were contacted with Signatone S-725-SRM tips as they are used for integrated circuits. In case of normal pressure only a meaningless scatter impedance plot with values in the G\( \Omega \)-range resulted. The quality of the measurement was greatly improved when additional force was applied to the contacting tips. Even in this manner, the impedance plot showed a discontinuity which disappeared after further adjustment of the tips. This emphasizes the necessity of good contacts to the microelectrodes.

The capacitance was 89 \( \pm 2.4 \,\text{pF (} \alpha = 0.97 \text{)} \), the resistance 41.9 \( \pm 1.0 \,\text{M}\Omega (\alpha = 0.065) \) and the Warburg impedance 8.6 \( \pm 0.6 \,\text{M}\Omega \) with an \( \alpha = 0.77 \pm 0.09 \) according to the fit to the equivalent circuit in Fig. 7.3. The origin of the unexpected Warburg impedance remains unclear. A possible cause could be molecular layers of water, which are always found on surfaces. The capacitance at 1 kHz measured at Siemens-Cerberus was 82.8 pF, with a conductance of 19 nS which equals a 52 M\( \Omega \) resistance. Below 0.1 Hz data points started to scatter, probably because the result-
Fig. 7.7 Microscope images of a 20µm-interdigitated microelectrode
Image of an uncoated, uncontacted electrode as produced by Siemens-Cerberus.

Fig. 7.8 Impedance spectrum of an uncoated 20 µm-interdigitated microelectrode
Spectrum was recorded with the Autolab FRA module, signal amplitude was 0.02 Vrms. Frequencies closest to the full decade have been tagged.
ing currents get too small. A low capacitance suggests that many digits are interrupted.

Capacities measured at 1 kHz were $81 \pm 21 \text{ pF}$ for the 40 µm-electrodes and $128 \pm 29 \text{ pF}$ for the 20 µm-electrodes. Resistances from the electrode connector to the end of a digit were typically 1.0-2.0 kΩ for 40 µm-electrodes and 2.2-3.3 kΩ for 20 µm-electrodes.

Silica glass has a relative dielectric constant $\varepsilon_r$ of 3.81 and a volume resistivity of 4-30 GΩ·cm [17]. The capacity of digits of the interdigitated microelectrode can be approximated by one of a linear, infinite conductor

$$C = \frac{2\pi\varepsilon_r\varepsilon_0 l}{\ln d}$$

which can be derived from the conductor’s potential [11]. $l$ denotes the total length of the digits, $d$ the distance between the digits’ centers and $\varepsilon_r$ the relative dielectric constant of the media in-between. The electric field between the two conductors corresponds to a dipole field which decays with $r^{-3}$, i.e. most of the capacity is concentrated very close to the digits. Based on the symmetry with respect to the plane through the digits, the relative dielectric constant

$$\varepsilon_r = \frac{\varepsilon_{\text{glass}} + \varepsilon_{\text{coating}}}{2}$$

is made out of equal contributions from the relative dielectric constants $\varepsilon_{\text{glass}}$ and $\varepsilon_{\text{coating}}$. $\varepsilon_{\text{coating}}$ is the relative dielectric constant of air ($\varepsilon \approx 1$) for the uncovered
electrode and the one of plasticized PVC ($\varepsilon \approx 4-8$ [15]) for the spin-coated electrode. The calculated values are in Table 7.2.

<table>
<thead>
<tr>
<th>Electrode size / µm</th>
<th>no of digits</th>
<th>$l$ / m</th>
<th>$C_{\text{air}}$ / pF</th>
<th>$C_{\text{PVC}}$ / pF</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>350</td>
<td>9.7</td>
<td>121</td>
<td>196-297</td>
</tr>
<tr>
<td>20</td>
<td>180</td>
<td>5.0</td>
<td>66.3</td>
<td>108-163</td>
</tr>
<tr>
<td>40</td>
<td>90</td>
<td>2.5</td>
<td>35.5</td>
<td>58-87</td>
</tr>
</tbody>
</table>

Table 7.2 Calculated capacities for interdigitated microelectrodes
Calculations according to (7.18) with $\varepsilon_{\text{coating}} 4-8$.

Electrode capacities essentially scale with the number of digits. The calculated capacities are mostly lower than the measured capacities, by about a factor of two. This suggests that the model is too simplistic, as expected. On the other hand, measured capacities could be artificially increased by e.g. adsorbed water. Such effects, however, are very difficult to quantitate.

The absorption of the interdigitated microelectrodes shows no qualitative distinction for the finger sizes of 10, 20 and 40 µm. The background spectra correspond to gold absorption. Minimum absorption for all finger sizes is at 515 nm. The significantly smaller absorbance for the 10 µm-electrode is due to incomplete formation of the digits, which let pass more light. There is no evidence for diffraction by the 10 µm-microelectrodes. The orientation of the microelectrode grid (vertical or parallel, i.e. perpendicular or aligned with the rectangular light beam) gives a different absorption, but obviously no systematic deviation. However, it can be expected that the perpendicular alignment is more robust.

The reported spectra were not background-corrected.

The membrane compositions, resistance and capacity of the empty interdigitated microelectrodes are summarized in Table 7.3. The membranes have been prepared by spin-coating 0.3 ml of the cocktail with ca. 140 mg plasticized polymer per ml THF, compare 8.2 Membrane preparation and characterization on p. 235. The membrane thicknesses were approx. 5 µm.

<table>
<thead>
<tr>
<th>Electrode</th>
<th>#</th>
<th>$R$ / MΩ</th>
<th>$C$ / pF</th>
<th>$w$(PVC)</th>
<th>$w$(DOS)</th>
<th>$w$(NI 1)</th>
<th>$w$(ETH 5418)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>F1</td>
<td>55.6</td>
<td>93.1</td>
<td>66.6</td>
<td>33.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>F4</td>
<td>52.6</td>
<td>82.8</td>
<td>ditto</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>F7</td>
<td>62.5</td>
<td>91</td>
<td>66.2</td>
<td>33.0</td>
<td>-</td>
<td>0.7</td>
</tr>
<tr>
<td>20</td>
<td>F11</td>
<td>34.5</td>
<td>137.8</td>
<td>ditto</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7.3 Parameters of the investigated membranes
R and C denote the resistance and capacity of the uncovered electrodes at 1 kHz.
The interdigitated microelectrode was made connectable by a clamp-glued double pin on the backside of the electrode. Contacts were attached to the interdigitated electrode after spin-coating. The contact pins could be plugged into a small adapter cable with a BNC connector on the other end. F29 used an interdigitated microelectrode recycled from earlier experiment, the original membrane was washed off with THF.

F1, F7, F13 and F21 were characterized two weeks after spin-coating. All their spectra show fully equilibrated states. Experiments with F29 were made 10 days after spin-coating. F4, F11, F17, F22 and F27 were measured 6 months after spin-coating. F22 had not been stored in the dark.

### 7.3.4 Measuring set-up

Experiments were carried out both at CCS and at facilities of Siemens-Cerberus in Männedorf, Switzerland. Similar set-ups where used with different absorption and impedance spectrometers as well as NO₂/humidity control systems, which constantly evolved during the experiments.

Relative humidity and temperature measurements at CCS were carried out with the rotronic A2 hygrometer hygrometer (rotronic, Bassersdorf, Switzerland). The sandwich electrodes were placed in a desiccator and allowed to equilibrate for an hour. 1 ml of pure nitrogen dioxide (quality 1.8 from PanGas, Kriens, Switzerland) was spiked with a syringe and its concentration calculated based on the spiked volume and the total volume of the desiccator; humidity was monitored.

Gas mixtures with NO₂ for the measurements at CCS in 7.5.1 Steady-state measurements with NO₂-sensitive membranes on p. 214 were prepared by a system of two mass flow controllers (MKS Instruments, Andover, MA, USA) built by Tomas Nezel. 1.1 ± 10% ppm NO₂ in synthetic air (Carbagas, Zürich) was mixed with synthetic air (quality 5.5, 20.5 ± 0.5 % O₂, <2 ppm H₂O, Sauerstoffwerke Lenzburg)

---

<table>
<thead>
<tr>
<th>Electrode</th>
<th>#</th>
<th>R / MΩ</th>
<th>C / pF</th>
<th>w(PVC)</th>
<th>w(DOS)</th>
<th>w(NI 1)</th>
<th>w(ETH 5418)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>F13</td>
<td>27.8</td>
<td>87.7</td>
<td>65.5</td>
<td>33.0</td>
<td>1.5</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>F17</td>
<td>38.5</td>
<td>81.4</td>
<td>ditto</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>F21</td>
<td>58.8</td>
<td>77.9</td>
<td>65.5</td>
<td>32.5</td>
<td>1.4</td>
<td>0.6</td>
</tr>
<tr>
<td>40</td>
<td>F22</td>
<td>52.6</td>
<td>145</td>
<td>ditto</td>
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<tr>
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<td>F29</td>
<td>45.9</td>
<td>92.0</td>
<td>65.9</td>
<td>32.1</td>
<td>1.4</td>
<td>0.6</td>
</tr>
</tbody>
</table>

**Table 7.3 Parameters of the investigated membranes**

R and C denote the resistance and capacity of the uncovered electrodes at 1 kHz.
which could be humidified, see Fig. 7.10a.

To control humidity the water-free synthetic air line was split up after the mass flow controller. The air of one branch was humidified by guiding it over a small water reservoir, which was subsequently mixed with dry air of the other branch. Tubing clamps allowed to adjust the ratio of the two flows and hence the humidity of the mixed flow. Humidity was measured at the outlet of the flow-through cell.

If nitrogen dioxide was used, the relative humidity was not measured on-line in order not to degrade the probe of the humidity monitor. Therefore, humidity of the air line was allowed to equilibrate at a given flow. The humidity monitor was detached and the nitrogen dioxide gas line switched on. Relative humidity was calculated assuming that the previously measured relative humidity was “diluted” according to the flow ratios, that nitrogen dioxide was initially completely dry (as specified) and that no water-consuming reactions take place. “Dry” denotes the
residual humidity of the synthetic air or pure nitrogen dioxide, i.e. ca. 0%. “Half humid” corresponds to roughly 35% relative humidity (not measured).

The measurements in 7.5.2 Timedrive measurements with NO$_2$-sensitive membranes on p. 220 were made with an improved [18] gas-mixing set-up at CCS, with two additional mass flow controllers which allowed to control humidity, see Fig. 7.10. 10.2 ± 2% ppm NO$_2$ in synthetic air (Carbagas, Zürich) was mixed with a humidified and a dry fraction of nitrogen (quality 5.5, AGA, Zürich) to give a gas flow of 200 ml/min with 0-0.5 ppm NO$_2$ and 0-93% RH. Nitrogen was humidified by guiding the nitrogen stream over the water surface in a 2-neck glass flask. Stainless steel tubing was used throughout, except for the last 10 cm before the flow-through cell, where tubing was made from Teflon. The NO$_2$-line could be disconnected from the gas mixture set-up by a manual valve.

Gas mixtures at Siemens-Cerberus were prepared in a similar fashion with mass flow controllers (Bronkhorst High-Tech B.V., Ruurlo, Netherlands) and were validated regularly by the company. 102 ppm NO$_2$ in synthetic air (PanGas, Kriens, Switzerland) was mixed with humidified synthetic air to give a gas flow of 200 ml/min with 0-1.5 ppm NO$_2$ and 0-90% RH. 2 mm i.d. Teflon tubing had been used for all connections after the mass flow controllers. The system was controlled by a self-written LabVIEW 5.0 program.

For the measurements at CCS conventional absorption flow-through cells have been adapted for the use with the microelectrodes in the Spekol 1100 diode array absorption spectrophotometer (Analytik Jena GmbH, Jena, Germany), see Fig. 7.11a. A cardboard inset held the glass plate with the microelectrode in place, the digits perpendicular to the beam. Please note that only 0.70 of totally 1.96 cm$^2$ of the micro-electrode’s surface was exposed to the gas mixtures. Spectra acquisition with the Spekol 1100 was controlled by a self-written LabVIEW 5.1 program.

Absorption spectra scattering of the Spekol 1100 was corrected according to an empirical function with the help of two support points. Please note that this treatment forces the spectrum through the two support points, usually 400 and 800 nm (isosbestic points of ETH 5418).

Impedance spectra were recorded with the Autolab Frequency Response Analyzer (FRA) in conjunction with the Autolab PGSTAT 20 (Eco Chemie, Netherlands) [19] in the frequency range of 50 kHz to 0.5, or 0.1 Hz, with 0.1 V amplitude. The instrument does not allow cable impedance compensation.

For the measurements at Siemens-Cerberus, the interdigitated microelectrode was held vertically by a clamp at the bottom of a small sample compartment in the Lamda 9 dual-beam absorption spectrophotometer, see Fig. 7.11b. The compartment was air-tight and fed by the gas flow from a connector at its top with an outlet
at the bottom. Recording a complete absorption spectrum in the range 300-860 nm took ca. 170 seconds. Impedance spectra for the range 100 Hz to 100 kHz were recorded with a HP 4274A Multi-Frequency LCR Meter (Hewlett-Packard, Palo Alto, CA, USA) with up to 1 V_{rms} amplitude, cable connections were compensated. A self-written LabVIEW 5.0 program, which controlled the HP 4274A via GPIB, made it possible to acquire impedance spectra at intervals of less than 10 s.

For both set-ups, connections to the flow-through cell after NO$_2$-addition were kept as short as possible, i.e. < 400 mm. Reference for all absorbance measurements was air.

The impedance spectra fitting was done by Wavemetrics IgorPro 3.15’s fitting engine. The obtained fit parameters are always plotted in the same fashion. On the
left side are the absolute values (modulus) of the respective CPE elements plotted as circle, square or triangle markers, on the right side are the corresponding phase vectors plotted as crosses versus relative humidity. The empty markers indicates the absence, filled markers the presence of nitrogen dioxide. The error bars show the standard deviation of the respective parameter as calculated by IgorPro; they were omitted if they were much smaller than the marker size.

### 7.4 Measurements with sandwich electrodes

For the first measurements with sandwich electrodes, capacity alone was measured at a set frequency of 1 kHz. The results suggested a large humidity contribution, but failed to give more information. Above all, there was no way to verify whether the expected reactions in the NO₂-sensitive polymeric membranes had actually taken place.

The 7-month old membranes P11 (0.6 % ETH 5418, 32.6 % PVC and 66.8 % DOS) and P19 (33.4 % PVC and 66.6 % DOS) were measured at approx. 44 % and at 1.0 % relative humidity. The impedance spectra are shown in Fig. 7.12. Three obvious outlier data points are removed.

The results differ from those made 2 months earlier with the HP 4274A. The resistance of P11 had increased by a factor of 2, whereas the impedance of P19 remained
unchanged. It is difficult to say whether the different measurement set-up, the different instrument or handling in-between (e.g. shipping) has caused these changes. Measurements in a desiccator have the big disadvantage that the total nitrogen dioxide concentration cannot be controlled very well. Adsorption of NO\textsubscript{2} to metal surfaces is a major problem for memory effects and reduces the effective nitrogen dioxide concentration.

The proposed sandwiched set-up allows no visual observation of the state of the membrane, which is a major drawback for mechanistic studies. The permeation velocity of the gases through the gold-coated frit is ill-defined and gives rise to a slow response. Thin spin-coated membranes produced a short-circuit and could not be measured in such a device. Thicker membranes tended to thinnen under pressure for about half an hour, thereby changing the geometrical capacity. The use of insulating spacers with a low permittivity could solve this problem.

### 7.5 Measurements with transparent interdigitated microelectrodes

#### 7.5.1 Steady-state measurements with NO\textsubscript{2}-sensitive membranes

In a first step, the magnitude of impedance effects was estimated with measurements of sensible membrane combinations in equilibrated states on 40 \textmu m interdigitated microelectrodes. The membrane compositions included blank, \textit{ETH 5418}, \textit{NI 1} and \textit{NI 1/ETH 5418}, all in PVC/DOS (2:1). All measurements, unless otherwise noted, have been made at CCS.

The absorption spectra of the blank membrane F1 (PVC/DOS alone) showed as expected no change, compare Fig. 7.13. There was no turbidity increase due to the uptake of water.

The membrane with ETH 5418 (F7 in Fig. 7.14) shows probably the most complex behavior. The initial variation of humidity has no effect on the absorbance, ETH 5418 remains deprotonated. With 0.5 ppm NO\textsubscript{2} there is no change within half an hour, but after further 30 min of dry 1 ppm NO\textsubscript{2} the absorption spectrum shows protonation of ETH 5418 due to either prolonged time, or decreased humidity.

ETH 5418 was partly deprotonated within a quarter of an hour when the membrane was purged by dry air without NO\textsubscript{2}. There was no further spectral change for the next two hours. Note that the isosbestic point around 550 nm had considerably shifted, which indicates the formation of a third species.
The membrane with NI 1 (F13, see Fig. 7.15) shows an unexpected behavior, though a judgement is difficult, because of the small absorbance changes. Upon exposure to nitrogen dioxide, absorbance around 370 and 550 nm decreases and does not recover when the membrane is purged by dry air again. There seems to be no humidity effect on the absorption spectra. Upon reaction with NO₂, an increase in the band at 560 nm would be expected, with a decrease below 535 nm. Tomas Nezel has observed similar effects with dry air.

A fully functional membrane with NI 1/ETH 5418 (F21, see Fig. 7.16) shows no deviation from the expected behavior. Upon nitrogen dioxide exposure, ETH 5418 is protonated, i.e. the absorbance band of the protonated form increases and the band of the deprotonated form decreases. The membrane shows only a small sensitivity to humidity.
Sample impedance spectra of F13 are shown in Fig. 7.17, the spectra of membranes with other compositions look qualitatively very much alike. The only exception is the impedance spectrum of F7 at the high relative humidity of 70.7%, see Fig. 7.20. Data points at frequencies below 1 Hz were distorted due to the very small resulting currents. Obvious outliers, mostly at these very low frequencies, are removed.

The model, which suits such spectra best, is the one depicted in Fig. 7.3 with two resistors, one capacitor and a Warburg impedance. The resistor $R_0$ was always orders of magnitude smaller than $R_1$ for the investigated membranes and was therefore set to 0 for fitting. All the other elements are modelled as Constant Phase Elements with the two parameters modulus and phase factor $\alpha$ (or $\xi$ for the Warburg impedance $\Omega$) as explained in 7.2.2 Fitting an equivalent circuit to impedance data on p. 199.
Fig. 7.17 F13 (NI 1 in PVC/DOS) impedance spectra
Tagged frequencies are those closest to the respective frequency decade.

Fig. 7.18 PVC/DOS (F1 & F4) impedance spectra fit
The first data point for F1 hints to hysteresis. $C_2$ for F7 is constant within fitting accuracy.
In the following, fit parameters for every membrane F1, F7, F13 and F21 are plotted against relative humidity. Remember that data was recorded in the *sequence* given in the corresponding absorption spectra. For comparison, humidity characterizations of the membranes with the corresponding identical composition F4, F11, F17 and F22 (all except F22 are 20 µm-interdigitated microelectrodes) measured at Siemens-Cerberus have been added to the respective plot. Note that these measurements were made 6 months after production when the membranes no longer showed an optical response to nitrogen dioxide. The limited frequency range allowed no determination of their Warburg impedance.

![Impedance spectra fit](image)

*Fig. 7.19 ETH 5418 in PVC/DOS (F7 & F11) impedance spectra fit*

Fit data of the impedance spectrum in Fig. 7.20 with the model in Fig. 7.21 is not shown.

Fit parameters for F1 and F4 (blank) are plotted in Fig. 7.18. For the high humidity data point no Warburg impedance contribution could be calculated because the large decrease in R1 had shifted the Warburg part to too low frequencies.

An increase in relative humidity seems to decrease both $\Omega$ and $R_1$. The large increase of the capacity $C_2$ above 35% relative humidity could be explained by a
Impedance measurements with NO₂-sensitive membranes

masking effect of a residual parallel capacity, such as the electrode-inherent capacity, or by an accelerated uptake of water.

F4 shows the same dependence on RH. Note that the Warburg impedance contribution cannot be determined very accurately because of the HP 4274A’s minimal frequency of 100 Hz.

Fig. 7.20 Impedance spectrum of F7 at 71% RH
Note that impedance spectrum fit quality must be judged in the Bode plots over the whole frequency range, not just 2 to 50 Hz where this fit is off. As always, a model with more parameters will give a better fit.

The fit parameters for F7 (ETH 5418) give - like the corresponding absorption spectra - an inconsistent picture. Ω seems again to decrease with increasing relative humidity, but the behavior of the resistance $R_1$ cannot be easily rationalized. The collapse of $R_1$ with the exposure of F7 to dry nitrogen dioxide seems to coincide with the appearance of a new species observed in the absorption spectra (compare Fig. 7.14).

The second measurement of F7 at 70.7% relative humidity gave a completely different impedance spectrum, see Fig. 7.20. A second semi-circle is discernible and overall impedance is much smaller.

Fig. 7.21 Equivalent circuit used for F7 at high RH
Obviously a different model has to be applied, with at least a further time constant. The data was found to reasonably fit the equivalent circuit model in Fig. 7.21, again with $R_0$ set to 0 and $\alpha(R_1) = 0$.

$R_1$ was determined as $221 \pm 22 \, \Omega$, $C_2 = 220 \pm 20 \, \text{nF}$ ($\alpha = 0.66 \pm 0.03$), $R_3 = 219 \pm 25 \, \text{k}\Omega$ ($\alpha = 0.07 \pm 0.01$), $C_4 = 100 \pm 14 \, \text{pF}$ ($\alpha = 0.99 \pm 0.01$) and $\Omega = 370.8 \pm 7.8 \, \text{k}\Omega$ ($\alpha = 0.484 \pm 0.008$). The big increase in bulk capacity with the respective decrease in bulk resistance might be due to a complete soaking with water, but is still unexpectedly high. It must be emphasized that attribution of any physical meaning to the elements of the equivalent circuit without carefully designed control experiments is completely arbitrary.

As for the absorption spectra, F13 (NI 1) gives very neat fitting data (Fig. 7.22). The bulk resistance $R_1$ is slightly decreasing with relative humidity for both with and without nitrogen dioxide. Exposure to nitrogen dioxide obviously induces a sudden drop in the bulk membrane resistance which cannot be observed with relative humidity variations only, at least in this RH-range. The capacities $C_2$ are in the same range as for F7 (ETH 5418), but behave more consistently in that nitrogen dioxide induces a small increase in capacity. Changes, however, remain within the standard deviations for individual $C_2$ fits. $\Omega$ decreases with increasing humidity, with rather small standard deviations. Humidity characterization of F13 after 6 months gave identical values for $R_1$ and slightly smaller values for $C_2$. Trends are reflected in the F17 data, though again, the Warburg impedances could not be determined.

F21 (NI 1/ETH 5418) in Fig. 7.23 yields at first glance essentially the same data as F13. The differentiation between the capacities is not as neat and the first data point for $R_1$ is expected to be lower. Note that F22 had not been stored in the dark and had turned transparent at the time of characterization, i.e. were bleached. The data for F22 shows the expected trend for $R_1$.

Some general trends can be clearly seen. Both $\Omega$ and $R_1$ decrease with increasing humidity, and there seems to be a further decrease during exposure to NO$_2$. In any case, a much broader data basis is needed to confirm the stated dependence of the individual equivalent circuit elements on the relative humidity.

### 7.5.2 Timedrive measurements with NO$_2$-sensitive membranes

The experiments in the previous sections could only show equilibrated states, because changes were much faster than the time needed to acquire an impedance spectrum. Kinetic information, however, is very valuable. We assume other reactions to happen prior to the protonation of ETH 5418, such as a dissociation, solvation or disproportionation of nitrogen dioxide in the membrane, or possibly the
reaction with NI 1. If, for instance, their follow-up reactions are slow, we would expect to see first a change in the impedance and afterwards a change in absorbance. With some carefully designed control experiments this kinetic data would be a big help in mechanism elucidation.

With the HP 4274A, a full impedance spectrum between 100 Hz and 100 kHz can be recorded every 7 s, which sets the temporal resolution of the impedance timedrive. Unfortunately, the Warburg impedance cannot be determined from this frequency range, compare the simulation in Fig. 7.5 on p. 197.

Such a timedrive experiment at Siemens-Cerberus with relative humidity changes and different nitrogen dioxide concentrations is shown for F29 in Fig. 7.24. No absorbance timedrive data is given, because the absorbance barely changed (maximum change at 517 nm was 0.005, no change at 667 nm), very similar to the initial state for F21 in Fig. 7.16 on p. 216. ETH 5418 essentially stayed fully deprotonated. We must conclude that the experiment for F21 could not be reproduced.
No absorbance changes were observed in the NIR-range from 800-3200 nm for relative humidity variations between 0 to 90%. Water has rather intense NIR absorption bands around 5200 cm\(^{-1}\) (~1.9 µm) and below 4000 cm\(^{-1}\) (2.5 µm), with an absorbance close to 1 for a 0.1 mm thick water layer [20]. For an approx. 5 µm thick membrane this means that we could expect a noticeable absorbance change, if the membrane was fully soaked with water. Obviously this was not the case. Nevertheless, this could be a method to quantitate water inside of much thicker membranes to possibly discriminate the impedance effects of water versus others, say nitrogen dioxide.

In the impedance timedrive there is an occasional overshooting of \(R_1\) and \(C_2\) upon media changes. The dependence of the equivalent circuit parameters on relative humidity is given in Fig. 7.25. In contrast to previous experiments (compare Fig. 7.18 on p. 217 and Fig. 7.22 on p. 221), where \(R_1\) had decreased and \(C_2\) increased, successive runs with the same relative humidity gave a higher \(R_1\) and lower \(C_2\). Another way to look at it is that relative humidity and NO\(_2\)-stress make
the membrane more insensitive, an observation shared by Tomas Nezel. If the relation is plotted for conductivity, i.e. \((R_1)^{-1}\), we obtain an exponential growth with a doubling in conductivity every additional 12% RH.

The question was now why the membrane had shown no NO\textsubscript{2}-response. The NO\textsubscript{2}-concentration had been confirmed by a Dräger NO\textsubscript{2}-kit (Drägerwerk, Lübeck, Germany). The function of ETH 5418, for instance, can be easily probed by protonation. When the membrane was quickly exposed to trifluoroacetic acid ETH 5418 got almost fully protonated, compare the spectra in Fig. 7.27. Trifluoroacetic acid is volatile enough (b.p. 71-73°C [21]) that it can be completely removed by evaporation within reasonable time. The timedrive had been recorded in the flow-through cell with a 100 ml/min gas flow at ambient RH = 32%, in order not have any humidity artifacts.

Note that there is also an absorbance increase around 350 nm, where ETH 5418 has an isosbestic point in methanol [5]. This suggests that a reaction with NI 1 occurred, compare also the absorbance of membrane with NI 1 in Fig. 7.15.
Upon protonation, impedance spectra show a large decrease in $R_1$ from 4.9 M$\Omega$ to 2.4 M$\Omega$, see Fig. 7.26. $C_2$ and W were unaffected.

Interesting is that $R_1$ by far does not go back to its original value, but stays around 3.3 M$\Omega$, even though the protonation is reversed by 80%. The changes in $R_1$ directly correlate to the absorbance at 675 nm.

We must conclude that not all the processes involved in protonation are fully reversible, even though TFA most probably had evaporated within 2 hours. Some new ionic species had been created and remained inside the membrane.

When the membrane was shortly gassed with 100 ppm NO$_2$, the resistance went down to 2.9 M$\Omega$. If NO$_2$-induced protonation increased conductivity in a linear fashion as for TFA, we would expect a slightly lower value of 2.75 M$\Omega$, based on the 75% of TFA protonation that was observed for NO$_2$. It is not clear whether NO$_2$ causes the protonation by its reaction with NI 1 as intended or whether this is due to a non-specific formation of nitrous acid in the membrane.

An earlier TFA protonation experiment with the 6 month-old F27 (also NI 1/ETH 5418 in PVC/DOS) at 40% RH without gas flow had decreased $R_1$ from 7.6 to
4.2 MΩ, and Ω from 2 to 0.5 MΩ, leaving $C_2$ unaffected at ~245 pF. Absorption spectra had shown that the same degree of protonation and deprotonation progress within the same time frame had occurred as for F29. In contrast, however, $R_1$ remained stable at 4.2 MΩ, and it was Ω which recovered to 2 MΩ.

### 7.6 Discussion

#### 7.6.1 Experimental issues

Most of the important questions that need to be addressed are very experimental in nature. This is not very astonishing since two different NO₂/humidity controls, flow-through cells, absorption spectrophotometers and impedance spectrometers were used, with both systems continuously evolving and being pushed to their limits.
It is important to note that measurements at CCS or Siemens-Cerberus yielded different impedance data for the very same membrane, especially for the capacity (compare F29 in Fig. 7.24 on p. 223 and Fig. 7.26 on p. 225). This has several reasons.

In experiments with the Spekol 1100, only a third of the whole interdigitated microelectrode was exposed to the media flow, the rest of the membrane being at a somewhat ill-defined state. This could have caused memory effects in the outer membrane reaches, which would explain the hysteresis effects seen in Fig. 7.18 on p. 217 and Fig. 7.22 on p. 221. Furthermore, we expect less of a dynamic range, because less of the membrane was affected by the sample gas changes.

The Autolab system does not allow, in contrast to the HP 4274A, cable capacity compensation, which presumably accounts for the systematically higher capacities obtained with the Autolab system. For this reason we suggest that the capacities determined with the HP 4274A are more accurate.

A third reason is the different frequency range. The Warburg impedance part cannot be determined with the HP 4274A, even though there must have been one for all membranes. At first glance this seems of minor importance, but for low-frequency data the Warburg contribution is compensated to a larger extent by the capacity and to a lesser extent by the resistance. This makes data difficult to compare.

Last but not least, each microelectrode has an inherent capacity and resistance, see Table 7.3 on p. 208. Both elements must be considered in parallel with those of the NO₂-sensor membrane model in Fig. 7.3 on p. 194. The microelectrode capacity of typically 90 pF is largely created by the glass support with \( \varepsilon = 4 \), compare the calculated capacity on p. 207. Thus, approximately half of the measured total capacity is invariant background of the microelectrode. The influence of the parallel microelectrode resistance of typically 50 MΩ is less critical, because measured resistances

![Fig. 7.27 Absorption spectra during protonation experiments with F29](image-url)
are only about 10% lower than without this contribution. Note that resistance and capacity values determined at 1 kHz (as given in Table 7.3 on p. 208) cannot be directly compared to those obtained from impedance spectra fits.

Experiments, which combine several spectroscopic techniques, are very demanding on infrastructure. In this case, without automation of media control and spectra acquisition, the time resolution of 7 s could never be gotten even close to. Unfortunately, either combination at CCS or at Siemens-Cerberus was not ideal. The HP 4274A had the needed speed and stability, but lacked the frequency range of the Autolab FRA module. On the other hand, the Spekol 1100 had the needed speed, but lacked stability and the spectral range of the Lamda 9.

The next step was to automate the data treatment and fitting, otherwise the thousands of impedance spectra generated in one single experiment cannot be handled.

Another issue, which must be stressed, is proper gas handling. Nitrogen dioxide quickly adsorbs to stainless steel, causing memory effects for subsequent experiments. Furthermore, the addition of NO₂ to humidified air must take place as close as possible to the flow-through cell, otherwise the effective concentration is greatly diminished.

It is not clear whether the impedance results have been affected by the quality of the interdigitated microelectrodes. Apart from the PVC/DOS membrane pair (F1/F4), the 20 µm-electrodes gave consistently lower capacities and higher resistances than the 40 µm-electrodes, though the opposite would be expected (see p. 208). This can be partly explained by membrane ageing, but the main cause are probably the many interrupted digits for the 20 µm-electrodes, as observed in optical microscopy, compare Fig. 7.7 on p. 206.

### 7.6.2 NO₂-sensitive polymeric membranes

Unclear is why the known sensor response of NI 1/ETH 5418 membrane could not be reproduced for the timedrive (or other) experiments. 

*Tomas Nezel* has identified possible influences, above all the humidity, nitrogen dioxide and temperature history, and the age of membranes. Possible explanations at this point would be pure speculation. A thorough discussion of these issues will be given in the thesis of *Tomas Nezel*.

An important consideration is that in contrast to the earlier measurement set-up as in Fig. 7.10a, where the membranes showed an optical NO₂-response, no really dry gas mixture could be produced with the set-up in Fig. 7.10b (i.e. RH < 5%).

Protonation experiments with volatile TFA have shown that ETH 5418 in the NI 1/ETH 5418 in PVC/DOS membrane can still be protonated and deprotonated, i.e. still assumes its designated transducer function. A non-complete recovery of either high initial resistance or Warburg impedance suggests that a reaction on a much
Discussion

longer time scale, possibly irreversible, has taken place in the membrane. Short gassing with the very high NO₂ concentration of 100 ppm shows that the membrane has lost mainly in sensitivity, though reactions at such large concentrations could be purely pH-induced by disproportionation of NO₂ with water.

If it was not ETH 5418 who failed, attention should focus on the other compound, NI 1. From the experiments, no conclusion can be drawn with respect to this point.

The design of the interdigitated microelectrode with the membrane on top does not allow to monitor processes at the membrane-air boundary, because there is no electrical field component perpendicular to the boundary. This means that only bulk processes or processes at the electrode/membrane interface are observed. The three elements capacitor, resistor and Warburg impedance are therefore most sensibly attributed with the membrane permittivity, resistance and diffusion of charged species.

All equivalent circuit elements show a dependence on both humidity and nitrogen dioxide. The most interesting one is probably the Warburg impedance. Ω-values are consistently smaller in the presence of NO₂ than in the absence for a given relative humidity. If this reflects the diffusion behavior of newly created ionic species, such as protonated ETH 5418 or nitrate, is pure speculation, but tempting. It is not clear whether Ω can still be determined at high relative humidities due to its small value is another issue.

Resistance shows a similar decrease as the Warburg impedance upon NO₂ exposure, whereas not much can be said with certainty for the capacitance.

There is little change in the equivalent circuit parameters if R₁ and C₂ were modelled ideally, i.e. with α₁ = 0 and α₂ = 1. Fitting typically yielded 0 < α₁ < 0.05 and 0.95 < α₂ < 1.00, i.e. reflected almost ideal behavior.

A glance at the impedance timedrive of F29 (Fig. 7.24 on p. 223) shows that the phase factor α and modulus are highly correlated. We have more ideal behavior of capacities at low relative humidities and more ideal behavior of resistances at high humidities. Stretching the logic of relaxation time distributions outlined on p. 199, the phase factors would yield a measure for the isotropy of the respective membrane parameters.

In any case humidity is the dominant cause for changes in all three equivalent circuit elements. There is an accelerated increase in capacitance and decrease in resistance for all membranes at relative humidities above 50%. Capacity changes for the observed NO₂-sensitive polymeric membranes in the range of 0-50% RH are very small (< 2 pF). This suggests that water uptake at high relative humidities is overproportional, that is, Henry’s law does not hold. In case of F7 the bulk resistance “collapsed” at 71% RH, compare Fig. 7.20. Membranes equilibrate within minutes at moderate RH, but may need up to half an hour for saturation at very high relative humidity values. Note that humidifying of measuring media also takes much longer
at high RH.
Absorption spectra show no or only very minor effects on humidity changes; these “invisible changes”, however, can be closely monitored by impedance spectroscopy. The overshooting in the impedance timedrive and hysteresis effects suggest that after all, there is some process happening in the membrane which obviously does not affect the absorption spectrum. Chromoionophores derived from Nile Blue, such as ETH 5418, are known to have solubility problems in plasticized PVC membranes [5]. Possibly humidity changes force a redissolution or precipitation of membrane compounds.

Note that most reported chemical sensors based on non-specific adsorption into polymers use capacity information only. For the NO$_2$-sensitive polymeric membranes, this is definitely not the optimal measuring mode.

If we take resistances and capacities values extrapolated to 0% RH for the membranes on the 40 µm-electrodes in 7.5.1 on p. 214, we obtain for the resistances

- blank (40 MΩ) >> NI 1 (9 MΩ) > ETH 5418 ≈ NI 1/ETH 5418 (~6 MΩ)

and for the capacities

- blank (40 pF) << NI 1, ETH 5418 and NI 1/ETH 5418 (120 pF).

The membranes are thick enough with respect to the digit’s size, so that differences in membrane thickness will not matter for the resistance. The larger resistance of the membranes with NI 1 compared to those with ETH 5418 is astonishing. We expect a smaller conductivity of ETH 5418 in its deprotonated, neutral state, than the one arising from the perchlorate counterion of NI 1. The data basis does not allow a definite conclusion.

It is very interesting that Golonka et al. have found the same relative humidity behavior for ceramics. They had investigated a planar thick-film sensor for relative humidity based on ZnCr$_2$O$_4$-TiO$_2$ ceramics probed by a platinum interdigitated microelectrode [22]. For their high relative humidity (RH > 90%) data, which look similar like the one in Fig. 7.20, except that the second semi-circle was more of a straight line, they had used a model with a total of 8 elements. They found that log($R$) was proportional to RH over the whole humidity range.

For the ETH 5418/NI 1 in PVC/DOS membranes we have found an slightly different behavior with log($R_1$) versus RH being downward-curved, which corresponds to an exponential increase in conductivity. Thus, for the NO$_2$-sensitive polymeric membrane, it is not the water alone, but the increased mobility of ionic species which increases conductivity in the membrane.

The stipulated mechanism by Nezel et al. of the N$_2$O$_4$ disproportionation to nitrite inside the membrane (see p. 191) has several consequences. First, the reaction
needs water to take place. Therefore, it should depend on relative humidity. Second, the stochiometry of the reaction changes. Rather than one chromoionophore per NI 1 there should be two, if all liberated protons are abstracted by chromoionophores. Tomas Nezel found no difference for the 1:1 or the 1:2 stochiometry NI 1:ETH 5418.

How much water approximately is taken up by the membrane? With the model of Brasher and Kingsbury we presume that water has a permittivity contribution proportional to its volume concentration with \( \varepsilon_{\text{water}} = 78.5 \) (at 25°C, [17]). If we assume that all membranes have equal permittivity contributions from glass and polymeric membrane, and membranes have a \( \varepsilon_r \)-range of 4-8 we obtain the values given in Table 7.4.

<table>
<thead>
<tr>
<th>RH/ %</th>
<th>( \Delta V/V (H_2O) )/ %</th>
<th>( \Delta C/C^a )/ %</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>0.3-0.6</td>
<td>2-3</td>
</tr>
<tr>
<td>80</td>
<td>3-15</td>
<td>25-150</td>
</tr>
<tr>
<td>coordinated water in NI 1</td>
<td>0.08(^b)</td>
<td>0.03-0.07</td>
</tr>
</tbody>
</table>

**Table 7.4 Water content in membrane calculated from capacity**

\( \varepsilon_r \)-range of 4-8 for all membranes and equal contribution of glass and polymeric membrane for overall permittivity.

- a. capacity change of whole interdigitated microelectrode
- b. based on calculations for NI 1 concentrations in [1]

We realize that the water content in the membrane at RH < 10% is roughly equimolar with respect to the reactive compounds, e.g. NI 1 with its coordinated water. For lower RH-values, a change in the reaction mechanism becomes very likely because of the scarcity of water. At very high relative humidities, there must be a considerable water content of well above several percent in the plasticized PVC.

A similar dissociation mechanism is perceivable for the orders of magnitude larger concentration of carbon dioxide. However, nitric acid (\( pK_a = -1.32 \) [23]) is much more acidic than carbonic acid (\( pK_a = 6.37/10.25 \) at 25°C [17]) which has a \( pK_a \) in the range of ETH 5418. Furthermore, there is no selective carbonate or hydrogen carbonate binding with any of the membrane components, which explains why no CO\(_2\)-interference has been found by Nezel et al. Sulfurous acid, on the other hand, is more acidic (\( pK_a = 1.81/6.91 \) at 18°C [17]), which would explain the slight SO\(_2\)-interference. Very acidic compounds, such as trifluoroacetic acid, trigger immediate protonation. Note that volatile electrolytes also diffuse much faster in hydrophobic polymers, though even nitric acid has an appreciable diffusion rate in PVC [7].

Impedance measurements would allow to quantitate the effects upon CO\(_2\) or SO\(_2\) dissolution and compare them with the NO\(_2\)-effects. Furthermore, the expected
dependence of the NO\textsubscript{2}-response on relative humidity variations could be examined.

Based on the available data, we suggest that NI 1 in a PVC/DOS membrane seems to be most promising for an exclusive impedance transduction. It featured the best separation of humidity and nitrogen dioxide effects without using the chromoionophore ETH 5418. Reversibility, however, is worse than with the NI 1/ETH 5418 combination and a transduction would only be possible in a limited RH-range.

### 7.7 Summary and Perspectives

A NO\textsubscript{2}-selective polymeric membrane has been characterized by absorption and impedance spectroscopy in the gas phase. The sensor membrane is based on a aquacyanocobalt(III)-cobyrinate derivative nitrite ionophore 1, which reacts selectively with nitrite. This reaction coincides with a protonation of the Nile Blue derivative ETH 5418, whose change in absorbance around 665 nm is observed. Earlier work in our group by Nezel \emph{et al.} demonstrated that a detection limit of 50 ppb can be achieved [1].

In this thesis, the first combined simultaneous impedance/absorbance measurements of reactive compounds in a plasticized polymer in the gas phase are described. Measurements were carried out with transparent interdigitated microelectrodes with digit widths of 20 and 40 µm. A blank poly(vinyl chloride) (PVC)/bis(2-ethylhexyl) sebacate (DOS) membrane and membranes containing the nitrite ionophore 1 and/or ETH 5418 based on [4] were investigated at varying relative humidities and NO\textsubscript{2} concentrations.

Impedance spectra in the range of 100 kHz to 0.1 Hz can be best represented by an equivalent circuit model with a resistor and a Warburg impedance in parallel with a capacity. The model can cope up to approx. 60% relative humidity, above which a complex model with additional time constants must be chosen. The three parameters have been attributed to bulk membrane properties. Typical values are 1-40 M\textOmega for the resistance, 40 to 140 pF for the capacitance and 0 to 10 M\textOmega for the Warburg impedance. In timedrive measurements, it was possible to achieve a time resolution of up to 7 s for the combined impedance/absorbance measurements.

Whereas absorption spectra show little to no relative humidity effect, the effect can be well monitored by impedance spectroscopy. There is a decrease in both membrane resistance and the Warburg impedance as well as an increase in capacitance with increasing relative humidity. The approx. 5 µm thick membranes equilibrate within minutes or, at most, half an hour with any environmental humidity. Water uptake above 50 to 60% relative humidity is greatly enhanced. The water content in the membrane was estimated to be below 1% (v/v) at ambient humidity up to 50%
relative humidity and around 10% for relative humidities close to 100%.
There is evidence that the NO$_2$-response of the membrane leads to a decrease in both Warburg impedance and resistance, with little effect on the membrane capacitance. No systematic investigation was possible, however, because the membrane response could not be reproduced. Protonation of ETH 5418 by trifluoroacetic acid or the effects of large concentrations of nitrogen dioxide can be easily correlated with decreasing resistance.

There are indications in membrane impedance spectra that relative humidity changes or protonation as a consequence of NO$_2$ exposure are not fully reversible.

Combined impedance/absorbance measurements can be a great help in elucidating the mechanisms of gas phase sensors based on reactive compounds in polymeric membranes. Impedance spectroscopy is well suited to monitor relative humidity changes or the creation of ionic species which are usually not accessible with absorption spectroscopy.

### 7.8 References


8 Appendix

8.1 Materials

Materials which have been used in the chapters 6 and 7 are listed therein. Chemicals which have been used in the other chapters are:

[Ru(dpp)$_3$](ClO$_4$)$_2$ had been synthesized by Daniel Freiner according to a synthesis kindly provided by Peter Belser, ETH$^T$ 3001 by Stefan Rásonyi, ETH$^T$ 3003 by Luzi Jenny at CCS, see 2.2 Synthesis of ETH$^T$ 3001 and 3003 on p. 11. Rhodamine 101 (Biochemika) and quinine sulfate dihydrate (purum) were both from Fluka (Buchs, Switzerland).

o-CPOE has been synthesized by Gerhard Mohr according to [1]; in the meantime it has become commercially available. 2-Nitrophenyl octyl ether (o-NPOE) was from Fluka (Selectophore). Polystyrene ($M_w$ 280’000) was from Aldrich (Gillingham, Dorset, UK), poly($\alpha$-methylstyrene), poly($p$-tert-butylstyrene), poly(4-methoxyxystrene), poly(2,4,6,-tribromostyrene), poly(2,6-dimethyl-$p$-phenylene oxide) and poly(bisphenol-A-carbonate) were from Scientific Polymer Products (Ontario, NY, USA), see Table 4.1 on p. 82 and Table 4.2 on p. 84.

Chloroform was from Riedel-de Haën (for analysis, stabilized with 1% ethanol), ethanol from Merck (absolute, pro analysis). Sulfuric acid (puriss p.a.), sodium sulfite were from Fluka (MicroSelect).

8.2 Membrane preparation and characterization

Cocktails were prepared in sealable 4 ml flasks with polyethylene (PE) heads. Compounds (150 to 200 mg in total) were weighed into the flasks on a AT 261 balance (Mettler-Toledo, Greifensee, Switzerland) with ±0.01 mg precision, which were then filled up to 2 ml solution volume with chloroform. All compositions given in percent are weight per weight. The cocktails were shaken on a IKA-Vibramax-VXR (IKA-Labortechnik, Staufen, Germany) until all components were dissolved. The usual method for membrane preparation was spin-coating with the device depicted in Fig. 8.1.

The glass wafer was placed on a custom-made holder mounted on the rotor within a metal receptacle. Insets as well as other holders make it possible to spin-coat most small-sized flat surfaces. Pure solvent was given into the receptacle which is largely closed off with a transparent lid to maintain a solvent-saturated atmosphere. This results in a smoother evaporation, leading to more regular membranes. Within 1 to 2 seconds, 0.3 ml of the air bubble-free cocktail were ejected from a 1 ml metal/
glass syringe placed approx. 5 mm above the center of the rotating glass wafer. The initial rotation speed was ca. 370 rpm, which was increased to ca. 980 rpm after several seconds. The backside of the glass wafers was always cleaned with the cocktail solvent to remove possible residues.

Changing the amount of cocktail had little effect on the thickness, i.e. the measured absorbance. A 2 ml-cocktail of 1.2% ETH T 3001 in 80.3% polystyrene/18.5% o-CPE dissolved in chloroform gave absorbances which followed the relation

\[ A = (0.126 \pm 0.007) - (0.009 \pm 0.004) \cdot (V_{\text{cocktail}}/\text{ml}) \quad (8.1) \]

for 6 cocktail volumes between 0.05 and 3.0 ml.

The exact figures are not that important, but two messages are clearly conveyed. The sensitivity to volume errors is rather small and - surprisingly - more cocktail actually means thinner membranes. Changing rotation speeds can help, but below ca. 270 rpm no complete coverage is guaranteed.

Membranes were spin-coated on glass wafers with a diameter of 35 mm and thickness of 2 mm. The glass wafers were manufactured by Bruno Nussberger of the Glasbläserei Hönggerberg, ETH Zürich. Effective thicknesses were 1.95 for quartz, 2.1 for the greenish and 1.8 mm for the whitish soft glass. The refractive index of the quartz glass is 1.45851 at \( \lambda = 589.6 \) nm and 1.45646 at \( \lambda = 656.27 \) nm, those for the soft glass are given as 1.513 to 1.523 in the wavelength range of 546 to 443 nm [2].
The membrane thickness has been measured with a TENCOR alpha-step 200 profilometer (San Jose, CA, USA) at the Laboratorium für Technische Chemie, ETH Zürich. Particularly plasticized membranes are difficult to measure because the stylus may penetrate the membrane, even with the minimum stylus weight of 2 mg applied. Measured thicknesses were 3.5 µm for a very soft membrane (39% o-NPOE), 5 to 6 µm for moderately plasticized polystyrene (plasticizer content < 17%) and 7.5 to 8.5 µm if the cocktail was standing for a while prior to use (solvent evaporation leads to more viscous cocktails). Refractive index measurements were carried out with a PLASMOS SD 2300 ellipsometer (Germany).

Membranes and cocktails were always stored in the dark when not used.

### 8.3 Instruments

#### 8.3.1 Luminescence spectrophotometer PE LS-50B

The luminescence spectrophotometer LS-50B from Perkin Elmer (Beaconsfield, UK) is a versatile instrument for routine use, though with certain limits regarding research use.

![Scheme of luminescence spectrophotometer PE LS-50B](image)

*Fig. 8.2 Scheme of luminescence spectrophotometer PE LS-50B*

The illustration is reprinted from the PE LS-50B manual.

The PE LS-50B uses a xenon flash lamp which has fairly high light intensities down to 300 nm. 230 nm is the practical lower limit of the spectral range although in the-
ory, measurements down to 200 nm should be possible. After passing the monochromator and excitation slit, a small part of the light beam is reflected onto a photomultiplier of the same type as the detector. This is done to internally reference the luminescence signal, which would otherwise vary due to different flash intensities, temperature and electronical effects (high voltage supply, analog-digital-converter). Experience shows that this works very well and makes signal intensities comparable within a few percent accuracy, even if the measurements were made several months apart. The spectrometer uses a Hamamatsu R-928 red-sensitive photomultiplier which extends the upper limit for measurements to about 850 nm. The specified wavelength accuracy is ±1 nm, wavelength reproducibility is ±0.5 nm.

There are variety of different bandwidth settings possible. Bandwidths for the excitation monochromator range from 2.5 to 15 nm and for the emission monochromator from 2.5 to 20 nm. The excitation bandwidth dictates signal intensity by a preset photomultiplier voltage.

The spectrometer has a built-in validation routine which can asses the S/N-ratio based on raman bands at 350 and 551 nm in a sealed water cuvette. Values obtained were usually around 440:1.

The instrument was either controlled by the commercial software FL Winlab or by a self-written LabVIEW 5.1 program, which fully controls the PE LS-50B via the serial interface.

Standard settings for all experiments were: excitation slit width of 2.5 nm, an emission slit width of 5 nm and an excitation wavelength of 460 nm. The emission wavelength was 620 nm with the 515 nm cut-off filter in place. If not otherwise noted, these settings applied.

All cuvettes used were 10 x 10 mm Suprasil™ cuvettes (Hellma, Müllheim, Germany) for luminescence spectroscopy if not otherwise mentioned.

Luminescence measurements on membranes were done in a usual way for solid samples with a 30°/60° excitation/emission geometry which directs reflected excitation light away from the emission monochromator. The membranes, which were cast on glass wafers, were positioned in self-built flow-through cells (third generation cell, compare Fig. 8.9 on p. 244). The flow-through cells could be securely placed with a snap-in mechanism. The membrane was facing the sample channel, excitation and emission light have to penetrate the glass support in order to reach the membrane. The set-up is depicted in Fig. 8.3.

Sychronous scans, i.e. (emission wavelength - excitation wavelength) = const, are very useful for the identification of different fluorescent species in a mixture due to their excitation/emission overlap bands. This function is implemented, but stray light levels were far too high as to give any useful spectra.
A gated photomultiplier can measure phosphorescence or chemiluminescence decay times in the range of 0.01 to 500 ms. The PE LS-50B cannot be used to determine lifetimes in the µs-range of the ruthenium(II) diimine complexes.

### 8.3.2 Life time measurements

All life time measurements were carried out by Paul Hartmann in April 1999 at AVL List GmbH in Graz, Austria. He performed the decay measurements with a PRA LN102 dye laser, pumped by a PRA LN103 nitrogen laser, and a fast PMT (Valvo 56 TUVP with 2-ns rise time), coupled to a Tektronix DSA 601A sampling oscilloscope according to [3]. The geometry of the decay measurements involved a 30° angle of incidence for the exciting laser pulse (\(\lambda_{\text{exc}} = 470\ \text{nm},\ \text{pulse width} \ t = 300\ \text{ps}\)) respective to the sample plane and a 90° angle between exciting pulse and emission optics. Emission was monitored through a Schott KV550 longpass filter and several Schott NG neutral filters. Measurements were performed at 25°C.

### 8.3.3 Self-referenced two-photodiode set-up

Principles for an ideal set-up which had been found in the book *Optoelectronics* [4] have been adapted with the help of Peter Eberhardt, electronics specialist at the Institut für Teilchenphysik, ETH Zürich. The idea was to reference the luminescence signal photodiode with an identical second photodiode which measures the excitation light from the LED, using the same circuitry. Much more elaborated set-ups have been described in the literature [5]. The ratio of the luminescence signal to the reference signal should eliminate LED fluctuations, temperature and electronical drifts. Fig. 8.4 shows the circuit which resulted.

Core of the circuit is a dual MOS-FET operational amplifier and the two photodiodes which are operated with a negative bias of -5 V. The bias voltage has been
dimensioned as small as possible because the dark current is directly proportional to it, whereas the photo-generated current is (in theory) independent of it. The positive voltage signal \( U_{\text{signal}} \) generated is

\[
U_{\text{signal}} = R_f \times I_{\text{photo}},
\]

i.e. directly proportional to the photodiode current, which in turn is proportional to the incident light intensity. The gain of the current/voltage conversion/amplification is in first approximation determined by the size of the feedback resistor \( R_f \). The feedback resistor together with a capacitor in parallel forms a low pass filter which suppresses high frequency contributions (< 25 Hz) in the signal.

A dual operational amplifier in the same integrated circuit is used, which minimizes fabrication-dependent and operational differences, such as supply voltage and temperature. An operational amplifier in MOS-FET technology is preferable to one in CMOS technology because there is no power dissipation, hence no heating of the IC, and only very little current drain (picoampères). The disadvantage of a larger off-set drift is more than compensated by the far larger thermic drift of the photodiodes and their dark current. Professional literature suggests further measures, e.g. a resistor tee rather than a single feedback resistor, which might further improve overall performance [6].

---

**Fig. 8.4 Schematic of self-referencing two photodiode set-up and voltage-stabilized LED power supply**

R1 and R2 correspond to the feedback resistor \( R_f \).
The conversion of -12 V to -5 V was necessary to provide a -5 V supply voltage with the existing +/-12 V/+5 V power supply, but adds no further functionality.

The LED voltage-stabilized power-supply in the bottom part of Fig. 8.4 is a design by Leo Weissberg, only the resistor R4 has been adapted to the different LED current.

A suitable photodiode was chosen from Siemens in cooperation with Ramon Rotiroti, the product manager of Siemens Schweiz for photodetectors, -transistors and -diodes. Eventually the photodiode BPW 34 S was chosen, due to the following criteria:

- high sensitivity around 650 nm (ca. 0.68 electrons per photon, 0.61 A/W)
- large surface area (2.65 x 2.65 mm)
- low dark current (ca. 1 nA at 5 V and 20°C)

The sensitivity characteristics for the photodiode BPW 34 S are given in Fig. 8.5. Feedback resistor values were chosen as 220 kΩ for the reference and 22 MΩ for the luminescence photodiode. The former was chosen to obtain a signal of half the operational amplifier’s saturation voltage, whereas the latter was as big as necessary to obtain a signal which could be reasonably measured with the HP 34420A Nanovoltmeter.

![Relative spectral sensitivity](image1)
![Photocurrent](image2)
Signal hubs were up to 35 mV between nitrogen and oxygen at signal levels of 140 to 260 mV. The signal noise was 0.04 to 0.06 mV, sometimes up to 0.1 mV. This gives at best a S/N-ratio of 875. High noise was always correlated with activity in the laboratory, due to accompanying electromagnetic and mechanical disturbances of the experimental set-up which could be deliberately instated.

Obviously the optical set-up is far from perfect. Adding light-collecting lenses or prisms for light collection together with a more careful, stable spatial arrangement of the LED and the photodiodes could greatly improve the design. Since the original aim of long-term stability measurements was thwarted by photobleaching and in view of far superior commercial instruments, the improvements were not pursued.
8.4 Flow-through cells

8.4.1 Flow-through cell design

The flow-through cells for the PE LS-50B luminescence spectrophotometer used in our group up to 1997 remained unsatisfactory mainly due to their large lumen volumes (110 rsp. 900 µl) which precluded the measurement of fast response times in liquids. A new cell was designed according to the following empirically derived guidelines:

- minimal volume of lumen
- constant cross-section along the flow-channel
- smooth geometry of the flow channel

The first criteria enables a fast exchange of the cell lumen and hence a quick response to concentration changes. Caspar Demuth had identified this as the exchange-determining step for the older flow-through cells. The two other criteria ensure that further dead volumes are avoided and that the complete lumen gets exchanged the quickest possible.

Earlier generations of flow-through cells (see Fig. 8.9) had a considerably large lumen, whereas their throughput was actually limited by the inner bore (φ 1.5 mm) of the channel ducts to either side of the cell. Hence it was decided to keep the channel cross-section close to the constant value of this bottleneck which corresponds to a cross-section of approximately 1.5 to 2 mm². A schematic drawing of the new cell is shown in Fig. 8.10. Three cells have been manufactured by the Workshop of Technische Chemie, ETH Zürich.

The cell is made from polypropylene (PP) which is very resistant to a wide range of chemicals. The o-ring (1 mm φ) around the flow-through section was custom-made.

Fig. 8.8 Characteristics of optical system
from silicon because of its unusual shape. The silicon material of the cell even withstands organic solvents, such as THF.

### 8.4.2 Flow-through cell performance

A very important parameter for a flow-through cell is the speed, with which the liquid or gas in the lumen gets exchanged. This can be measured by fast switching between a fluorescent (e.g. 10⁻³ M solution of quinine sulfate in 0.1 N sulfuric acid) and a non-fluorescent solution (water). The speed with which the full luminescence intensity level (i.e. cell completely filled with fluorescent solution) is established, is a measure for the speed of the liquid exchange. Another possibility are pH-sensitive optodes which have already been successfully applied to rapid flow-through analysis [7].

Typical response times were around 11s for a 95%-change either way. The signal-to-noise (S/N) ratio of the quinine solution was approximately 225:1 at linear velocities of 2 cm/s. Without pumping an about three times lower S/N ratio would be obtained. This suggests that flow instabilities degrade the S/N ratio. Pumping velocity changes (2 to 20 cm/s) had no influence on signal level, signal stability or response time (at these high flow rates). Large air bubbles can reside for several minutes within the cell before they are eventually carried away by the liquid flow. Switching from gas to liquid causes similar instabilities.

This experiment contrasted to the visual observation when switching between dyed/colorless solutions. Liquid exchange seemed to take place almost instantaneously. If the cell was in a vertical position (as used in PE LS-50B) with a bottom-up flow, only very small air bubbles - if any - were observed at the rectangular channel sec-
tion where the actual measurement takes place. Large air bubbles tended to get stuck in the trapezoidal channel below the thin-layer section until they were suddenly purged by the flow. This mechanism was not as efficient in the case of a top-down flow, which made a bottom-up flow the direction of choice.

In the case of gaseous media the flow-through cell is no longer time-limiting. Sample rates of at least 10 Hz are needed to accurately evaluate response times for gases. This is too fast for most commercial luminescence spectrophotometer, including the PE LS-50 B. With the help of a self-built set-up similar to the one in 8.3.3 Self-referenced two-photodiode set-up on p. 239 and manual gas switching this can be easily done. Fig. 8.11 shows an example of a response curve of a membrane with ETH$^T$ 3001 in polystyrene/o-CPOE (1.2%, 80.2% and 18.5% respectively).

The preparation of variable oxygen content mixtures with the set-up described 8.5.1 Media handling on p. 246 has considerably longer settling times than the
8.5 Oxygen measurement set-up

8.5.1 Media handling

A schematic with the whole media handling system (both for gas and oxygenated water) is given in Fig. 8.12, a picture is shown in Fig. 8.13. The whole set-up including mass flow controllers, valves, peristaltic pumps and level switches was controlled by a self-written LabVIEW 5.1 program.

Copper and stainless steel tubing has been used up to the flow-through cell. The system was supplied by 50 l gas bottles with nitrogen 5.5 and oxygen 5.5 at 200 bar (both from AGA, Zürich). Pressurized air from the house supply system cannot be accurately regulated and has only been used to air-saturate water.

For gas mixing two mass flow controllers of Type 1179 from MKS Instruments (Andover, MA, USA) were used [8]. The total volume flow for nitrogen was 200 ml/min which amounts to sufficiently high linear flow velocities of above 25 cm/s in the 2 mm inner diameter tubing. The mass flow controllers can be operated from 0 to 95 % relative humidity (non-condensing). Accuracy of the gas flow control is ±1 % in the range from 2 to 100 % of the maximal gas flow rate.

After the mass flow controllers 3 mm stainless steel tubing (i.d. 2 mm) was used up to the mixing T-union and to the two-fold solenoid valve, including the connection to the six-fold solenoid valve from Cole-Parmer Instrument Company (Vernon...
Gas washing bottle, peristaltic pump and bath circulator were used for measurements in oxygenated water.

Fig. 8.12 Gas handling system schematics

Gas washing bottle, peristaltic pump and bath circulator were used for measurements in oxygenated water.

**Materials and Equipment**

- **Gas Sources**
  - Oxygen (O₂) 5.5 bar, 50 l
  - Nitrogen (N₂) 5.5 bar, 50 l

- **Pumps and Valves**
  - Peristaltic pump
  - Mass flow controllers (0, 1)
  - 2-fold solenoid valve
  - 6-fold solenoid valve

- **Connections**
  - Ø 1/4” connections
  - Outer diameters in mm, approx. lengths in m

- **Central Gas Supply System**
  - Flow-through cell
  - Bath circulator
  - Waste outlet

**Operation**

1. Oxygen (O₂) from central gas supply system enters the system at 5.5 bar.
2. Nitrogen (N₂) from central gas supply system enters the system at 5.5 bar.
3. Gas handling system includes gas washing bottle, peristaltic pump, and bath circulator.
4. Oxygen and nitrogen are mixed through the system.
5. The mixed gas is directed to the flow-through cell and waste outlet.
6. The system also includes mass flow controllers for precise gas flow adjustment.

**Diagram Key**

- Stainless steel
- Copper
- Tygon™ tubing

---

**Notes**

- Outer diameters in mm, approx. lengths in m
- Central gas supply system

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**Appendix**

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For practical reasons a standard protocol had been adopted to characterize the membranes, including excitation and emission spectra. This ensures reproducible, well documented measurement conditions. A standard measurement is shown in Fig. 8.14. The emission/excitation spectra which are recorded at the tagged positions in Fig. 8.14 are shown in Fig. 8.15.
For all measurements in gas a third generation flow-through cell (see Fig. 8.9 on p. 244) was used. No response times could be determined because the settling time of ca. 3 s of the gas mixing was well above membrane response times, compare 8.4.2 Flow-through cell performance on p. 244.

Oxygen partial pressures reported here are based on an assumed constant environmental pressure of 1000 mbar and calculated from the measured oxygen/nitrogen...
flows, not the *set* flows. Intensity measurements for the Stern-Volmer plots have been made at 14 different oxygen partial pressures between 0 and 1 bar. Please note that *dry* gases have been used for the characterization, with experimental relative humidity always < 4%. Ambient temperatures were 24 to 28°C.

### 8.5.2 Measurements in oxygenated water

A careful preparation of oxygenated water and its fast transport into the flow-through cell is very critical to obtain proper quenching data in water.

Henry’s law can be used to calculate the oxygen concentration in water because first, the mole fraction of oxygen in water (~2·10⁻⁵) is small enough and second, at 1 bar there is only a very small difference between fugacity and partial pressure [9]. Therefore, the oxygen concentration in water is proportional to the partial pressure. For 1 bar [O₂] at 25°C we obtain 8.13 mg/l. Note the -0.14 mg·l⁻¹/°C (i.e. -1.8%/°C) temperature dependence of the oxygen concentration. Any dissolved electrolyte will lower the oxygen concentration.

The main goal was to reach a steady state in the gas washing bottle which was used for the gas saturation of the water. Any larger addition of water at a given moment would inevitably vary both temperature and gas concentration. A second concern
was that the water level determines the water pressure resistance to the gas flow, which can result in less vigorous bubbling and a larger saturation time for higher water levels.

Special 400 ml gas washing bottles with a sideward stopcock were made by Evelyne Trüb of the Glasbläserei Hönggerberg, ETH Zürich. A stopcock with a 8 mm olive was attached 2 cm above the glass frit level. Through this connection the gas washing bottle could be continuously fed with fresh water in the zone of greatest turbulence, as not to disturb the attained gas saturation equilibrium. A level switch controlled a peristaltic pump, which delivered water as soon as the level switch reacted. This made it possible to control the water level within a few millimeter.

In a second gas washing bottle water was air-saturated with pressurized air from the in-house gas system; water was supplied by a communicating tube from a 10 l-water container with no special level control. Both gas washing bottles were thermostated to 25 ± 1°C with a EX-210 bath circulator (NESLAB, Portsmouth, NH, USA).

Deionized water had been used for all measurements in oxygenated water. A 10 g/l sodium sulfite solution was used as a chemically prepared oxygen-free solution. Experiments has shown that its use distorts measurements, at least the response behavior. The response from oxygenated water to degassed water was slower than changing to sodium sulfite solution. For the reverse step from sodium sulfite solution to oxygenated water the behavior was also qualitatively different with a slower initial and faster final decrease compared to the faster change from degassed solution to oxygenated water.

As for the gas measurements, a standard protocol had been established. Periods of 1000 s duration with 100%, 80%, 60%, 40%, 20%, 0% oxygen (v/v) bubbled through the water were alternated with 1000 s periods with air-saturated water. The experiment was terminated with a 1000 s-period with the sodium sulfite solution. Oxygen partial pressures given in figures and Stern-Volmer constants calculated from this data correspond to the oxygen content in the oxygen/nitrogen gas mixture in equilibria with water at an assumed constant pressure of 1 bar. For all the Stern-Volmer plots the 0% oxygen concentration was taken as $I_0$, even though luminescence levels for the sodium sulfite solution were consistently slightly higher.

Oxygenated water was pumped by a Gilson Minipuls 3 (Gilson, Villiers le Bel, France) at 4.0 ml/min, which corresponds to ~2.2 m/min linear velocity. All connections were primed prior to the experiment start. The Gilson Minipuls 3 used 1.14 mm i.d. PVC flow tubing (Elkay Products, Shrewsbury, MA, USA) for the ca. 500 mm long pumping section and TYGON tubing R3603, 1.6/3.2 mm (Cole Parmer) for all other plastic tubing. Please note that the Gilson Minipuls 3 was
placed after the flow-through cell in order to minimize the distance between gas washing bottles and flow-through cell.

The need for a fast transport of the sample solution is best illustrated with the dependence of luminescence on oxygen partial pressure at different flow rates in Fig. 8.16.

![Fig. 8.16 Flow rate dependence of oxygen saturation in water](image)

Please note that curves intersect approx. at air oxygen partial pressure.

The longer the delay between gas saturation and luminescence measurement in the flow-through cell, the larger the desaturation, i.e. equilibration with air. Keep in mind that the fastest flow rate of 4 ml/min corresponds to a time delay of less than half a minute along one meter of 90% stainless steel tubing and 10% TYGON tubing which has a low oxygen permeation. Prior to optimization even flow rates of 0.1 ml/min had resulted in complete air equilibration.

As a whole oxygen saturation were close to critical. The large flow rates exchange the gas washing bottle volume within 100 min with a rather small gas flow of 200 ml/min to saturate such a volume.

Please note that the luminescence of even fully oxygenated water, i.e. in contact with 100% oxygen, is higher than the luminescence for nitrogen for the same membrane. This is an experimental artifact, caused by the refractive index induced by water.

Contrary to gas experiments, a second generation flow-through cell was used for experiments in water. At the very high flow rates necessary to prevent desaturation of the water frequent air bubbles had given rise to a very unstable signal with the third generation cell.
8.5.3 Experimental limitations

With the long-term experiment described in 5.3.1 Bleaching in the luminescence spectrophotometer on p. 126 an astonishing large noise of 7.2 (1.0%) and 4.4 (1.3%) for the nitrogen and oxygen signals, respectively, had been found. A closer look revealed a clear day pattern. These two series, minus their linear offsets, are displayed in Fig. 8.17. Their absolute values are 751.6 and 335.9, respectively.

Signals start rising at 18h in the evening until 9h in the morning when they start decreasing again. The nitrogen and oxygen series are obviously highly correlated and seem to depend linearly on one another. A least-squares fit reveals that the amplitude in the oxygen signal is 0.56 of the nitrogen signal, whereas the absolute signal values give a ratio with a similar value of 0.45.

The most plausible explanation of this daily pattern is the temperature-dependence of the intramolecular luminescence quenching. With lower temperatures during night luminescence increases whereas during the day with higher temperatures the luminescence decreases. This clearly shows the limits of unthermostated measurements.

Thermostating both sample media as well as the glass wafer proved to be impossible with the employed polypropylene flow-through cells.

A very delicate issue is the calculation of detection limits, a figure of merit which is usually given. According to the Union of Pure and Applied Chemistry (IUPAC), the limit of detection (LOD) is defined as

$$\text{LOD} = 3 \times \text{noise} + \text{background},$$  \hspace{1cm} (8.3)
where signal noise is defined as
\[
\text{noise} = 2 \times \text{rms(residual signal)}.
\] (8.4)

Despite apparently clear definitions there is a wide room for manipulation. Signal noise is efficiently diminished by averaging during or after data acquisition; noise eventually only depends on the time resolution which is requested. Various instrumental parameters influence noise. Larger bandwidths for instance increase the light level, which allows to use lower photomultiplier voltages, resulting in lower noise. Drift spans, such as the temperature drifts discussed above, which are two orders of magnitude larger than signal noise, make the calculation of detection limits completely arbitrary. Therefore, no estimates of detection limits are reported. However, they can be easily calculated for any existing instrument based on the membrane absorbance, quantum yield and the Stern-Volmer constants; the same applies to the dynamic range.

### 8.6 Data treatment

IgorPro (Wavemetrics, Inc., Lake Oswego, OR, USA) has been used for all data treatment. All errors reported in this work are standard deviations of the respective quantities.

Self-written programs for NO₂/RH-control with analog mass flow controllers, control of Bronkhorst mass flow controllers via DDE server, control of digital MKS mass flow controllers via RS-485, interfacing of the Spekol 1100 absorption spectrophotometer, Uvikon 942 absorption spectrophotometer, PE LS-50B luminescence spectrophotometer and Gilson Minipuls 3 peristaltic pump via RS-232, data acquisition from HP 4274A Multi-Frequency LCR Meter, PREMA 5000 Scanner, HP 34420A Nanovoltmeter via GPIB have been written in LabVIEW (National Instruments, Austin, TX, USA).

IgorPro procedures and LabVIEW programs are available at request.

### 8.7 References


**Glossary**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
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<tbody>
<tr>
<td>AcN</td>
<td>acetonitrile</td>
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<tr>
<td>ATR</td>
<td>Attenuated Total Reflection</td>
</tr>
<tr>
<td>a.u.</td>
<td>arbitrary units</td>
</tr>
<tr>
<td>AE</td>
<td>Auxiliary Electrode</td>
</tr>
<tr>
<td>bpy</td>
<td>2,2’-bipyridine (2,2’-dipyridyl)</td>
</tr>
<tr>
<td>CCS</td>
<td>Centre for Chemical Sensors</td>
</tr>
<tr>
<td>χ²</td>
<td>chi-square</td>
</tr>
<tr>
<td>CE</td>
<td>Counter Electrode (also AE)</td>
</tr>
<tr>
<td>CPE</td>
<td>Constant Phase Element</td>
</tr>
<tr>
<td>o-CPOE</td>
<td>o-cyanophenyl octyl ether (1-cyano-2-octyloxy benzene)</td>
</tr>
<tr>
<td>CV</td>
<td>Cyclic Voltammetry/Voltamogram</td>
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<tr>
<td>DOA</td>
<td>dioctyladipate</td>
</tr>
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<td>DOS</td>
<td>bis(2-ethylhexyl) sebacate</td>
</tr>
<tr>
<td>dpp</td>
<td>4,7-diphenyl-1,10-phenanthroline</td>
</tr>
<tr>
<td>3-dpp</td>
<td>4,7-bis(4-propylphenyl)-1,10-phenanthroline</td>
</tr>
<tr>
<td>8-dpp</td>
<td>4,7-bis(4-octylphenyl)-1,10-phenanthroline</td>
</tr>
<tr>
<td>ε</td>
<td>molar decadic absorption coefficient <em>or</em> dielectric constant (permittivity)</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray Ionization</td>
</tr>
<tr>
<td>ETH¹ 3001</td>
<td>tris(4,7-bis(4-propylphenyl)-1,10-phenanthroline)-ruthenium(II) perchlorate</td>
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<td>ethanol</td>
</tr>
<tr>
<td>Φₗ</td>
<td>luminescence quantum yield</td>
</tr>
<tr>
<td>FT-ICR</td>
<td>Fourier Transform Ion Cyclotron Resonance</td>
</tr>
</tbody>
</table>
GCE  Glassy Carbon Electrode
GPIB  General Purpose Interface Bus
i.d.  inner diameter
KTTFPB  potassium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate
L  ligand
LC  Ligand-Centered (spectroscopic state)
LO  N-oxide of ligand
$\lambda_{\text{max}}$  wavelength of absorbance (abs) or emission (em) maximum
MALDI  Matrix-Assisted Laser Desorption/Ionization
MC  Metal-Centered (spectroscopic state)
MeOH  methanol
MLCT  Metal Ligand Charge Transfer (spectroscopic state)
MS  Mass Spectrometry
$n_D$  refractive index
NHE  Normal Hydrogen Electrode
NI 1  nitrite ionophore 1
NIR  Near Infrared
NMR  Nuclear Magnetic Resonance
o-NPOE  o-nitrophenyl octyl ether (1-nitro-2-octylxy benzene)
O  oxidized species
$^1\text{O}_2$  singlet oxygen
phen  1,10-phenanthroline
phenO  1,10-phenanthroline-N-oxide
$pK_a$  $-\log$(acid ionization constant)
PAMS  poly($\alpha$-methylstyrene)
PC  poly(bisphenol-A-carbonate)
PDPO  poly(2,6-dimethyl-p-phenylene oxide)
PMOS  poly(4-methoxystyrene)
p$O_2$  oxygen partial pressure
PS  polystyrene
PtBrS  poly(2,4,6-tribromostyrene)
PtBuS  poly($p$-tert-butylstyrene)
PVC  poly(vinyl chloride)
R  reduced species
RE  Reference Electrode
RH  Relative Humidity
SCE  Standard Calomel Electrode
S/N  signal-to-noise
TBPA  tetrabutylammonium perchlorate
$\theta_c$  critical angle
$\tau_0$  luminescence lifetime in absence of quencher
$t_{90\%}$  time for a 90% signal change
THF  tetrahydrofurane
$T_g$  glass transition temperature
TLC  Thin Layer Chromatography
TFA  trifluoroacetic acid
$w$  percent weight/weight
WE  Working Electrode
$\Omega$  modulus of Warburg impedance
Chemical structures

Fig. 8.18 Ruthenium(II) diimine complexes and common ligands
Fig. 8.19 Polymer and additives
Fig. 8.20 Dyes and special compounds
Index

A
Absorbance spectroscopy
  glass wafer absorbance background 50
  reproducibility of membrane measurements 50
Actinometry 123
Admittance 193
Anodic peak potential 171
Arrhenius plot 153
ATR crystal 125

B
Bis(2-ethylhexyl)sebacate, see DOS
4,7-Bis(4-octylphenyl)-1,10-phenanthroline 13
4,7-Bis(4-propylphenyl)-1,10-phenanthroline 14
Bode plots 198
Brasher and Kingsbury model 202

C
Centre for Chemical Sensors 10
Changchun Institute of Applied Chemistry 162
Charge transfer 8
Chi-square 76
(2-Chloroethyl)-(4-octylphenyl)ketone 12
(2-Chloroethyl)-(4-propylphenyl)ketone 14
Chromoionophore 189
Clark electrode 1
Cobalt(II) porphyrins 68
Coextraction mechanism
  pH sensitivity 190
  principle 189
Companies
  Bosch Telecom 191
  Glasbläserei, ETH Hönggerberg 167, 251
  Precision Sensing GmbH 68
  Siemens Building Technologies - Cercberus Division 192, 204
  Workshop Technische Chemie, ETH Zürich 243
Conductor, linear, infinite
  capacity 207
Constant Phase Element (CPE) 193, 194
  effect of phase factor 199
o-CPOE
  absorbance spectrum 146
  characterization 85
  IR spectrum 136
  quenching effect 89
Critical angle 41
Crystal field 7
Cyclic voltammetry
  quasi-infinite conditions 172
  thin-layer situation 172

D
Dielectric constant 204
Dielectric loss factor 204
Diffusion
  ions in hydrophobic polymers, of 191
  stepwidth 152
DOS 190
  use as plasticizer 191
Dual Mode Sorption Theory 80
Dye leaching 99
Dye migration 81
Dynamic quenching 71

Einstein-Smoluchowski equation 152
Electrochemical reversibility 171
Electroneutrality condition 189
Electrospray Ionisation Mass Spectrometry (ESI-MS) 142
Emission spectra correction
  with quinine sulfate 39
  with Rhodamine B 39
  with standard light source 37
Energy gap law 8, 25
ETH 5418 189, 204
  absorbance characteristics 191
  absorbance spectra 215
ETH\textsuperscript{T} 3001
  absorbance spectrum 20
  bleaching in ethanol 133
  emission spectrum 20
  ESI mass spectrum 144
  excitation spectrum 20
  excitation/emission plot 91
  IR spectrum (solid) 19, 136
  luminescence lifetime (in EtOH) 24
  luminescence lifetime (in plast. PS) 86
  luminescence quantum yield 22
  MALDI mass spectrum 141
  solubility 17
  structure (calculated) 17
  synthesis 14
ETH\textsuperscript{T} 3003
  absorbance spectrum 20, 22
  \textsuperscript{1}H NMR data 14
  lipophilicity 111
  luminescence lifetime (in EtOH) 24
  luminescence lifetime (in plast. PS) 86
  synthesis 13
ETH\textsuperscript{T} 4012 129
(Ethylendiamine)bis(2,2'-bipyridine)ruthenium(II)
  photobleaching 145
Europt(r)ode 2000 111
Excitation spectra correction 36
Exponential distribution 76
Extensive quantity 67

F
Fluorescence 8
Förster energy transfer 153
Fourier Transform Ion Cyclotron Resonance (FT-ICR) 143
Friedel-Crafts reaction 12
Full Width at Half Maximum (FWHM) 76

G
Gamma distribution 77
Glass transition temperature 80

H
Henry’s law 80, 203, 250
Hund’s rules 7

I
+I effect 9
Impedance 193
  complex representation 194
  geometrical construction 195
Impedance data fitting 199
Impedance spectroscopy 193
  charge-transfer 201
for ion-selective electrodes 201
ideal measuring range 198
simulated spectra 197

Impedance spectrum
   explanation of fit parameter representation 212, 216

Instruments
   AT 261 balance 235
   Autolab Frequency Response Analyzer 211
   Autolab PGSTAT 10 170
   Autolab PGSTAT 20 170, 211
   BAS100B Electrochemical Analyzer 163
   Bruker Vector 33 FT-IR spectrophotometer 125
   Finnigan TSQ 7000 ESI mass spectrometer 125
   Gilson Minipuls 3 peristaltic pump 254
   GREISINGER electronic GTH 1200A digital thermometer 53
   Hitachi U-3210 49, 169
   HP 34420A Nanovoltmeter 241, 254
   HP 4274A Multi-Frequency LCR Meter 254
   IKA-Vibramax-VXR 235
   Nabertherm L3 heating chamber 103
   Napco Model 8000 Autoclave 104
   Neslab EX-210 bath circulator 251
   Newport Digital Power Meter 122
   PE LS-50B luminescence spectrophotometer, see PE LS-50B
   Perkin Elmer Lambda 900 UV/VIS/NIR Spectrometer 49
   PLASMOS SD 2300 ellipsometer 237
   PREMA 5000 Scanner 254
   rotronic A2 hygrometer hygrometer 209
   Spekol 1100 absorbance spectrophotometer, see Spekol 1100
   Spex Fluorolog Spectrometer 48
   TENCOR alpha-step 200 profilometer 237
   TN-6500 Rapid Scan Spectrometer 165
   Uvikon 942 absorbance spectrophotometer, see Uvikon 942
   VARIAN DMS 90 UV Visible Spectrometer 166

Intensive quantity 67
Interdigitated microelectrodes 204
capacity calculation 207
capacity model 207
Internal conversion 7, 8
Intersystem crossing 7, 8
Isosbestic point 128

J
Jablonski diagram 7

K
Kramers-Kronig transformation 57, 198
KTTFPB 189, 190, 192

L
Langmuir sorption 80
\(^3\)LC state 9
LED
   photobleaching effects 127
Levenberg-Marquardt algorithm 199
Limit of detection 253
Lognormal distribution 77
Luminescence intensity
   measurement 67
Luminescence lifetime
   measurement 67
   modulated measurement method 67
   pulsed measurement method 67
Luminescence quantum yield 8
   in dry salts 23
   in oxygen optodes 109
water effect 53
Luminescence quantum yield determinations
solution level changes 53
Luminescence quenching
distribution-based models 75
Gaussian model 75
log-Gaussian model 75
Multi-component model 73
simple model 72
temperature dependence 23
two-site model 96, 109
vibrational 23
Luminescence spectroscopy
membrane inhomogeneity 47
membrane measurements artifacts 33

M
Matrix-Assisted Laser Desorption/Ionisation (MALDI-MS) 140
Maxwell-Boltzmann distribution 77
\(^3\)MC state 7, 9, 148, 153
Membranes
marks for positioning 49
Mercury lamp
characterization 122
emission spectrum 122
membrane bleaching, for 123
solution bleaching, for 124
MICROX I oxygen meter 68
\(^1\)MLCT state 8
\(^3\)MLCT state 7, 9
Modulus 194
MOS-FET operational amplifier 239

N
N,N’-dioxides 149
NI 1 189, 190
absorbance 190
absorbance spectra 216
NIR-VIS spectroscopy 131
Nitrite ionophore 1, see NI 1
Nitrogen dioxide
disproportionation reaction with water 191, 229
Nitrogen dioxide sensor 189
cross-sensitivities 191
equivalent circuit model 194
equivalent circuit model at high RH 219
Non-radiative decay 7, 8, 25
Normal distribution 76
N-oxide 149
\(o\)-NPOE
absorbance spectrum 146
characterization 85
density 91
quenching effect 90
Nyquist plot 198

O
Oxidative quenching 9
Oxygen
diffusion constant in polymers 81
diffusion constants 152
Oxygen gradient measurements 68
Oxygen optodes
absorbance mode 68
high sensitivities 109
plasticization effects 109
sensor design 68
sterilization 104, 111
\( \pi \) bonding orbital 7
\( \pi^* \) antibonding orbital 7
Particle size effects on color 110
PdOEPK 109
PE LS-50B 122

- comparison with Spex Fluorolog 48
- cut-off filter artifacts 54
- description 237
- emission correction calibration factors 39
- emission spectra calibration 37
- LabVIEW control 254
- luminescence background estimation 35
- photobleaching effects 126

Penetration depth (ATR-IR) 135
Permittivity 204

Persons
- Adlkofe, Klaus 199
- Belser, Peter 18, 174, 235
- Demuth, Caspar 189, 243
- Dubois, Fréderic 140
- Eberhardt, Peter 239
- Freiner, Daniel 10, 23, 235
- Gramlich, Volker 16
- Greter, Oswald 125
- Hartmann, Paul 86
- Heinz Erni 38
- Jenny, Luzi 10, 235
- Lehmann, Edda 125
- Linhoff, Michael 58
- Lü, Taiping 101
- Mohr, Gerhard 235
- Nezel, Tomas 191, 209, 227
- Nussberger, Bruno 167, 236
- Rásonyi, Stefan 10, 235
- Simon, Wilhelm 191
- Tomas Nezel 227
- Trüb, Evelyne 251

Weissberg, Leo 241
Weissmüller, Gilberto 199
Phase factor 194
Phase shift 193
Phosphate buffer 100
Phosphorescence 7, 8
Photobleaching 119
- alternative theories 149
- calculation of absorbed light 130
- mechanism 147
- polymer matrix 145
Photodiode
- bias voltage 239
- characteristics of BPW 34 S 241
- dark current 240
- light power calculation 128
Photoproducts 119
Polycyclic aromatic hydrocarbons (PAH) 1
Polymer dyeing 81
Polymers
- cellulose acetate (CA) 83
- LSR 2030 101
- poly(2,4,6-tribromostyrene) 82, 92
- poly(2,6-dimethyl-p-phenylene oxide) 84, 92
- poly(4-iodostyrene) 82
- poly(4-methoxystyrene) 82, 92
- poly(4-methylstyrene) 82
- poly(α-methylstyrene) 82, 92
- poly(bisphenol-A-carbonate) (PC) 84, 92
- poly(methyl methacrylate) 83
- poly(p-tert-butylstyrene) 82, 92
- poly(styrene-co-methyl methacrylate) 82
- poly(tetrafluoroethylene) 83
- poly(vinyl chloride), see PVC
- polyacenaphtaline 83
- polyacrylonitrile 81
polyetherimide (PEI) 111
polysiloxanes 83
polysiloxanes, see polysiloxanes
polystyrene, see polystyrene (PS)
poly(ether sulfone) (PSU) 111
polyvinylcarbazol 83
RTV-2K silicone 101
Siloprene K 1000 84
Siloprene LSR 2030 101
Polysiloxanes
IR bands 101
Polystyrene (PS)
absorbance spectrum 146
density 92
effects on \( T_g \) 80
fluorescence 34
phosphorescence 34
plasticization effects on mechanical stability 85
processing temperatures 81
refractive index 92
solubility parameter 85
PtOEPK 109
PVC
impurities 203
R
Radiative decay 8
Raman scattering 34
RC member 196
Reductive quenching 9
Reference electrode 168
Refraction 41
Refractive index corrections
for cuvette 46
formula 45
model for membrane on glass 41
simulation for membrane 44
values for polymer membranes 45
Reichardt’s Dye 99
Relative humidity sensor

ceramics 229
Rhodamine 101
   absorbance spectrum 53
   luminescence quantum yield standard 51
Rhodamine B
   dye migration 111
   quantum counter behavior 36
   quantum yield standard 22
Ru(bpy)\(_3\)
   luminescence quenching mechanism in solution 9
   photolysis in water 119
Ru(cage)\(_3\) 148
Ru(dpp)\(_3\)
   adsorption 54
   crystal structure 16
   electrochemiluminescence yield 23
   photobleaching 120
S
Sandwich electrodes 192, 204
Scanning Confocal Laser Microscopy 111
Self-absorption in the membrane 131
Signal noise 254
Singlet oxygen
   formation in the quenching process 9
   lifetimes 152
Skraup synthesis of quinolines 12
Snell’s law 41
Software
   EQUVCRT 199
   FL Winlab 238
   IgorPro 75, 199, 212
   kram2dn1.exe 58
   LabVIEW 254
Sybyl 6.3 16
Spekol 1100 254
Spin-orbit coupling 8, 25
Static quenching 72
Steam sterilization 104
Stern-Volmer constant 72
statistical considerations 96
Stern-Volmer kinetics 71
Stern-Volmer plot 72
difference between lifetime and intensity data 74
Stokes shift 1, 68
Sulfite 99
Superoxide radical anion 9
T
Tempering 103
Three-state model 24
Time constants 198
Transient 67
Tuning 9
Two-site model 73
U
Uvikon 942 49, 51, 54
control by LabVIEW 254
provisions for spectroelectrochemistry 169
W
Warburg impedance 194, 196, 202
Water
NIR absorption 222
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