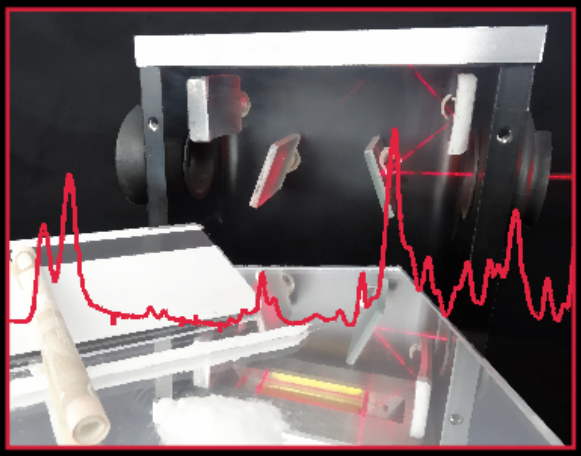
Diss ETH No. 21461

Infrared Spectral Analysis of Drugs in Human Saliva

Kerstin M.-C. Hans



Diss. ETH No. 21461

Infrared spectral analysis of drugs in human saliva

A dissertation submitted to

ETH Zurich

for the degree of

Doctor of Sciences

presented by

Kerstin Meta-Catherina Hans
Dipl. Phys., University of Konstanz
born April 20 1983
citizen of Germany

accepted on the recommendation of

Prof. Dr. Markus W. Sigrist
Prof. Dr. Jérôme Faist
PD Dr. Heinz-Detlef Kronfeldt

2013

For Meta and Cäcilia

Contents

Abstract

Infrared spectroscopy is gaining more importance in analyzing biological samples and monitoring environmental parameters. The analysis of environmental gases or investigation of cancer tissue are examples for the application of infrared spectroscopy. This thesis addresses three research questions with impact on society: the analysis of cocaine in saliva, the detection of caffeine in saliva and beverages and the investigation of saliva from patients with generalized aggressive periodontitis.

The first project regarding the detection of cocaine in saliva aimes for a table-top set-up with the possibility to be miniaturized to pave the path to a hand-held, compact device. Such a device would allow for (semi-)quantitative, on-site analyses of cocaine and would provide risk assessment, e.g. people driving under the influence of cocaine. This research question was examined in detailed studies to first explore the optimum wavelength: regions of strong cocaine absorption and minimum spectral interference with every day products, masking agents, common medicine, cutting agents of cocaine and saliva in different situations. The most suitable spectral range was found around $1750\,\mathrm{cm}^{-1}$. A one-step extraction technique was developed and evaluated to reduce the large background absorption by saliva. Samples were analyzed with Fourier-transform infrared (FTIR) spectroscopy and a quantum cascade laser (QCL) set-up. In addition, transmission and attenuated total reflectance (ATR) spec-

troscopy were used for the analysis. The limit of detection (LOD) for the FTIR-ATR spectroscopy is $\sim 1\,\mu g\,ml^{-1}$ and can be further improved by preconcentration to a few $100\,ng\,ml^{-1}$. This LOD is necessary to identify a person abusing cocaine. The newly designed QCL set-up has a current LOD for transmission and ATR spectroscopy of $< 10\,\mu g\,ml^{-1}$ and $\sim 3\,\mu g\,ml^{-1}$, respectively. Further stabilization of the QCL set-up is required to lower these detection limits.

The second topic is directed toward the detection of caffeine in fluids. The influence of caffeine on many diseases, such as Alzheimer's and Parkinson's disease, is currently under investigation. So far, test-persons in studies analyzing the correlation between Alzheimer's or Parkinson's disease have to abstain from caffeine-containing beverages, which is problematic due to the social relevance of coffee and tea. The number of test persons might increase significantly if caffeine intake could be monitored with a hand-held, compact device instead. Therefore, a minaturizable one-step extraction technique for caffeine from beverages and saliva was developed. The extracts were investigated with FTIR-ATR spectroscopy. Even without any pH stabilization qualitative data on caffeine concentrations in various beverages could be derived.

The third project concerns the study of patients suffering from generalized aggressive periodontitis (G-AgP). Periodontitis is a disease with high prevalence. But until today, a method predicting the individual progress of the disease has not been found. Such a method should estimate the risk of a patient for bone loss within a certain time. The identification of spectral differences in saliva between patients with G-AgP and healthy volunteers might allow early diagnostics and could lead to improved prognoses. The investigation were conducted with FTIR-ATR spectroscopy and potential differences in the spectra were evaluated with principle component analysis (PCA) in the spectral range from 1230 to $1180\,cm^{-1}$. In addition, the difference between the absorbance at 1206 and $1196\,cm^{-1}$

was analyzed with the analysis of variance (ANOVA). Although the number of test persons with 10 persons in total was still small, both methods yield promising results to differentiate between the sample groups. Since the investigated spectral range is rather narrow a quantum cascade laser based device could be built for chair-side tests.

In conclusion, the analysis of all three research questions showed promissing potential for miniturisation and future on-site applications.

Zusammenfassung

Die Infrarotspektroskopie wird immer häufiger zur Charakterisierung von biologischen Proben und von wichtigen Umweltparametern angewendet. Zum Beispiel können mit dieser Methode Krebszellen charakterisiert und die Konzentration von Umweltgasen wie CO_2 bestimmt werden. In dieser Arbeit wird Infrarotspektroskopie genutzt um die folgenden drei gesellschaftsrelevanten Themen genauer zu untersuchen: Der Nachweis von Kokain im Speichel, der Nachweis von Koffein im Speichel und in Getränken und die Charakterisierung von Speichel von Patienten mit generalisierter aggressiver Parodontitis.

Für das erste Thema, welches den Nachweis von Kokain in Speichel betrachtet, soll ein Versuch aufgebaut werden, dessen Komponenten gegebenenfalls miniaturisiert werden können. Dies könnte zu einem tragbaren, kompakten und einfach zu bedienenden Analysegerät führen. Ein solches Gerät könnte vor Ort Diagnosen ermöglichen, z.B. für die Polizei oder Ambulanz. Für diese Untersuchungen wurde zuerst nach einem geeigneten Wellenlängen Bereich für den Nachweis von Kokain im Speichel gesucht. Der Wellenlängenbereich soll eine hohe Absorption von Kokain und möglichst wenig Absorption von anderen Substanzen aufweisen. Um diesen Bereich zu identifizieren wurden Alltagsprodukte, Schnittmittel von Strassenkokain, gebräuchliche Medikamente, maskierende Substanzen[1] und

[1] Substanzen die von Drogenkonsumenten genommen werden, um Dorgentests

Speichel von Testpersonen in unterschiedlichen Situationen untersucht. Die Untersuchungen ergaben, dass der Wellenlängenbereich um $1750\,\mathrm{cm}^{-1}$ am Besten zum Nachweis von Kokain im Speichel geeignet ist. Ferner wurde eine einstufige Extraktionsmethode entwickelt und untersucht, die die Nachweisgrenze von Kokain im Speichel deutlich senkt. Alle Proben wurden mit einem Fourier-Transform-Infrarot- (FTIR) Spektrometer oder einem Quantenkaskadenlaser- (QCL) Aufbau untersucht. Die spektroskopischen Methoden der gedämpften Totalreflektion (engl. attenuated total reflection - ATR) und Transmission wurden verwendet. Die gemessene Nachweisgrenze für Kokain im Speichel unter Verwendung von FTIR-ATR Spektroskopie liegt bei $\sim 1\,\mu\mathrm{g\,ml}^{-1}$ und kann durch Aufkonzentrierung auf einige $100\,\mathrm{ng\,ml}^{-1}$ verbessert werden. Diese Nachweisgrenze ist notwendig, um Menschen die unter direktem Einfluss von Kokain stehen zu identifizieren. Für den QCL-Aufbau wurde eine Nachweisgrenze von $< 10\,\mu\mathrm{g\,ml}^{-1}$ für die Transmissionsmessungen und von $\sim 3\,\mu\mathrm{g\,ml}^{-1}$ für die ATR-Messungen ermittelt. Um tiefere Nachweisgrenzen zu erreichen, müsste der QCL-Messaufbau besser stabilisiert werden.

Das zweite Thema beschäftigt sich mit dem Nachweis von Koffein im Speichel und Getränken. Der Einfluss von Koffein auf den Krankheitsverlauf von z.B. Alzheimer- und Parkinson-Krankheit ist Gegenstand aktueller Forschung. Für diese Studien müssen die Testpersonen für gewöhnlich auf den Konsum von Koffein verzichten. Dieser Verzicht stellt ein großes Problem dar, da das Konsumieren von Getränken, wie Kaffee und Tee, von großer sozialer Bedeutung ist. Die Anzahl der Testpersonen für solche Studien könnte sich erheblich vergrößern, falls anstatt eines Verzichts auf Koffein, der Konsum gemessen werden könnte. Für solche Studien wird ein tragbares, kompaktes Analysegerät benötigt, um die Koffeinkonzentration des jeweiligen Getränkes zu messen. Wiederum wurde ein Versuchaufbau realsiert, dessen Komponenten gegebenenfalls minia-

zu verfälschen.

turisiert werden könnten. Zur Erhöhung der Nachweisgrenze von Koffein wurde ferner ein einstufiges Koffeinextraktionsverfahren entwickelt. Alle Extrakte wurden mittels FTIR-ATR Spektroskopie analysiert. Die Messergebnisse waren auch ohne pH-Stabilistation qualitative wertvoll. Der Koffeingehalt der untersuchten Extrakte aus Kaffe, Tee und Guarana wurde erfolgreich bestimmt. Eine Stabilisation der pH-Werte wird zu quantitativen Ergebnissen führen.

Das dritte Thema beschäftigte sich mit dem Speichel von Patienten mit generalisierter aggressiver Parodontitis (G-AgP). Parodontitis ist eine Krankheit mit hoher Prävalenz. Jedoch gibt es noch keine Methode die zuverlässige Prognosen über den Krankheitsverlauf einer Einzelperson machen kann. Von besonderem Interesse bei dem Krankheitsverlauf ist die Risikoabschätzung bezüglich eines raschen Knochenverlustes. Ein messbarer Unterschied in den Speichelspektren von gesunden und an G-AgP erkrankten Menschen könnte zu einer früheren Diagnose der Krankheit und verbesserten Prognose ihres Verlaufs führen. Der Speichel wurde mit FTIR-ATR Spektroskopie untersucht. Die Spektren wurden in dem Intervall von 1230 bis 1180 cm^{-1} mit der Hauptkomponentenanalyse (engl. principal component analysis - PCA) untersucht, welche Hinweise auf eine Unterscheidung der beiden Gruppen identifizierte. Des Weiteren wurde die Differenz in der Absorption für die Wellenlängen 1206 und 1196 cm^{-1} bestimmt und mit der Varianzanalyse (engl. analysis of variance - ANOVA) diskriminiert. Diese Analyse bestätigte, dass die beiden Gruppen sich signifikant unterscheiden trotz der geringen Anzahl (10) der Testpersonen. Auf Grund der geringen spektralen Intervallbreite, kann ein QCL-Messaufbau für zukünftige Untersuchungen. eingesetzt werden. Dadurch könnte der Messaufbau zu einem tragbaren Gerät verkleinert werden, welches für den Einsatz in Arztpraxen geeignet wäre.

Alle drei untersuchten Themen zeigten vielversprechende Resultate. Die verwendeten Analysemethoden besitzten das Potential zur Verkleinerung des Messaufbaus in ein kompaktes, tragbares und ein-

fach zu bedienendes Gerät für den Einsatz vor Ort.

Symbols and abbreviations

Symbols

The symbols are explained in detail in the text. This table serves as quick reference to the symbols. Symbols that are used twice in this thesis, are clarified by the context they appear in.

Symbol	Description
α	Absorption coefficient
$\alpha_{0.1}$	Calculated absorption coefficient at $\sim$1756 cm^{-1} of cocaine at a concentration of 0.1 $\mu\mathrm{g\,ml}^{-1}$
α_d	Dissociation degree
$\alpha_{\mathrm{H_2O}}$	Absorption coefficient of water at $\sim$1756 cm^{-1}
α_{TCE}	Absorption coefficient of tetrachloroethylene at $\sim$1756 cm^{-1}
A	Fitting parameter for the determination of the concentration
A	of cocaine
A	Absorbance
A^-	Brønsted base
$\mathbf{B}$	Magnetic field
c	Speed of light

Symbol	Description
c(HA)	Concentration of the substance HA
c_0(HA)	Concentration of the substance HA before dissociation
c_{cal}	Calculated concentration of the solute in the extract
$c_{initial}$	Initial concentration of the solute in the liquid, e.g. cocaine in saliva before extraction
c_o(HA)	Concentration of the substance HA in 1-octanol
c_w(HA)	Concentration of the substance HA in water
d	Penetration depth of the light into the sample for one single reflection
D	Distribution coefficient
η	Extraction efficiency
$\mathbf{E}$	Electric field
HA	Brønsted acid
I	Light intensity
$\mathbf{k}$	Wave vector pointing in the direction of the wave propagation
κ	Attenuation factor
k	Magnitude of the wave vector
k	Intercept of the linear regression to calibrate the cocaine concentration in the extract
K_a	Acid dissociation constant
K_{app}	Distribution coefficient
K_{ow}	Partition coefficient
K_W	Ion product of water
λ	Wavelength of the light
l	Optical path length
m	Slope of the linear regression to calibrate the cocaine concentration in the extract
n_{Sa}	Refractive index of the sample
n_{ZnSe}	Refractive index of the ZnSe
$\tilde{\nu}$	Wavelength in cm^{-1}
n_c	Complex refractive index
P	Partition coefficient
pH	$-\log_{10}\left(c(\mathrm{H_3O^+})/(\mathrm{mol}\cdot\mathrm{l}^{-1})\right)$

Symbol	Description
pK_a	$-\log_{10}(K_a)$
ω	Angular frequency of the light (in vacuum)
r	Correlation coefficient
σ	Standard deviation
s_A	Spectrum of a reference samples
s_D	Spectrum to correct for changes in the experimental conditions
s_E, s_F	Spectral characteristics of impurities of the samples
s_{fit}	Linear combination of measured spectrum fitted to the measurements
s_m	Measured spectra of the extracts
$\mathfrak{T}$	Temperature
t	Time
T	Transmission
Θ	Angle of total internal reflection

Abbreviations

The Abbreviations are explained in detail in the text. This table only serves as a quick reverence.

Abbreviation	Explanation
AME	Anhydroecgonine methyl ester
ANOVA	Analysis of variance
ATR	Attenuated total reflection
BE	Benzoylecgonine
BOP	Bleeding on probing
C_2H_5OH	Ethanol
CAL	Clinical attachment loss
CAS	Chemical Abstracts Service
CGCI	Narrow bore capillary gas chromatography
COC	Cocaine
COE	Cocaethylene

Abbreviation	Explanation
conc.	Concentration
CRDS	Cavity ring-down spectroscopy
cw	Continuous wave
DAD	Diode array detection
DDS	Drug Detection System
DFB	Distributed feedback
DFG	Difference frequency generation
EME	Ecgonine methyl ester
EC	Ecgonine
ELISA	Enzyme-linked immunosorbent assay
FTIR	Fourier-transform infrared
FWHM	Full width of half maximum
G-AgP	Generalized aggressive periodontitis
GC	Gas chromatography
GCF	Gingival crevicular fluid
HPLC	High-pressure liquid chromatography
ICL	Interband cascade laser
IR	Infrared
LC	Liquid chromatography
LOD	Limit of detection
MAE	Microwave assisted extraction
MS	Mass spectroscopy
MS/MS	Tandem mass spectroscopy
NCOC	Norcocaine
NOA	Norland Optical Adhesive
OPO	Optical parametric oscillators
OSE	One-step extraction
PC	Principal component
PCA	Principle component analysis
PCR	Polymerase chain reaction
PDMS	Polydimethylsiloxane
PPD	Pocket probing depth
QCL	Quantum cascade laser
RB	Reference beam

Abbreviation	Explanation
S	Solute
SAMHSA	U.S. Substance Abuse and Mental Health Services Administration
SB	Sample beam
SERS	Surface-enhanced Raman scattering
SF	Salivary flow
SPE	Solid-phase extraction
St.COC	Street cocaine
TCE	Tetrachloroethylene
UV	Ultraviolet

Part I

Introduction, motivation and theory

Chapter 1

Introduction

This thesis comprises three major research questions:

- Infrared spectroscopy of cocaine in saliva with the help of a one-step extraction technique (major part).
- Spectral determination of caffeine concentration in beverages aided by a one step-extraction technique.
- Differentiation between infrared spectra of saliva from healthy test persons and patients with general aggressive periodontitis (G-AgP).

Psychoactive drugs are often involved in severe accidents, e.g. the number of accidents most likely caused under the influence of drugs and/or medicine increased by 50 % in Switzerland between 1992 and 2010 [1]. A sensitive quantitative drug detection method that can be applied to an easily accessible body fluid such as saliva is thus of utmost importance. In the frame of the nano-tera project IrSens [2] we developed an infrared-based table-top sensor that could be miniaturized to a hand-held device which would support risk assessment on-site and might make secondary, expensive laboratory test obsolete. At first the infrared wavelength interval with the least spectral

interference and a high cocaine absorption was investigated. Secondly, an extraction method was developed that can be implemented on microfluidic chips. Thirdly, the limit of detection for cocaine in saliva was identified and the method was validated with street cocaine samples. For these analyses two methods, i.e. transmission and attenuated total reflection (ATR) spectroscopy, were investigated. The experiments were performed with a Fourier-Transform infrared (FTIR) spectrometer (cf. chapter 9) and a quantum cascade laser set-up (cf. chapter 11).

The second research topic concerns the caffeine concentration in beverages. This question is of interest since moderate caffeine consume is associated with prevention for Alzheimer's and Parikson's disease [3] (cf. chapter 12). The development of a tabletop set-up that could be miniaturized into a hand-held device, might enlarge the data set significantly needed to increase the knowledge of these relations. Currently, test-persons in these studies have to abstain caffeine-containing beverages during the study, which is very difficult due to the social significance of coffee and tea. If the test person could monitor his or her caffeine consume with a compact device instead, the number of test persons would increase significantly. A simple one-step extraction method was developed that can be implemented on a microfluidic chip. In addition, caffeine-containing beverages as well as saliva samples after the caffeine consume and extracts from both were analyzed with FTIR spectroscopy (cf. chapter 13).

The last research topic was embedded in an cooperation with the Institute of Oral Biology at the University of Zurich. Periodontitis is a wide spread oral disease [4] that is also associated with an increased risk of coronary heart disease [5] (cf. chapter 14). It is important to better understand this disease to project its development and assess the risk of rapid bone loss. In this thesis saliva samples from patients with generalized aggressive periodontitis (G-AgP) were investigated as a first step for spectral differences in saliva samples for

patient with and without G-AgP. Spectral and statistical analysis demonstrate the feasibility of differentiation (cf. chapter 15). In addition, porphyromonas gingivalis bacteria, which are involved in the progression of periodontitis, were spectrally analyzed for their detection in saliva.

This thesis is divided into 5 parts: theory, introduction and results of the cocaine study, introduction and results of the caffeine study, introduction and results of G-AgP trial and the conclusion. In the first part an introduction to the theory is given. The cocaine, caffeine and G-AgP parts give an introduction to the theme and the results. Finally the results are summarized in the last part.

Chapter 2

Infrared spectroscopy

Infrared (IR) spectroscopy is a widely used technique to analyze solid, liquid and gaseous samples. Most fundamental molecular vibrations occur in the mid-infrared (mid-IR, wavelengths range: 2.5 to 25 µm or 4000 to 400 cm^{-1}) and the richest chemical information can be found there [6]. In the near-IR spectral range (wavelengths: 0.8 to 2.5 µm or 12500 to 4000 cm^{-1}), overtones and combinations of the fundamental vibrations dominate the spectral response. Additionally, some electronic absorptions are located in the near-IR spectral range. In the far-IR (wavelengths: 25 to 500 µm or 400 to 20 cm^{-1}) vibrations of heavy atoms, lattice modes of solids and some rotational absorptions of small molecules dominate the absorption spectrum [6].

Infrared spectroscopy has many applications. Currently, it is used more often to characterize biological samples. One example is its use to characterize various types of diseases such as cancer tissue [7] or Alzheimer's disease in human grey matter samples [8]. The oxygenation of periodontal tissue was analyzed with a near-infrared spectrometer [9]. Oral films and saliva of healthy humans were analyzed with Fourier Transform Infrared (FTIR) spectroscopy [10–12].

Its major advantages are the highly selective, quantitative analysis and the non-destructive investigation of samples.

In this chapter the Beer-Lamberts law and some techniques to investigate absorption spectra are briefly discussed.

2.1 Beer-Lambert's absorption law

The Beer-Lambert's law describes the attenuation of light due to absorption[1]. It can be explained with the Maxwell equations and the introduction of a complex refractive index n_c.

The Maxwell equations for light propagation in a homogeneous medium without any sources and with the dielectric constant ε are [13]:

$$\nabla \cdot \boldsymbol{E} = 0 \tag{2.1}$$

$$\nabla \cdot \boldsymbol{B} = 0 \tag{2.2}$$

$$\nabla \times \boldsymbol{E} + \frac{1}{c}\frac{\partial \boldsymbol{B}}{\partial t} = 0 \tag{2.3}$$

$$\nabla \times \boldsymbol{B} - \frac{\varepsilon}{c}\frac{\partial \boldsymbol{E}}{\partial t} = 0 \tag{2.4}$$

where $\boldsymbol{E}$ indicates the electric, $\boldsymbol{B}$ the magnetic field, c the speed of light in vacuum and t the time. Applying the rotation operator on equation 2.3 gives:

$$\nabla \times (\nabla \times \boldsymbol{E}) = \nabla \times \left(-\frac{1}{c}\frac{\partial \boldsymbol{B}}{\partial t}\right) \tag{2.5}$$

The left hand side of equation 2.5 is then:

$$\nabla \times (\nabla \times \boldsymbol{E}) = \nabla \underbrace{(\nabla \cdot \boldsymbol{E})}_{=0 \text{ (see equation 2.1)}} - \nabla^2 \boldsymbol{E}$$

$$\Rightarrow \nabla \times (\nabla \times \boldsymbol{E}) = -\Delta \boldsymbol{E} \tag{2.6}$$

[1] This chapter was inspired by [13].

The right hand side of equation 2.5 can be simplified to:

$$\nabla \times \left(-\frac{1}{c} \frac{\partial \boldsymbol{B}}{\partial t} \right) = -\frac{1}{c} \cdot \frac{\partial}{\partial t} \underbrace{(\nabla \times \boldsymbol{B})}_{= \frac{\varepsilon}{c} \frac{\partial \boldsymbol{E}}{\partial t} \text{ (see equation 2.4)}}$$

$$\nabla \times \left(\frac{1}{c} \frac{\partial \boldsymbol{B}}{\partial t} \right) = -\frac{\varepsilon}{c^2} \frac{\partial^2}{\partial t^2} \boldsymbol{E} \tag{2.7}$$

Hence, the equation for the electric field results from equations 2.6 and 2.7:

$$\left(\frac{\partial^2}{\partial \boldsymbol{x}^2} - \frac{\varepsilon}{c^2} \frac{\partial^2}{\partial t^2} \right) \boldsymbol{E}(\boldsymbol{x}, t) = 0 \tag{2.8}$$

where $\boldsymbol{E}(\boldsymbol{x}, t)$ indicates the electric field vector at the place $\boldsymbol{x}$ and at the time t. The solution to equation 2.8 is:

$$\boldsymbol{E}(\boldsymbol{x}, t) = \boldsymbol{E}_0 e^{2\pi i n \boldsymbol{k} \boldsymbol{x} - i\omega t} \tag{2.9}$$

where $\boldsymbol{E}_0$ indicates the amplitude of the electric field vector, $n = \sqrt{\varepsilon}$ the refractive index of the medium, ω the angular frequency of the light (in vacuum), λ the wavelength of the light (in vacuum), and $\boldsymbol{k}$ the wave vector pointing in the direction of the wave propagation. The magnitude of the wave vector k, also known as wavenumber, is given as:

$$2\pi n k = n \frac{\omega}{c} = \frac{2\pi n}{\lambda}. \tag{2.10}$$

An analogue equation to equation 2.8 can be calculated for the magnetic field $\boldsymbol{B}(\boldsymbol{x}, t)$. Thus the solutions, the two plane waves, cannot be independent of each other.

Since

$$(\nabla \times \boldsymbol{E})_{x_1} = \frac{\partial E_{x_3}}{\partial x_2} - \frac{\partial A_{x_2}}{\partial x_3} = (i2\pi n(k_{x_2} E_{x_3} - k_{x_3} E_{x_2})) = i2\pi n \, (\boldsymbol{k} \times \boldsymbol{E})_{x_1}$$

it follows that $\boldsymbol{B}$, $\boldsymbol{k}$ and $\boldsymbol{E}$ are perpendicular to each other:

$$\boldsymbol{B} = \frac{2\pi c n}{\omega} \boldsymbol{k} \times \boldsymbol{E}. \tag{2.11}$$

According to equation 2.11 the only difference for a plane wave to propagate through vacuum or a medium is the speed of light being reduced to c/n [13].

This model lacks the concept of absorption which can be incorporated by introducing a complex refractive index n_c [13]:

$$n_c = n + i\kappa \tag{2.12}$$

where n indicates the refractive index and κ the attenuation factor. Inserting this relation into equation 2.9 yields:

$$\boldsymbol{E}(\boldsymbol{x}, t) = \boldsymbol{E}_0 e^{-2\pi\kappa \boldsymbol{k}\boldsymbol{x}} e^{2\pi n \boldsymbol{k}\boldsymbol{x} - i\omega t} \tag{2.13}$$

The first exponential factor of equation 2.13 indicates the attenuation of the wave caused by the absorption, the second exponential factor reflects the oscillating nature of the wave propagating through space. The light intensity is proportional to the square of the electrical field. Therefore, the light intensity $I(x)$ decays with:

$$I(x) = I(0)e^{-4\pi\kappa k x} \tag{2.14}$$

where $I(0)$ indicates the initial intensity of the light. This equation is also known as Beer-Lambert's law and describes the attenuation of light that propagates through an absorbing medium [13].

When the absorption of a substance is regarded independent of the optical path length, often the absorption coefficient is calculated for the substance. The absorption coefficient α is defined as:

$$T = e^{-\alpha \cdot l} \Longrightarrow \alpha = -ln(T)/l \tag{2.15}$$

where T is the transmission and l the optical path length. The absorption coefficient α is dependent on the wavelength $\tilde{\nu}$. From equation 2.14 we can establish the following relationship:

$$\alpha_{\tilde{\nu}} = -4\pi\kappa k.$$

Examples for α are given in sections 8.2, 9.4.1 and 9.4.5.

The absorbance is also often used to characterize a sample. Due to historical reasons, the absorbance A of a sample is defined as:

$$A = -\log_{10}(T) = -\log_{10}(I(x)/I(0)) = 4\pi k\kappa l \log_{10}(e)$$

where T indicates the transmission ($T = I(x)/I(0)$) [13].

2.2 Fourier-transform infrared (FTIR) spectrometer

Most Fourier-Transform InfraRed (FTIR) spectrometers are based on the principle of a Michelson interferometer. A thermal source provides a broad spectral wavelength range. The light is guided to a beam splitter. After splitting the beam, each partial beam is reflected from one mirror, before the beams are once more combined (cf. figure 2.1). An interference pattern is created. One of the mirrors moves continuously (in the rapid scan mode), which causes the interference pattern to vary in time. A detector is recording the interference pattern. Afterwards, the computer is calculating the Fourier transformation yielding the spectrum of the source. If a sample is placed in front of the detector its transmission can be recorded [6]. The maximal spectral resolution of FTIR spectrometer depends on the model. For the analysis of liquids and solids with relatively broad absorbance a resolution between of 0.5 and 4 cm^{-1} is sufficient (for the FTIR spectrometer used in this thesis see chapter 8).

2.3 Laser spectroscopy

Sometimes tunable lasers or laser systems are used instead of FTIR spectrometers. Besides the obvious advantages like coherent, mono-

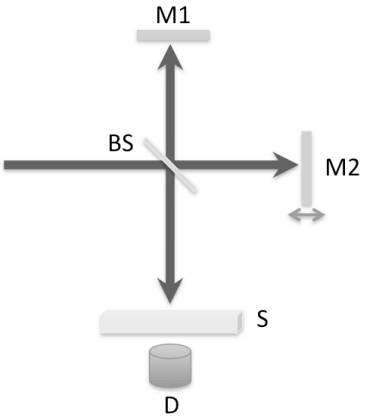


Figure 2.1: **Scheme of a Michelson Interferometer.** The light is entering from the left and detected with the detector on the bottom of the scheme. This technique is used inside an FTIR-spectrometer. (Abbreviations: BS- beam splitter; M1- mirror; M2- moving mirror; S- sample; D- detector)

chromatic light, they often offer a higher resolution than FTIR spectrometer. However, lasers only offer a much smaller spectral range for the analysis or not continuous tuning, such as CO and CO_2 lasers [14].

Other lasers do not cover the especially interesting region between 3 and 6 µm at room temperature. For example interband InGaAs/Sb lasers do not cover larger wavelengths than 3 µm at room temperature [14, 15] and most lead salt lasers require cryogenic temperature [14]. Similar problems bear interband cascade lasers (ICL) which require cryogenic cooling above ∼4 µm wavelength [14].

Some laser systems use non-linear optical effects to enable large tuning ranges. Examples are optical parametric oscillators (OPO) or difference frequency generation (DFG) [15]. These systems are usually rather complex and exhibit lower efficicency than other lasers [14, 15].

However, Quantum cascade lasers and new developments in optically-pumped lead salt lasers [16] represent promising sources. At the cost of a limited tuning range they allow a miniaturization of IR spectrometers.

2.3.1 Quantum cascade lasers

Quantum cascade lasers (QCLs) were first demonstrated in 1994 [17]. They are directly driven by current like diode lasers, but differ significantly in the underlying physics. They use intersubband transitions of electrons to generate photons [15]. Unlike in diode lasers, the emission wavelength in these unipolar semiconductor lasers is not determined by the energy gap between conduction and valence band but rather by the layer thickness. These intersubband transitions were described with the following words:

"Electrons streaming down a potential staircase sequentially emit

photons at the steps. The steps consist of coupled quantum wells in which population inversion between discrete conduction band excited states is achieved by control of tunneling." [17]

A few years after their discovery their application for spectroscopy was investigated [14]. Since a good control over the emission frequency is needed for spectroscopic applications, improvements were implemented on the laser devices like distributed feedback gratings (DFB) or external cavities. More information about quantum cascade lasers can be found in [14, 15].

Overall, QCLs are one of the most promising laser sources for spectroscopic applications.

2.4 Spectroscopic techniques

Different spectroscopic techniques were implemented over the years. Transmission spectroscopy is the most straightforward implementation for the investigation of light absorbing samples. This spectroscopic scheme is suitable for samples with medium absorbance strength like gases at higher concentrations or liquids (with a small absorption coefficient).

Samples with lower absorption coefficients or small optical path length (like thin films), are often analyzed with multi-pass cells (e.g., gases at low concentrations [18]) or with cavity ring-down spectroscopy (CRDS) [19]. These techniques enhance the signal in elongating the interaction path of the light with the analyte. However, most solids and liquids absorb too strongly to implement these techniques.

Evanescent waves can be used in versatile ways to investigate highly and weakly absorbing samples. Weakly absorbing samples are often investigated with evanescent-wave cavity ring-down spec-

troscopy or waveguide absorption spectroscopy [19]. Whereas, strongly absorbing samples (e.g., liquids or solids) are often studied with attenuated total reflection (ATR) spectroscopy [19].

In this thesis liquid and solid samples were investigated with both transmission and ATR spectroscopy.

Attenuated total reflection spectroscopy

Attenuated total reflection (ATR) spectroscopy is very suitable for the analysis of samples with strong absorbance. Light is guided into a medium with a high refraction index, e.g., a ZnSe crystal (n=2.4) and totally reflected once or several times. Each time the light is reflected at the boundary of the crystal surface and air, an evanescent wave is created (cf. figure 2.2). This evanescent wave is decaying exponentially and penetrates into the sample on top of the surface. If the sample is absorbing, the outgoing light is attenuated [12].

A transmission signal can be gained by measuring the intensity of the outgoing light without and with sample. The optical path length (i.e. the corresponding thickness of the sample) is dependent on the wavelength, the refractive index of the ZnSe crystal and the refractive index of the sample. As explained by Popov and Lavrent'ev the penetration depth d can be calculated [20]:

$$d = \frac{\lambda}{2\pi n_{ZnSe}\sqrt{sin^2\Theta - (n_{Sa}/n_{ZnSe})^2}} \tag{2.16}$$

where λ indicates the wavelength, n_{Sa} the refractive index of the sample, n_{ZnSe} the refractive index of the ZnSe crystal and Θ the angle of total internal reflection [20].

The penetration depth for, e.g., water in the infrared wavelength range[2] is $\sim 1\,\mu m$. The resulting small path length allows the inves-

[2] calculated with the refractive index data from Downing and Williams [21]

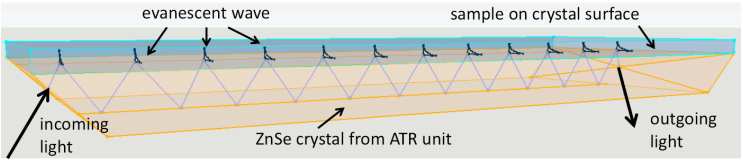


Figure 2.2: **The creation of an evanescent wave inside a ZnSe crystal.** Light is entering the crystal from the left side. It is reflected 12 times inside the crystal creating an evanescent field. The light can penetrate samples placed at the crystal's surface. The outgoing light is attenuated if the sample is absorbing [12].

tigation of strongly absorbing substances.

Chapter 3

Basic chemical principles

This chapter introduces the chemical principles of the extraction technique developed in this thesis and explained in detail in section 7.1. First a brief introduction to the law of mass action is given. Afterwards, the acid-base theory is explained to understand the behavior of the analysed drugs in water. Finally, the partition coefficient is introduced. Examples with calculated data can be found in sections 9.4.2 and 9.5.1.

3.1 Law of mass action

In this section the law of mass action, the equilibrium constant and the pH are defined[1]. All chemical reactions reach an equilibrium after a certain time. The state of equilibrium is shown with a chemical reaction equation as follows:

[1] This section is inspired by [22].

$$a\mathrm{A} + b\mathrm{B} \quad \rightleftharpoons \quad x\mathrm{X} + z\mathrm{Z} \tag{3.1}$$

where A, B, X, Z denote the substances involved in the chemical reaction while a, b, x, z indicate the stoichiometric ratios. The law of mass action describes the ratio of the educt and product concentrations [22]:

$$\frac{c^x(\mathrm{X}) \cdot c^z(\mathrm{Z})}{c^a(\mathrm{A}) \cdot c^b(\mathrm{B})} = K$$

where c indicates the concentration of the corresponding substance and K is a specific constant for the chemical reaction displayed in equation 3.1. As an example for a chemical equilibrium the autoprotolysis of H_2O is displayed with its law of mass action:

$$2\mathrm{H_2O} \rightleftharpoons \mathrm{HO^-} + \mathrm{H_3O^+} \quad \Longrightarrow \quad \frac{c(\mathrm{H_3O^+}) \cdot c(\mathrm{HO^-})}{c^2(\mathrm{H_2O})} = K \tag{3.2}$$

Since the equilibrium is strongly on the left side of the reaction depicted in equation 3.2 and the concentration of H_2O in pure water is:

$$c(\mathrm{H_2O}) = \frac{1000\,\mathrm{g\,l^{-1}}}{18.015\,\mathrm{g\,mol^{-1}}} = 55.51\,\mathrm{mol\,l^{-1}},$$

it can be included into the constant K in equation 3.2:

$$c(\mathrm{H_3O^+}) \cdot c(\mathrm{HO^-}) = K_W$$

where K_W represents the ion product of water with

$$K_W = 10^{-14}\,\mathrm{mol^2\,l^{-2}} \text{ at } 25\,^\circ\mathrm{C} \text{ [22].}$$

Since the concentration of oxonium ions H_3O^+ and hydroxide ions HO^- is equal in water, the concentration at the equilibrium is:

$$c(\mathrm{H_3O^+}) = c(\mathrm{HO^-}) = 10^{-7}\,\mathrm{mol\,l^{-1}}$$

The pH-value is defined as $\mathrm{pH} = -\log_{10}\left(\frac{c(\mathrm{H_3O^+})}{mol \cdot l^{-1}}\right)$. Hence, a pH of 7 is neutral, every pH>7 is considered basic and every pH<7 acidic [22].

3.2 Brønsted-Lowry acid-base theory

The Brønsted-Lowry acid-base theory describes a general acid base reaction as follows[2]:

$$HA + H_2O \rightleftharpoons H_3O^+ + A^- \tag{3.3}$$

where HA and A^- represent any conjugated acid-base pair. In the Brønsted acid-base theory, an acid is defined as a molecule that can liberate a proton (proton donator) and a basis as a molecule that can accept protons [22]. In this case HA is the Brønsted acid and A^- is the conjugated Brønsted base. The strength of an acid is characterized by the ability to donate a proton (to dissociate itself). As a consequence, a strong acid at equilibrium in water (like HCl) shows a much greater concentration of the basic form (Cl^-) than of the acidic form (HCl). The opposite statement is true for a strong base: the concentration of HA (in the chemical reaction in equation 3.3) is much higher than the concentration of A^-. In conclusion, a strong acid has a weak corresponding base and vice versa [22].

Weak acids are not fully dissociated when dissolved in water. For example acetic acid CH_3CO_2H forms with water the following equilibrium:

$$CH_3CO_2H + H_2O \rightleftharpoons H_3O^+ + CH_3CO_2^-$$

Hence, the law of mass action is:

$$\frac{c(H_3O^+) \cdot c(CH_3CO_2^-)}{c(CH_3CO_2H) \cdot c(H_2O)} = K.$$

For low concentrations of CH_3CO_2H the concentration of H_2O can be regarded as constant (cf. section 3.1). Therefore, the constants can be combined to $K_a = K \cdot c(H_2O)$:

$$\frac{c(H_3O^+) \cdot c(CH_3CO_2^-)}{c(CH_3CO_2H)} = K_a \tag{3.4}$$

[2]This section is inspired by [22].

where K_a is the acid dissociation constant. Often the dissociation degree α_d is used to describe an acid. It denotes how much of the acid is dissociated:

$$\alpha_d = \frac{c(CH_3CO_2^-)}{c_0(CH_3CO_2H)} = \frac{c(CH_3CO_2^-)}{c(CH_3CO_2H) + c(CH_3CO_2^-)}.$$

Here, c_0 represents the initial concentration of acetic acid molecules added to the water, i.e. $c_0(CH_3CO_2H) = c(CH_3CO_2H) + c(CH_3CO_2^-)$. α_d can be calculated with the help of K_a. The concentration of the conjugated base is equal to the concentration of H_3O^+. In addition, $c(H_3O^+)$ is equal to the difference between the total concentration $c_0(CH_3CO_2H)$ and the concentration of the not dissociated acetic acid $c(CH_3CO_2H)$:

$$c(H_3O^+) = c(CH_3CO_2^-) = c_0(CH_3CO_2H) - c(CH_3CO_2H)$$

Hence, the law of mass action in equation 3.4 can be rewritten to:

$$\begin{aligned} K_a &= \frac{c(H_3O^+) \cdot c(CH_3CO_2^-)}{c(CH_3CO_2H)} = \frac{c^2(CH_3CO_2^-)}{c_0(CH_3CO_2H) - c(CH_3CO_2^-)} \\ \Rightarrow 0 &= c^2(CH_3CO_2^-) + K_a \cdot c(CH_3CO_2^-) - K_a \cdot c_0(CH_3CO_2H) \end{aligned}$$

$$\Rightarrow c(CH_3CO_2^-) = -\frac{1}{2}K_a + \sqrt{K_a^2/4 + K_a \cdot c_0(CH_3CO_2H)}$$

The negative part of the solution is not considered since negative concentrations have no physical meaning.
The acid dissociation constant can be connected to the pH in taking the negative decade logarithm of equation 3.4 :

$$\begin{aligned} \frac{c(CH_3CO_2^-)}{c(CH_3CO_2H)} &= K_a/c(H_3O^+) \qquad (3.5) \\ \Rightarrow -\log_{10}(K_a) + \log_{10}(c(H_3O^+)) &= -\log_{10}\left(\frac{c(CH_3CO_2^-)}{c(CH_3CO_2H)}\right). \end{aligned}$$

This yields the Henderson- Hasselbalch equation [22]:

$$pH = pK_a - \log_{10}\left(\frac{c(\mathrm{CH_3CO_2H})}{c(\mathrm{CH_3CO_2^-})}\right) \tag{3.6}$$

where $pK_a = -log_{10}(K_a)$. With this equation the pH value of the solution can be calculated [22].

3.3 Partition coefficient

Many organic solvents are not miscible with each other or with water[3]. When a third compound (the solute) is added, it often dissolves in both liquids in a specific ratio. This ratio is called the partition coefficient P or K_{ow}:

$$K_{ow} = P = c_{\mathrm{solventI}}/c_{\mathrm{solventII}} \tag{3.7}$$

where c_{solventI} indicates the concentration of the solute in the first solvent and $c_{\mathrm{solventII}}$ the concentration of the solute in the second solvent [23]. However, when the solute dissociates very strongly, equation 3.7 only holds for one configuration of the solute and its according K_{ow} value. Other problems are encountered when the concentrations of the solute are very high. In the latter case the solute-solute interactions can no longer be neglected [23].
Water is a very polar solvent and solves therefore polar solutes very well. Whereas many organic solvents are less polar and thus only miscible with water to a limited extent. Liquids that solve badly in water are called hydrophobic.
Historically the most often used solvents to investigate the partition coefficient are n-octanol and water. In general there are 4 symbols used for the partition coefficient: P, K_{ow}, and D, K_{app}. Traditionally P and K_{ow} are used when a single molecular species is distributed, at low concentration, into two solvents:

$$P = K_{ow} = \frac{c_o(\mathrm{S})}{c_w(\mathrm{S})}$$

[3]This section is enspired by [23]

where $c_o(\mathrm{S})$ indicates the concentration of the solute S in octanol and $c_w(\mathrm{S})$ indicates the concentration of the solute S in water. D or K_{app} is often used when the solute is partly or completely ionized in the aqueous phase. Usually it is assumed that the dissociated solute does not enter the organic solvent phase. Since this is not fully true a correction of K_{ow} has to be made. If the pH of the solution is close to the negative logarithm of the acid dissociation constant pK_a the distribution between the two solvents can be calculated as follows:

$$K_{app} = \frac{c_o(\mathrm{HA})}{c_w(\mathrm{HA}) + c_w(\mathrm{A}^-)} \tag{3.8}$$

where K_{app} denotes the distribution coefficient, $c(\mathrm{HA})$ indicates the concentration of the associated solute (in water or octanol) and $c_w(\mathrm{A}^-)$ the concentration of the dissociated solute in water.

Dividing K_{ow} by K_{app} leads to:

$$K_{ow}/K_{app} = 1 + \frac{c_w(\mathrm{A}^-)}{c_w(\mathrm{HA})}. \tag{3.9}$$

Equation 3.5 can be written as:

$$c_w(\mathrm{A}^-)/c_w(\mathrm{HA}) = K_a/c_w(\mathrm{H_3O^+}) = 10^{pH-pK_a} \tag{3.10}$$

Thus, applying the logarithm to the basis 10 to equation 3.9 and inserting equation 3.10 into it yields:

$$\log_{10} K_{ow} = \log_{10} K_{app} + \log_{10}\left(1 + 10^{pH-pKa}\right)$$

Most often K_{ow} and K_a are given in the literature so K_{app} can be calculated:

$$\log_{10} K_{app} = \log_{10} K_{ow} - \log_{10}\left(1 + 10^{pH-pKa}\right) \tag{3.11}$$

More information about the octanol-water partition coefficient can be found in [23]. Calculations for cocaine and cutting agents of street cocaine are based on the described theory and can be found in sections 9.4.2 and 9.5.1.

Chapter 4

The body fluid saliva

The chosen matrix for the testing of cocaine and diagnosis of periodontitis in this thesis is saliva. It represents the body fluid that is easiest to access and to collect for analysis. This chapter introduces where saliva is formed, what it consists of and under which circumstances saliva changes. Experimental studies on this topic can be found in section 9.3.

4.1 Saliva production

Saliva is a mixture of fluids produced by the salivary glands, the gingival fold, oral mucosa transudate. It contains mucous of the nasal cavity and pharynx, non-adherent oral bacterial, food remainders, desquamated epithelial and blood cells. In addition, traces of medication or other chemical products can be found [24].

It consists to $\sim$ 99 % of water with various electrolytes (e.g., sodium, potassium, calcium, chloride, magnesium, bicarbonate, phosphate), proteins (e.g., enzymes, immunoglobulins, mucosal glycopro-

teins, albumin, polypeptides, oligopeptides), glucose and nitrogenous products (e.g. urea and ammonia) [24]. The composition is listed in table 4.1.

Table 4.1: **Composition of human saliva.** (Abbreviations: '+'- $< 1\,\%$ of the total protein amount or detected but not estimated; '++'- between 1 and 5 % of the total protein amount; '+++'- between 5 and 15 % of the total protein amount; '++++' - $> 15\,\%$ of the total protein amount; DHEA- dehydroepiandrosterone; PRPs- proline-rich proteins; PRG- proline-rich glycoprotein; EP-GP- proteins related to a salivary glycoprotein; U/ml- describes the active units per ml of the noted protein; CFU- colony forming units)

substance	concentration
water	$\sim 99\,\%$ [24]
sugars	5 to $10\,\mu g\,ml^{-1}$ [24]
glucose	0.02 to $0.08\,\mu mol\,l^{-1}$ [25]
electrolytes	
Na^+	2 to $26\,mmol\,l^{-1}$ [26]
K^+	13 to $40\,mmol\,l^{-1}$ [26]
Ca^{2+}	0.5 to $2.8\,mmol\,l^{-1}$ [26]
Cl^-	
Mg^{2+}	0.15 to $0.6\,mmol\,l^{-1}$ [26]
HCO_3^-	
PO_4^{3-}	2.9 to $6.5\,mmol\,l^{-1}$ [25]
total protein	300 to $810\,\mu g\,ml^{-1}$ [25]
	18 to $32\,\mu g\,ml^{-1}$ [27]
Mucins	++++ [28]
Acidic PRPs	++++[28]
Basic PRPs	++++[28]
Basic PRG	+++ [28]
Cystatins	++ [28]
Statherin	++[28]

to be continued

continued table 4.1

substance	concentration
EP-GP	+[28]
VEGh	+[28]
Lactoferrin	+[28]
Lactoperoxidase	+[28]
Haptocorrin	+[28]
β-Microseminoprotein	+[28]
Albumin	+[28]
Zn-α2 Glycoprotein	+[28]
α-Amylase	13 to 139 U/ml [25]
	++++ [28]
Lysozyme	+[28]
Kalikrein	+[28]
sIgA	40 to 139 $\mathrm{mg\,l^{-1}}$ [25]
	+++ [28]
IgG	+[28]
IgM	+[28]
Histamins	+[28]
Cortisol	9.77 to 18.87 $\mathrm{mmol\,l^{-1}}$ [25]
	0.4 to 10.2 $\mathrm{ng\,ml^{-1}}$ [27]
Testosterone	29.3 to 281 $\mathrm{pg\,ml^{-1}}$ [27]
DHEA	7.3 to 1081 $\mathrm{pg\,ml^{-1}}$ [27]
progestins	100 to 800 $\mathrm{pmol\,l^{-1}}$ [26]
corticosteroids	0.09 to 0.27 $\mathrm{mg\,ml^{-1}}$ [26]
urea	3.8 to 5.6 $\mathrm{mmol\,l^{-1}}$ [25]
Thiocyante (SCN^-)	0.83 ± 0.42 $\mathrm{mmol\,l^{-1}}$ [11]
Acetaldehyde[1]	< 0.5 μM [29]
bacteria (S. salivarius)	10^8 CFU/ml [30]
nicotine	up to ~ 3000 $\mathrm{ng\,ml^{-1}}$ [31]
caffeine	up to 8250 $\mathrm{ng\,ml^{-1}}$ [32]
	up to ~ 44 $\mathrm{mmol\,mol^{-1}}$ [33]

[1] rises significantly if alcohol is consumed

The major function of saliva is to cover, moisturize and lubricate the oral cavity, i.e. the mouth. The protection of the oral cavity is achieved with the help of mucins[2]. Mucins preserve the salivary viscoelasticity, regulate selectively the adhesion of microorganisms (bacterial or fungal colonization) to the oral tissue, protect against dehydration and proteolytic attacks[3] by microorganisms. In addition they help with the mastication[4], speech and deglutition[5] [24]. Additional functions of saliva include buffering the pH, maintenance of tooth integrity, antibacterial activity, taste and digestion [34].

At rest the salivary production is kept to a minimum. External stimuli of salivary production include mechanical, gustatory, olfactory or pharmacological stimuli. They are responsible for 80 to 90 % of the salivary fluid production. The fluid production ranges from 1 to 1.5 l per day for a healthy human [24]. The pH of saliva can vary between 5.3 and 7.8 [34].

4.2 Variations of salivary flow

A healthy adult has stimulated salivary flow (SF) rates between 1 to 3 $\mathrm{ml\,min^{-1}}$, whereas unstimulated flow rates range between 0.1 to 0.35 $\mathrm{ml\,min^{-1}}$. High variation from person-to-person can be monitored in the flow rates. Therefore, no judgment of "healthy" or "abnormal" salivary flow based on only one measurement can be made [24].

Salivary flow is influenced by many factors, e.g. the daily cycle. Low salivary flow occurs during sleep, whereas higher rates are monitored during food intake. The flow is also changing with season. In

[2] proteins with high carbohydrate content

[3] attacks that would break proteins into their constituents, i.e. smaller polypeptides or amnio acids

[4] chewing

[5] swallowing

winter higher salivary flow rates are measured than during summer [34].

Bodyposture, lighting and smoking can change salivary flow. Higher rates are recorded for standing or lying persons than for persons in a sitting position. Test persons in a dark room or blind folded persons show a lower salivary flow rate than the control. Higher flow rates are also found with smokers [24].

Individual hydration has an influence on salivary flow as well as certain medicine intake. Physical exercise changes the composition of the saliva. The usage of alcohol-containing mouthwash or alcohol intake does change the salivary secretion [24, 29] and leads to high alcohol metabolite levels, e.g. acetaldehyde [29].

Food intake in general can have a variaty of effects. The consumption of poppy seeds for example leads to raised morphine levels in saliva (up to 200 ng/ml) [35].

Part II

Cocaine

Chapter 5

Cocaine

This chapter provides the social relevance for drug tests, gives a brief history of the drug cocaine, explains the effects and metabolites of cocaine. In addition, it introduces cocaine street samples and current cocaine tests[1].

5.1 Motivation

The consumption of illegal drugs can cause hardship in society. A certain classification of illegal drugs is necessary to estimate potential harm. Parameters for this analysis can be physical harm and chances of addiction for the individual consumer. When those criteria are multiplied it is possible to order the drugs from least harmful to most harmful. Heroin and cocaine were found to be the most harmful drugs measured by these criteria [43].

Animal studies showed the severe influence of cocaine on the animal behavior. In an experiment two groups of rats could self-administrate either cocaine or heroin. In this experiment, cocaine

[1] Part of this chapter was previously published in [12, 36–42]

showed to be the drug with the higher fatality rate. The rats would alter their regular sleeping and eating rhythms. After 30 days into the experiment 90 % of the rats were dead, in the heroin group 36 % died [44].

Additional problems arise from accidents most likely caused under the influence of drugs and/or medicine. The number of these accidents increased by 50% in Switzerland between 1992 and 2010 [1]. Other European countries report this problem, too [45–47]. The effects of cocaine on driving are described in chapter 5.3.

5.2 A short history of cocaine

This section provides a brief account of the origin of the plant and the history of the consume in America and Europe.

5.2.1 The plant

The coca bush grows to a height of a few meters and is naturally found in South America and on the Indonesian Islands. The plant is easy to recognize with its spiraled, soft leaves and red bark. The botanical name is Erythroxylon (Erythros=red, xylon=wood). Its flowers are grouped and light yellow, whereas the fruit is a small, red stone fruit [48].

The cocaine is found in the leaves. Its concentration depends on the average temperature of the days during the maturing time of the plant. A temperature range between 15 °C and 20 °C without abrupt changes, an altitude between 600 and 1800 m and a very humid climate are the best conditions for good growth. Coca bushes can be harvested 4 times a year. The harvested leaves are dried on brick earth and keep their green color. They can be shipped like tobacco leaves. [48]

Besides alkaloids the coca leaves contain calcium, iron, vitamin A, B2 and E [49].

5.2.2 Early consume in South America

The oldest hints for the consume of coca leaves were found in Ecuador. A lime basin and figures of people consuming coca leaves are dated back to 3000 BC to a culture called *Valdivia*.. The oldest coca leaves were found in Peru from 1300 BC. In the Andes coca leaves were used for religious or healing rituals. The early coca leave consumption was a privilege for people with higher status or used as medicine. The medical application is still common in regions where regular medical care is not established [49].

The Spanish conquerors found the chewing of coca leaves within a religious context widely spread in 1531-1534. The Catholic Church banned its usage in 1551. Sixteen years later the church allowed the chewing again since the conquest of the land by the Spanish unbalanced the production of comestible goods. Chewing coca leaves reduces hunger and stimulates the people. Thus, the stimulation was used to increase the productivity of the gold and silver miners. As a result, a whole coca industry developed with profit comparable to the gold or silver mines [49].

In the 17th and 18th century cocaine stimulated only modest interest in Europe, which rose with the first isolation of cocaine from the coca leaves in 1860 and an enthusiastic report about the effects of cocaine. Cocaine was applied as anesthetic replacing morphine and also consumed as luxury food, e.g., in combination with vine ("Vin Mariani"). First warnings against cocaine and its potential for addiction were voiced by Erlenmeyer in 1885. In 1905 novocaine (also known as procaine) was discovered as local anesthetic and replacement drug for cocaine [49].

In conclusion, cocaine was a far spread drug, in the nineteen-

twenties. It was easily accessible through pharmacies and medical doctors as well as commonly illegally traded. In 1914 the Harrison Act tried to control the trade of opiates and cocaine in the US and prohibit the usage as recreational drug. These measures did not reduce the consumption of cocaine, but increased its price. Towards the end of the thirties the consume of cocaine decreased. Reasons for this decline are most likely the discovery of legally traded amphetamines [49].

During the so-called psychedelic revolution of the 1960s the cocaine consume increased dramatically. First it was viewed as "the champagne of drugs" and mainly consumed by the rich members of the society, but in the 1970s and 80s its use spread far beyond them. In 1972 9 % and in 1982 even 25 % of all eighteen to twenty-five year old persons had tested at least once cocaine. During the 1990s the traffic of cocaine was mainly controlled by the Colombian cartels and Mexican organizations whereas in Europe the Italian Mafia and the Russian organized crime were in control of its trade [50].

Today, the possession, trade and consume of cocaine is forbidden in Europe and North America.

5.2.3 Consume

At the beginning of the cocaine consume the coca leaves were chewed by first removing the petiole (stem) of the coca leaves. Then they are chewed shortly into a round bite of coca leaves. Afterwards an alkaline substance (e.g., chalk, sea shells, bark...) is added to the bite for an enhanced recovery of the cocaine from the leaves. Thereafter, the bite is clamped between cheek and jaw and no longer chewed. It is kept there till the bite dissolves [51]. The cocaine recovery from the leaves is enhanced due to the alkaline substance but studies show that the amount of absorbed cocaine by the chewer[2] is reduced as

[2]The amount of cocaine that enters the blood stream of the chewer is reduced.

well. Most of the cocaine is transformed to ecgonine[3] by saliva and stomach acid. Ecgonine is 80 times less toxic than cocaine [49].

Hundred grams of coca leaves can supply the daily demand of calcium, iron, vitamin A, B2 and E. The daily chewed doses was in average 60 g and thereby contributed to a better nutrition of the chewer [49]. Other traditional ways of consume of coca leaves are sniffing or eating [49].

Today, cocaine is usually sniffed, smoked (inhaled) or injected intravenously. It is often sold as cocaine hydrochloride by pharmaceutical companies. This substance is colorless, scentless, bitter tasting and water solvable. Illegal cocaine, i.e. street cocaine, is usually *cocaine base*, *free-base* and *crack* (see also section 5.5). *Cocaine base* is found in grey crystals and is a highly impure intermediate product in the synthesis of cocaine. It is found in South America and lower classes of the USA. *Free base* is purified from intermediate products and hydrochlorides.

Crack also known as *rock* is a mixture of *free base*, baking soda (natron or sodium carbonates) and water. After boiling the ingredients the mixture is cooled down very quickly. This method allows to increase the effect of cocaine on humans. The name origins from the crackle that is audible during the smoking of crack [49]. Daily doses of cocaine consumers vary between 0.1 g and 30 g of cocaine per day [48].

5.3 Effect of cocaine

Cocaine stimulates an euphoric feeling ("high") by enhancing dopamine release in the brain of consumers. This cocaine induced "high" lasts 20 to 40 min [48, 49, 52]. The positive feeling is turned into a depression after the impact of the drug wears off. The cocaine

[3] one of the metabolites of cocaine, see chapter 5.4

depression facilitates the development of a psychological addiction with the first consume.

The feelings of hunger, thirst, being awake and sleeping are disturbed. Because of these side effects consumers might develop a cocaine psychosis, which can lead to permanent damage. Other effects reported by cocaine users are paranoid delusions, fearfulness, suspicious behavior and auditory hallucinations [53].

Cocaine does influence the driving behavior. Examples for these changes are: increased nervousness, greater alertness and impaired concentration. There have also been reports of reckless and reduced driving ability [54]. Additionally, it can lead to speeding, poor impulse control, turning in front of other vehicles, high risk behavior and even loss of the control of the vehicle [55].

Reasons for exposing oneself to cocaine can be the wish to upgrade a social gathering, enhancing the productivity at the workplace, rising the mood, improvement in performance of sports or sex, self-medication, curiosity, peer pressure and ideological motivation, like breaking with a society that does not approve the consume of cocaine [56]. Most of the named expectations are not fulfilled by cocaine.

Not every consumer of cocaine will become addicted after the first consume. Some individuals are better in resisting the regular usage of cocaine than others. Research in this area showed the following potential criteria to prevent cocaine addiction: external circumstances like access to information and education, access to a working health care system, good quality of living, good atmosphere at the workplace, social networking, satisfying career chances, social support and social control. All these criteria can be helpful to prevent addiction to cocaine or for therapy from addiction. There are also some individual criteria that help preventing cocaine addiction. Examples are risk awareness, personal care about healthy

behavior, confidence in self-efficacy[4], active problem tackling, experience in overcoming challenges, social skills, religious engagement, autonomous judging and emotional stability [56].

Combination with other drugs

If cocaine is consumed with other drugs like caffeine or nicotine its effect might be enhanced since both legal drugs enhance brain dopamine release. The co-usage of nicotine usually results in chain smoking.

The liver is producing an additional psychoactive substance, cocaethylene, when cocaine is consumed in combination with alcohol. Cocaethylene is detectable in urine and blood 100 minutes after the consumption of cocaine and alcohol. It causes euphoria like cocaine but is longer lasting (up to 2.5 hours) and it is more toxic as well [52].

Sometimes cocaine is consumed in combination with marijuana. It has been reported that this can prolong the time of the effect of the drugs [57].

Cocaine is also often consumed in combination with heroin or morphine. This combination is called *speedball*. Then the effects are enhanced compared to the individual drugs and the risk of addiction is increased [58, 59].

5.4 Metabolites of Cocaine

After the consumption of cocaine its chemical degradation begins resulting in several substances. How quickly this degradation takes place was investigated in people (*in vivo*) and *ex vivo* for samples

[4]confidence in being capable to reach certain goals in taking action

from cocaine-addicted people. The degradation time in collected samples (*ex vivo*) seems to differ. Cone *et al.* claim that recovery rates of 90% of cocaine from saliva are possible after 4 days refrigerated storage or even longer if citric acid is added to the saliva sample [60]. Other scientists assessed that it is very quickly degraded into ecgonine [49].

Many studies investigated the degradation of cocaine in human test persons in various body fluids by administrating cocaine in a controlled environment and by sampling blood, plasma, urine and saliva in regular intervals (e.g. [61–68]). Most studies investigated single cocaine administration [64–66], but repeated administrations were also investigated [61–63]. These studies focus usually on a few metabolites and their development over time. The major metabolites of cocaine are benzoylecgonine (BE), ecgonine methyl ester (EME), norcocaine (NCOC). When cocaine is consumed with alcohol cocaethylene (COE) is found, too. When cocaine is smoked, anhydroecgonine methyl ester (AME) is found in all body fluids as well.

As example the results of one study is shown. This study investigated the development of cocaine, benzoylecgonine (BE) and anhydroecgonine methyl ester (AME) concentrations over time in saliva and urine [64]. The results for the temporal development of the cocaine concentration is displayed in figure 5.1. The study was based on 6 test subjects [64]. The high variation from person-to-person is obvious.

Other researchers investigated samples collected from road-side-tests [71] or from people in clinics [72–74]. Additionally post mortem investigations were done [70, 75, 76]. These studies often analyze many metabolites but do not show the development of their concentrations with time.

The degradation pathway of some metabolites is shown in figure

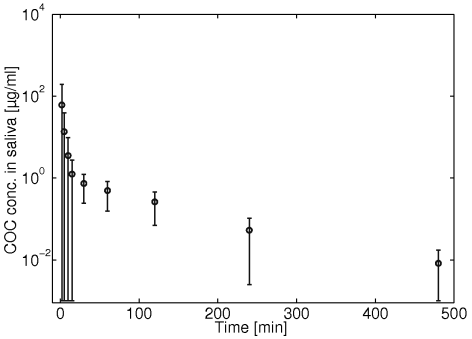


Figure 5.1: **Cocaine concentration in saliva over time.** The average cocaine concentration in saliva and its corresponding standard deviation are displayed after administrating the test persons a single dose of 40 mg cocaine. The data is acquired from [64]. (Since the value zero cannot be displayed on a log-scale the value was set to 10^{-3})

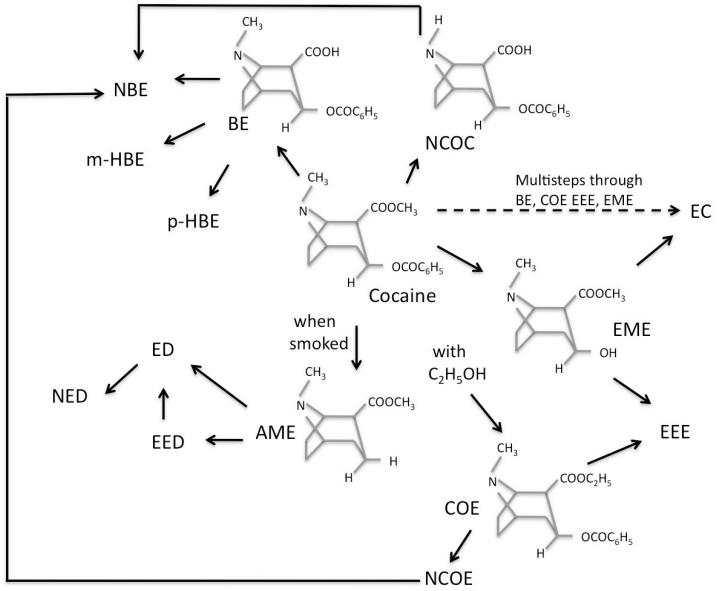


Figure 5.2: **Metabolites of Cocaine.** Degradation of cocaine in humans according to [69, 70]. Cocaine and its major metabolites are displayed in their molecular structure. (Abbreviations: BE- Benzoylecgonine; NCOC- Norcocaine; EC- Ecgonine; EME- Ecgonine methyl ester; COE- Cocaethylene; C_2H_5OH- Ethanol; COE- Cocaethylene; AME- Anhydroecgonine methyl ester; EED- Ethyl ecgonidine; ED- Ecgonidine; NED- Nor-ecgonidine; p-HBE- p-Hydroxybenzoylecgonine; m-HBE- m-Hydroxybenzoylecgonine; NBE- Benzoylborecgonine; EEE- Ecgonine ethyl ester; NCOE- Norcocaethylene)

5.2. To compare the data of the metabolites in diverse body fluids the highest concentrations found in literature are listed in table 5.1. The data emphasize the strong dependence of the concentrations of cocaine and its metabolites on the body fluid. For saliva, cocaine itself shows far higher concentrations than its metabolites during a high which is why most tests analyzing saliva target cocaine.

Various body fluids were used for the analysis of drugs or metabolites but the most often used and most reliable matrices in living people are saliva, urine and blood. Most on-road-side tests are using saliva as matrix for their drug tests since it is easy to collect and can be collected by non-medical staff (in contrast to blood). In addition, fewer privacy issues are concerned compared to urine collection. Under laboratory conditions blood or plasma samples are most often encountered.

5.5 Cutting agents of street cocaine

Cocaine is sold in different forms on the street (cf. section 5.2.3) and many different cutting agents are encountered in street cocaine. They can be categorized as diluents and adulterants. Diluents are pharmacologically inert cutting agents like sugars, or baby powder whereas adulterants have an effect on humans like the local anesthetic lidocaine.

Street samples are analyzed for multiple purpose. First, the sentence for the possession of drugs depends on the concentration of drugs in the seized powder. Second, some adulterants are a higher threat to health than cocaine itself. Third, the economics of the illegal drug market, e.g., origin of the drug, can be determined by the composition of the street sample [85].

In 1973, 73 % of all the seized street cocaine samples contained

Table 5.1: **Concentrations of cocaine and metabolites in various body fluids.** CAS numbers and molecular formulas are from [77, 78]. Concentrations displayed in italic font were found in plasma and concentrations in brackets were found post-mortem. (Abbreviations: m.c.- max. concentration in saliva/urine/blood; mm- major metabolite; m- metabolite)

metabolite	CAS	chemical formula	class	m.c. saliva [ng ml^{-1}]	m.c. blood [ng ml^{-1}]	m.c. urine [ng ml^{-1}]	references
Cocaine (COC)			parent drug	500 000 [64]	*1 351* [62] (2 982 [75])	∼700 000 [63] (415 594 [75])	all plus [79]
Benzoylecgonine (BE)	519-09-5	$C_{16}H_{19}NO_4$	mm	5 046 [62]	*5 389* [62] (5 723 [75])	524 000 [70] (830 377 [75])	[60, 62, 65–70] & [72–76, 80–84]
Norcocaine (NCOC)	18717-72-1	$C_{16}H_{19}NO_4$	mm	1 784 [62]	*18* [68]	42 [70] (132 [70])	[62, 64–66, 68–70] & [72–74]
Benzoylnor-ecgonine (NBE)	41889-45-6	$C_{15}H_{17}NO_4$	m of BE &NCOC	4.7 [72]	*385* [62] (175 [70])	72 172 [70] (61 024 [70])	[62, 66, 69, 70] & [72–74]
Ecgonine Methyl Ester (EME)	7143-04-1	$C_{10}H_{17}NO_3$	mm	22 197 [62]	*3 073* [62] (2 430 [75])	33 003 [73] (191 078 [75])	[60, 62, 65–70] & [72–74, 80, 82–84]
Cocaethylene (COE)	529-38-4	$C_{18}H_{23}NO_4$	only with C_2H_6O	342 [84]	*7* [66] (7 [70])	68 [70] (2 576 [70])	[67, 69, 70] & [72–74, 80, 84]
Norcoca-ethylene (NCOE)	137220-02-1	$C_{17}H_{21}NO_4$	m of COE			14 [70] (9 [70])	[69, 70, 72–74]
Ecgonine Ethyl Ester (EEE)	70939-97-8	$C_{11}H_{19}NO_3$	m of EME and COE		7 [66] (52 [70])	3 648 [73] (4 931 [70])	[70, 72–74]
Anhydroecgonine Methyl Ester (AME)	43021-26-7	$C_{10}H_{15}NO_2$	only if smoked	4 374 [64]	*8* [66] (42 [76])	224 [70] (2 030 [76])	[69, 70, 72–74] & [76, 84]
Ecgonidine (ED)	484-93-5	$C_9H_{19}NO_2$	m of AME		(773 [76])	2 937 [70] (7 452 [76])	[70, 76]
Ethyl Ecgonidine (EED)	73045-45-1	$C_{11}H_{17}NO_2$	m of AME			(39 [70])	[70]
Nor-Ecgonidine (NED)	150283-68-4	$C_8H_{11}NO_2$	m of AME			163 [70] (75 [70])	[70]
Ecgonine (EC)	481-37-8	$C_9H_{15}NO_3$	m of EME, BE, COE, EEC	25.9 [72]	(2 167 [75])	341 187 [70] (68 346 [75])	[70, 73, 82, 84]
m-Hydroxybenzoyl-ecgonine (m-HBE)	129944-99-6	$C_{16}H_{19}NO_5$	m of BE		*17* [68] (31 [70])	1 525 [70] (2 456 [70])	[68, 70, 72, 73]
p-Hydroxybezoyl-ecgonine (p-HBE)	90899-22-2	$C_{16}H_{19}NO_5$	m of BE	336 [62]	*85* [66] (88 [70])	6 939 [70] (5 409 [70])	[62, 68, 70, 72, 73]
m-Hydroxycocaine	71387-58-1	$C_{17}H_{21}NO_5$		<few [72]	*13* [68]	88 [73]	[66, 68, 72, 73]
p-Hydroxycocaine	89339-16-2	$C_{17}H_{21}NO_5$		609 [62]	*13* [68]		[62, 66, 68, 72, 73]

no adulterants in the USA, whereas 27 % contained at least one of the following adulterants: procaine, amphetamine, caffeine, phencyclidine. In addition, the following diluents were found: sugars, inositol, mannitol, talc, flour and cornstarch. Even these pharmacological inert substances bear great risk when injected intravenously. They can result in emboli and obstruct the blood flow to many vital organs [86].

From 1985 to 1993 street samples were collected in Spain. At the beginning of the study lidocaine dominated as an adulterant but towards the end of the study caffeine was the most common one. Pricetam and procaine were secondary to the principal adulterants [87].

In 1995 common adulterants found in street cocaine samples in Spain were caffeine, procaine.HCl, lidocaine.HCl and piracetam. Common diluents were glucose, lactose, sucrose, starch and mannitol [88]. In 1995, an analysis of street cocaine samples in the UK found an average cocaine concentration of 51 %. Furthermore, the average concentration of crack cocaine in crack was found to be 85 %. The most often used adulterants were lidocaine[5], paracetamol, procaine, benzocaine and caffeine. The most common diluents were glucose and other sugars [89]. From 1996 to 1997 the following adulterants were found in Rome (listed in descending likelihood of occurrence): lidocaine, caffeine, phenacetine, dipyrone, phenmetrazine, ephedrine, nor-aminophenazone, salicylamide, procaine, aminophenazone, diphenidramine, phendimetrazine, phenobarbital and phentermine. The most common diluents were sugars [90].

In 2001 caffeine, lidocaine and prilocaine were the most common adulterants in Brazil. Starch and sugars were found as diluents [91]. Street cocaine samples were also analysed in the Netherlands from 1999 to 2007. The following adulterants were found, listed

[5]also known as lignocaine

in descending likelihood: phenacetin, caffeine, lidocaine, procaine, dilatiazem, levamisol, hydroxyzin [92]. In France the following adulterants were detected in 2006: phenacetin, caffeine, paracetamol, diliazem and lidocaine [93]. A study in 2008 classified caffeine, anhydrous D-glucose, mannitol, lactose, maltose, talc powder, flour, and baby formula as common cutting agents [94].

In conclusion, phenacetin and caffeine are of the most common adulterants today. Local anesthetics are also very common adulterants. The most common diluents are starch and sugars. A spectral analysis of selected adulterants and diluents can be found in section 9.2. Additionally, a spectral analysis of steet samples can be found in section 9.5.

5.6 Cutoff concentration of cocaine in saliva

For deciding whether a sample is positive or negative on a drug test a cutoff limit has to be set. This means all samples that have a concentration above this limit are positive and all below are negative. So far Europe does not have a consistent cutoff level.

In UK the cutoff for cocaine and metabolites in oral fluid are at $30\,\mathrm{ng\,ml^{-1}}$ and the confirmation cutoff[6] is $15\,\mathrm{ng\,ml^{-1}}$ [95]. In Sweden a zero-concentration limit for controlled substances in the blood of drivers is in force since 1st July 1999 [71, 96].

The U.S. Substance Abuse and Mental Health Services Administration (SAMHSA) suggests cutoffs for cocaine and metabolites in oral fluid of $20\,\mathrm{ng\,ml^{-1}}$ and confirmation cutoffs of $8\,\mathrm{ng\,ml^{-1}}$ [95] (as metabolites ecgonine methyl ester and benzoylecgonine are usually

[6]The confirmation cutoff concentration is the concentration used at the second laboratory test, if the initial test was positive.

measured, some methods also investigate cocaethylene [67]).

Law enforcement in most countries only accepts laboratory results from blood tests. Therefore, the current cutoff concentration for cocaine in saliva is still subject to discussion. As mentioned above in section 5.3 the duration of a cocaine "high" is 20 to 45 min [48, 49, 52]. The average cocaine concentration in saliva after 30 min is $700 \pm 500\,\mathrm{ng\,ml^{-1}}$ (see chapter 5.4, figure 5.1 [64]). Recently, the EU-project DRUID suggested an equivalent cutoff concentration for cocaine in saliva of $170\,\mathrm{ng\,ml^{-1}}$ compared to the cutoff of $10\,\mathrm{ng\,ml^{-1}}$ in blood [97]. This accounts for the much higher concentration of cocaine in saliva than in blood (see section 5.4).

5.7 Current measurement methods

There are many ways to detect cocaine in different body fluids (like urine, saliva, blood, plasma) and also solids (like nails [94], hair [98], teeth [99]). This section introduces some of the detection schemes with their limits of detection[7].

The methods are divided in 3 categories: laboratory techniques like gas chromatography-mass spectroscopy (GC-MS), on-site tests (field tests) mostly based on immunoassays and new tests which are trying to bridge the gap between the complex and expensive laboratory techniques and the field tests. Depending on the category the tests are characterized by different criteria (cf. figure 5.3). The on-site tests need to be quick and compact, whereas laboratory tests have no need for transportation and can afford longer measuring times. New tests should require only little training and give quantitative or at least semi-quantitative (i.e. order of magnitude) results.

[7] parts of this chapter have been previously published in [12, 36]

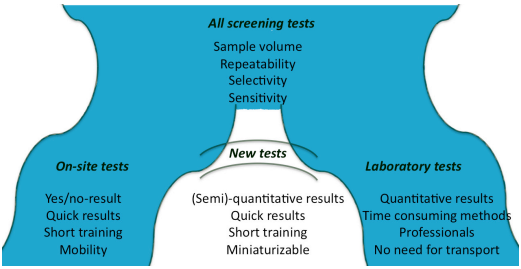


Figure 5.3: **Criteria for drug tests of the three categories:** Laboratory tests, on-site tests and new tests bridging the gap between the two others [36].

5.7.1 Standard laboratory tests

The most common laboratory analysis method for cocaine is gas or liquid chromatography (GC/LC) combined with (tandem) mass spectroscopy (MS). Often the body liquid samples need to be prepared by solid-phase extraction (SPE) [71, 74, 76, 84, 99–107]. The limit of detection (LOD) for GC/MS analysis were found between 1 to $5\,\mathrm{ng\,ml^{-1}}$ for cocaine and its metabolites in body fluids [74, 108]. Other gas chromatographic techniques for the detection of cocaine are for example SPE GC-flame ionization detection [80] or narrow bore capillary gas chromatography (CGCI) [85].

High-pressure liquid chromatography (HPLC) reaches LODs of $20\,\mathrm{ng\,ml^{-1}}$ for cocaine and BE [108], whereas the limit of detection of LC-MS is observed between 0.5 to $1\,\mathrm{ng\,ml^{-1}}$ [71, 106, 109] for only $100\,\mu\mathrm{l}$ samples of oral fluid [109]. Similar LODs are reported for LC-MS/MS [72, 80, 81, 110–115]. High performance liquid chromatography with diode array detection (HPLC-DAD) achieves a limit of detection at $10\,\mathrm{ng\,ml^{-1}}$ for cocaine, cocaethylene and benzoylecgonine in saliva [80, 116].

5.7.2 Common on-site tests

Most often immunoassays are used as on-site tests. They are based on an antigen-antibody reaction. Immunoassays are usually sold in test-stripe form and are very compact. They offer binary results (positive/negative), thus providing no quantitative information. Some of the tests have the potential for improvement in terms of reliability [117, 118]. Unfortunately, the success rate of some of the tests can be as low as 75 % [109]. Positive on-site test results need to be confirmed with laboratory tests on blood or plasma.

Many different brands of on-site tests are currently on the market. They are usually characterized by their cutoff concentration. For example DrugWipe® (Securetec) test [119, 120] is characterized by a cutoff concentration of $50\,\mathrm{ng\,ml^{-1}}$ for cocaine in sweat and saliva [119]. The test has the size of a pen and is one of the most often used on-site drug tests for cocaine, cannabinoids, benzodiazepines, amphetamines and methamphetamines. It can be acquired with a reading device to minimize human error [119] and has an acquisition time of approximately 2 min [121]. When assessed in the field the reports differ. A study confirmed the DrugWipe cutoff without any false results [120, 122]. Others found an accuracy of 98.6% [123] or under 80% [124].

The OraLab® test (Varian) is characterized by a cutoff concentration of $20\,\mathrm{ng\,ml^{-1}}$ for cocaine in saliva (manufactor statement). One advantage of this test is that half of the sample can be stored separately for confirmation tests. Test results must be interpreted 10 to 15 min after application (collection time 3 min). The accuracy of the OraLab Test for cocaine was 98.3% in this study [109].

Another test by Draeger offers a cutoff concentration of $5\,\mathrm{ng\,ml^{-1}}$ for cocaine in saliva samples [109]. The DrugTest 5000 is available

with an analyzer which reads the test result and interprets it into a positive or negative outcome. The accuracy of the DrugTest for cocaine varies between 97.6 % [109] and 96.9 % [125]. The testing time is approximately 17 min and the success rate[8] for a test result is 90 % [109].

At the moment the Cozart® company is selling two different products for on-site saliva drug tests: RapiScan® and DDS (Drug Detection System). The Cozart® DDS oral fluid collection device has collection times between under 10 s and 105 s and their cocaine recovery with the Cozart® DDS Buffer was 100% [126]. The manufacturer's cutoff concentration is assessed to $10\,\mathrm{ng\,ml^{-1}}$ but could not be confirmed by other studies [117]. The Cozart® RapiScan® can scan up to 5 substances at once (amphetamine, cocaine, opiate, benzodiazepine and either cannabionoide or methadone). The analysis time was between 3 and 12 min [127]. Numbers for the accuracy differ between 99.4 % [128], 96.6 % [129] and 100 % [130].

For the Uplink® test (OraSure) the cutoff concentration was determined to $200\,\mathrm{ng\,ml^{-1}}$ (by the manufacturer) for cocaine and could not be confirmed (only 1 in 10 positive samples were tested correctly) [122]. Nevertheless, according to the manufacturer the test can detect benzoylecgonine as well (cutoff $10\,\mathrm{ng\,ml^{-1}}$) which was confirmed in this study [122].

The Oratect® (Branan) has a cutoff of concentration of $20\,\mathrm{ng\,ml^{-1}}$ for cocaine which was confirmed with artificial samples [122]. UltiMed's SalivaScreen has a manufacturers cutoff at $30\,\mathrm{ng\,ml^{-1}}$, but failed to detect 7 out of 10 times samples with a cocaine concentration of $40\,\mathrm{ng\,ml^{-1}}$ [117].

In conclusion, there are many commercial providers for cocaine tests. Their limit of detection and runtime is compared in table 5.2.

[8] The probability to gain any test result.

Table 5.2: **Comparison between commercial on-site tests.** The measurement time, accuracy and cutoff concentrations are compared between the tests. (Abbreviations: cutoff conc.- cutoff concentration; COC- Cocaine; BE- Benzoylecgonine; COE- Cocaethylene; EME- Ecgonine methyl ester; n.m.- not mentioned)

test	target	cutoff conc. $[\mathrm{ng\,ml^{-1}}]$	accuracy	time	reference
Securetec Drugwipe	COC	50	>90%	3-10 min	[119, 122]
Varian OraLab	COC	10-20	98.30%	15 min	[109, 117]
OraSure Uplink	BE	10	high	n.m.	[122]
	COC	200	$\sim$ 10%	n.m.	[122]
Branan Oratect	COC	20	confirmed	n.m.	[122, 128]
UltiMed SalivaScreen	COC	40-30	65%	13 min	[117]
Draeger	COC	5	>90%	17 min	[109]
Draeger	BE	200	n.m.	n.m.	[131]
DrugTest 5000	COE	500	n.m.	n.m.	[131]
	EME	10000	n.m.	n.m.	[131]
cozart RapiScan	COC	300	n.m.	4 -13 min	[127]
	BE	30	96-100%	n.m.	[127, 129, 130]
	COE	200	n.m.	n.m.	[127]
Cozart DDS	BE	30	n.m.	3 min -6 min	[132]
	COC	100	n.m.	3 min -6 min	[132]
	COE	65	n.m.	3 min -6 min	[132]

So far, none of the easy-to-use commercial on-site-tests are able to measure quantitatively. They only provide a positive or negative result with respect to the cutoff concentration.

5.7.3 Nonstandard and new tests

These tests are based on various principles. Radioimmunoassays for example were used to investigate urine [133] and hair [134]. Nuclear magnetic resonance-studies [85, 135] were utilized to differentiate the different components of illicit cocaine [85]. Spin immunoassays show a sensitivity for benzoylecgonine of $1\,\mu g\,ml^{-1}$ (30 seconds measurement time) [136]. Thin-layer chromatography has a limit of detection for methylecgonine of $1\,\mu g\,ml^{-1}$ [137].

Very often the validation of cocaine involves optical schemes combined with immunoassays. For example the evanescent wave flow fluorometer utilizes monoclonal antibodies and a fluorescent benzoylecgonine derivate to reach a limit of detection (LOD) of $5\,ng\,ml^{-1}$ in their flow buffer (no experiments with body fluids reported) [108]. A similar technique was used by the flow immunosensor achieving an LOD of $5\,ng\,ml^{-1}$ in an acquisition time of less than a minute. But no experiments with body fluids were reported [133]. Enzyme-linked immunoassays are based on a change in absorbance when cocaine binds to the antigen. This method achieves LODs in the $ng\,ml^{-1}$ range but requires extensive sample preparation [133]. A fiber optic biosensor utilizing a competitive fluorescence immunoassay achieved an LOD of $50\,ng\,ml^{-1}$ for benzoylecgonine (a cocaine metabolite) in urine [138].

Sometimes aptameric sensors are combined with optical methods. Aptameric sensors exploit a strain of oligonucleotides, which are designed to specifically bind to a certain molecule. The binding induces a conformational change in the oligonucleotides, which is detected by various methods, e.g. fluorescence. The most important optical techniques are bulk fluorescence assays [139] and

fluorescent aptameric sensors [140]. The latter achieved an LOD of 121.3 ng ml^{-1} in serum [140]. Surface-enhanced Raman scattering spectroscopy (SERS) [141], fluorescence [142] and quantum dots [143] in combination with aptameric sensors lead to even lower LOD, but these techniques were not applied to body fluids, so far.

Raman spectroscopy is very often performed to detect cocaine on solid samples like finger nails [94], natural or synthetic fibers (clothes) [144], cyanoacrylate-fumed fingerprints [145], passenger bags on airports [146] and rum bottles[9] [147]. Metal-doped sol-gels in combination with Surface-Enhanced Raman Scattering (SERS) were used for the detection of drugs in saliva. The method achieved an LOD of 250 μg ml^{-1} in saliva [148]. Another study combined SERS with photonic crystals. The acquisition time was between 10 and 15 sec. Cocaine and benzoylecgonine were successfully identified (cocaine at a concentration of 30 μg/ml; no body fluids), but the limit of detection has not been given [149]. Raman spectroscopy in combination with Kerr gated fluorescence was also used for the laboratory analysis of street samples [150].

Other spectroscopic techniques were applied on body fluids and solid samples, e.g. Terahertz spectroscopy for solid samples [151–153]. Cocaine in urine was investigated with UV spectroscopy after a liquid-liquid extraction yielding an LOD of 1.25 µg ml^{-1} [154]. Infrared spectroscopy was used to analyze solid street samples [85, 88, 155].

Some of the introduced methods are summarized in table 5.3. The most important methods to investigate body fluids will be compared to the work described in this thesis in section 9.4.9.

[9] Sometimes cocaine is smuggled dissolved in ethanol. With Raman spectroscopy the content of the sealed bottle can be analyzed.

Table 5.3: **Detection methods for cocaine and/or metabolites.** (Abbreviations: AME- Anhydro ecgonine methyl ester; COC- Cocaine; BE- Benzoylecgonine; COE- Cocaethylene; EME- Ecgonine methyl ester; apt.- aptameric sensor; evan.- evanescent wave; n.m.- not mentioned; spec.- spectroscopy)

method	target	LOD ng ml^{-1}	time	reference
GC-MS	COC	1	$>$ 51 min	[74]
	BE	5	$>$ 51 min	[74]
High pressure LC	COC +BE	20	n.m.	[108]
LC-MS	COC + BE	0.5	$>$ 37 min	[109]
LC-MS/MS	COC + BE	0.5	very long	[110]
HPLC-DAD	COC+BE+COE	10	n.m.	[80, 116]
Raman-spec.	COC + BE	250 000	10 min	[148]
	COC (BE+EME+AME)	1-3200 μM	10 min	[141]
	COC (+BE)	30 000	15 sec	[149]
spin immunoassay	BE	500 - 1 000	30 sec	[136]
thin layer chrom.	ME	1 000	n.m.	[137]
flow immunosensor	COC	5	1 min	[133]
immunoassay evan.	COC + COE	5	5-12 min	[108]
UV spec.	BE	1.25	n.m.	[154]
fluorescent apt.	COC	121.3	$>$ 120 min	[140]

5.8 Current extraction techniques

Many of the above mentioned techniques need sample preparation to reduce the background signal of the body fluid. Most often solid phase extraction (SPE) is applied. The term SPE includes a variety of protocols. But it usually requires expensive and bulky equipment for the extraction. Centrifuges, temperature-controlled evaporators and complex filtering devices like conditioned columns, cartridges or plates are implemented in the extraction process [84, 156–158]. In addition, SPE is time consuming and needs specially trained staff, hence, it is not suitable for testing in the field.

An alternative extraction method for cocaine in saliva is Microwave Assisted Extraction (MAE). This technique involves special equipment like centrifuges, too. It was validated as a sample preparation technique for High Pressure Liquid Chromatography with diode array detection [159].

5.9 False results on drug tests

Drug testing of employees at the workplace is conducted with increasing frequency. These tests are either performed on fixed or randomly chosen dates to ensure that the workers are always "clean"[10]. In the USA even sport coaches at schools are allowed to test the children for drug consume. More and more products are offered for parents to secretly test their children for drug abuse. As an example, the product "Hair Confirm Standard 7-Drugs" from "Test medical symptoms @ home, Inc." only requires the parents to cut a strip of hair of their children and to send it in for analysis.

Hair test results are strongly questioned by a study which investigated children of cocaine-using mothers. The adults had aver-

[10] i.e. not under the influence of drugs

age cocaine and benzoylecgonine concentrations of $2.4\,\mathrm{ng\,mg^{-1}}$ and $0.39\,\mathrm{ng\,mg^{-1}}$, respectively, in hair. Their children had concentrations of $2.4\,\mathrm{ng\,mg^{-1}}$ cocaine and $0.74\,\mathrm{ng\,mg^{-1}}$ benzoylecognine in their hair [81]. Hence, without any further investigations the conclusion would be that the children consumed equally or even more cocaine than their parents, even so they were passive consumers. Most likely the cocaine got in contact with the hair of the children, where it dissolved with a fluid like sweat and was then absorbed by the hair [81, 98]. Hence, a cocaine contaminated desk, fingers or toys provide a simple explanation for these test results. As a consequence, the results of a positive hair drug test do not reflect the cocaine consume of a person, but might rather hint on cocaine contamination in his or her environment.

Urine or saliva tests are more reliable than hair tests as less contamination can occur. None of the children in the case described above had detectable drug traces in the urine (cut off at 300 ng/ml BE) in contrast to the adults [81]. Drug tests based on the analysis of saliva are better suited for detecting recent drug abuse.

Today, there is a big market for products promising cocaine and other drug users that their drug test will be negative if they use a specific product to clean body fluids or hair of any toxins, e.g., shampoos, dissolvable pills, mouthwashes, diets and herbal miracle teas [160–162].

In principle there are two groups of masking substances. The first group is aimed to dilute the body fluid or increase the metabolism. For example diuretics or extensive consume of liquids are recommended, as well as, exercising to increase the metabolism. The second group are masking substances that result either in a positive test that can be explained (e.g. consume of poppy seeds leads to an increased morphine level) or hinder the proper function of the on-site test. An example for the attempt to omit correct results is the usage of vinegar to change the pH of the body fluid [160–162].

Most of these "suggestions" concern masking and therefore falsifying urine tests, but some might be applied to saliva as well, which is why some of these substances should be investigated.

In conclusion, checking the pH-value seems to be a good step when working with body fluids in general. Also the effect of mouthwashes has to be taken into account when testing for saliva especially when it has been applied < 10 min before the test. Rinsing the oral cavity with water before sampling seems to be necessary. Investigation of stimulated saliva might be of advantage, to minimize endogenous influences. Saliva can be stimulated by chewing paraffin (might filter drugs out) or sucking a sour candy (changes the pH).

Certainly, the mouth of the person has to checked before obtaining the sample so that a masking or dilution of the sample is impeded.

Chapter 6

Saliva acquisition

In the following section the saliva sampling protocol is described. Saliva production is influenced by multiple factors[1]. Some of these factors were investigated experimentally in section 9.3, others are summarized in chapter 4. Since the circumstances prior and during sampling are of great importance, a questionnaire about the recent oral history had to be answered by each participant taking part in our studies. All tests were conducted with the approval of the ethic committee of the ETH Zurich. The information sheet for participants, the questionnaire and the consent form for participants can be found in the Appendices A, B and C, respectively.

Almost all saliva samples were taken from people with an empty stomach to keep variations as small as possible. Volunteers were asked to brush their teeth at least 30 min before sampling. Brushing the teeth helps to form a comparable basis for the tests. The minimum of 30 min delay between the brushing and salivating was necessary to minimize the influence of the different toothpastes on saliva. Before each sampling the test person was asked to rinse

[1] In addition to the named references, parts of this chapter have been published previously [12, 36–41].

her/his mouth with tap water for at least 30 s. After disgorging the tap water and swallowing empty twice, the sampling started. Samples were taken in the morning hours, in bright light and an upright sitting position. All those factors are influencing the saliva production (cf. chapter 4). Samples were collected in a glass test tube.

Two different groups of saliva samples were analyzed in this part of the thesis. The first group consisted of saliva acquired under different circumstances. These investigations started with the analysis of the infrared (IR) spectrum of saliva from a person with an empty stomach. Afterwards the influence on the IR spectrum of saliva of externally introduced changes in the oral cavity like for example the consumption of food was studied. Six different situations were analyzed for one to two test persons. The results and details concerning these investigations can be found in section 9.3 (details can also be found in [12, 37]).

The second group of saliva samples was used to validate the cocaine extraction process. Most of these samples were taken from test persons with an empty stomach. The sample volume was between 7 and 30 ml to provide sufficient sample volume for comparison specimens and the extraction procedure (cf. section 7.1.2 and [36–38]). In total 8 persons (2 female and 6 male) participated in the saliva study. Their age ranged from 25 to 64 years. Some of these test persons were asked to salivate once with an empty stomach and a second time after an externally induced change in the oral cavity, like for example the consumption of food or the smoking of a cigarette (for results see section 9.4.1 and 9.4).

The saliva acquisition for the caffeine study was essentially the same. Details about the saliva acquisition for the periodonitits trial can be found in section 14.2.

Chapter 7

Sample preparation

This section discusses the one-step extraction for cocaine followed by a description of the preparation of the water-based samples[1].

7.1 Extraction of cocaine

Detecting drugs in saliva or other water based solutions at low concentrations is very challenging. Saliva consists of $\sim 99\,\%$ of water and water absorbs strongly in the infrared spectral range. Even when saliva samples are dried on top of the ATR crystal, major absorption features of the saliva remain and interfere with the caffeine or cocaine spectrum (cf. section 9.4.1 and figure 9.10). Hence, a small change in the absorbance has to be detected on a strong absorbance background signal[2]. An extraction is expected to improve the determination of cocaine concentrations. In the following the criteria for the solvents selected for the extraction method as well as the sample preparation are described.

[1] Parts of this chapter have been published previously [12, 36–42].
[2] caused by the saliva

7.1.1 Criteria for the choice of the solvent

Three criteria for the extraction procedure were defined: simplicity, quick extraction and no use of bulky laboratory equipment. The idea was to be able to confine the extraction process in a mobile, stand-alone, hand-held device based on a micro-fluidic chip. Therefore, a one-step extraction process was developed and tested for several solvents.

Solvents considered for extraction were chosen based on the following criteria:

- low water solubility
- lower absorbance than water in the spectral range of high cocaine[3] absorption between $1770\,\mathrm{cm}^{-1}$ and $1700\,\mathrm{cm}^{-1}$ and around $1270\,\mathrm{cm}^{-1}$ [12]
- higher solubility of cocaine[4] in the solvent than in water (ideally high partition coefficient)
- compatible with saliva (e.g. no protein extraction)
- liquid between $-10\,^{\circ}\mathrm{C}$ and $40\,^{\circ}\mathrm{C}$ (for ideal storage conditions)
- compatible with the material used for the production of the micro fluidic channel

Based on these criteria the following solvents were chosen to test cocaine extraction: 1-octanol, iso-octane, n-heptane, dibutyl ether, toluene, dichloroethane and tetrachloroethylene[5]. Since Norland optical adhesive 81 (NOA 81) was chosen as material for the microfluidic extraction device its compatibility with the selected solvents was tested, as well [163].

[3] or high caffeine absorption between $1720\,\mathrm{cm}^{-1}$ and $1650\,\mathrm{cm}^{-1}$
[4] or caffeine for caffeine extraction
[5] also known as tetrachloroethene or perchloroethene

For the extraction the cocaine was dissolved in saliva and then extracted with the solvents. Details of this extraction process are described in the following section. The results of the extraction can be found in section 9.4.

7.1.2 Cocaine extraction

In this thesis saliva samples spiked with cocaine or metabolites were investigated. No administration of cocaine was conducted. Often spiked samples are used to validate an extraction process [74, 82, 84]. It allows to ensure reproducibility of the extraction process since the concentration in the samples is known and not subject to person-to-person variations (see chapter 5.4). Two sample preparation methods were developed and are explained in this section.

In the first method saliva was filtered with sterile syringe filters (0.2 µm, Sarsted). Cocaine was weighed[6] into a glass tube and then mixed with the filtered saliva (cf. figure 7.1). A part of the filtered and unspiked saliva was kept as first comparison specimen. An ultrasonic bath supported the dissolving of the drug or metabolite in saliva. During the 30 min of sonication, the sample was surrounded by ice to prevent heating. The second comparison specimen was then set aside (saliva spiked with cocaine) and the other part of the sample was diluted with saliva to the desired concentration. From each spiked saliva sample 1.6 ml were mixed with 1.6 ml of the solvent used for the extraction. The sample was mixed by manually shaking for at least 30 seconds. After the self-separation of the liquids the saliva and the solvent phase were measured to compare the concentration of cocaine in both liquids. For some solvents a third phase formed consisting of stable droplets in one of the liquids (cf. figure 7.2). For some solvents these droplets could be destroyed by either adding isopropanol, by applying an ultrasonic bath or by cen-

[6] Different scales were used, but most samples were weighed with a Mettler Toledo Classic AB 265-S.

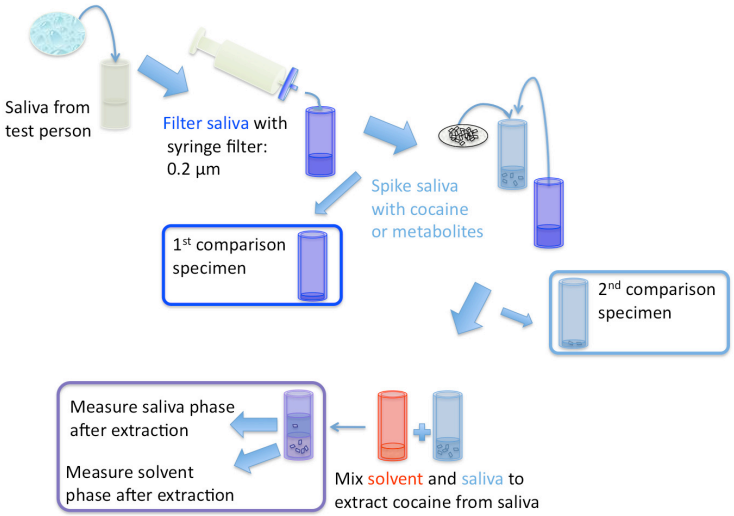


Figure 7.1: **First extraction method.** Saliva is filtered with syringe filters and mixed with cocaine. Then the cocaine is extracted from the saliva with a solvent. Afterwards the solvent and saliva phase are separated and analyzed. The spectra are compared with the spectra of the pure and spiked saliva samples (comparison specimen).

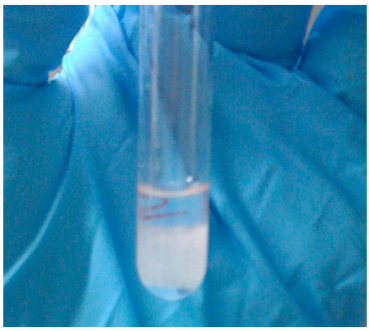


Figure 7.2: **Octanol saliva mixture.** Mixing Octanol with saliva formed stable droplets that formed a third, milky phase between the saliva and the octanol phase. The picture was taken 11 days after mixing.

trifugation of the samples (for more details see section 9.4).

The extraction method was successful, but the measurements showed high variations in the absorbance signal. The variations were most likely enhanced by the uncertainty in the weighing process of the cocaine (most likely caused by vibrations or pressure changes in the room), or by the changes in the pH of the saliva (cf. chapter 3). Therefore two changes of the extraction method were implemented (cf. figure 7.3). First, the cocaine was no longer weighed. Instead of weighing the cocaine, a calibrated cocaine solution in acetonitrile (Lipomed) was purchased and pipetted[7] into the test tube. The acetonitrile evaporated over night at ambient temperature and pressure. As a result a thin film of cocaine was visible at the bottom of the test tubes. These films were dissolved with the filtered saliva in an ultrasonic bath (30 min) while the sample was surrounded by

[7] Pipettman 50-200 µl

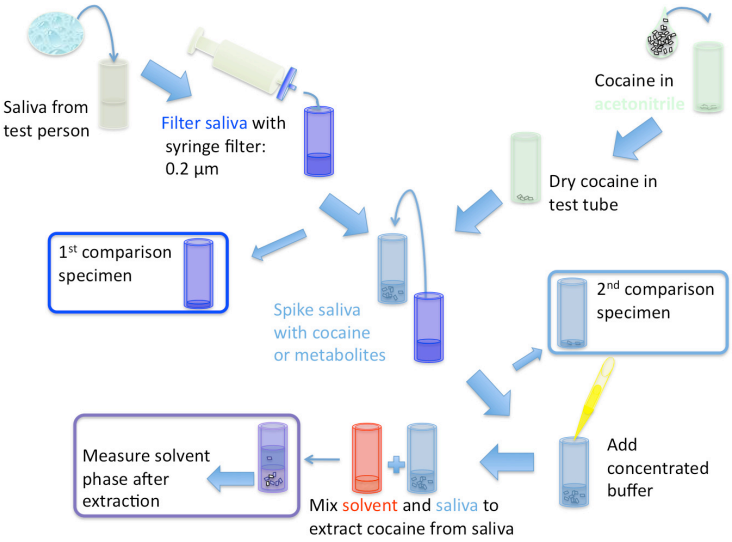


Figure 7.3: **Second extraction method.** Saliva is filtered with syringe filters, mixed with cocaine. A pH buffer is added to the saliva cocaine mixture. Then the cocaine is extracted with a solvent. Afterwards the solvent phase is separated and measured. The spectra of both comparison specimens are measure to detect potential irregularities in the saliva [36].

ice to prevent heating. The second change was a stabilization of the pH with a concentrated buffer (Titrisol). The mixing ratio of saliva : buffer was 9 : 1. Afterwards the extraction solvent was added. A measurement of the saliva after the extraction was not possible anymore due to the drastic change in the pH. Afterwards the extraction solvent was added in a 1 : 1 ratio to the saliva. The differences in the results of the two extraction methods are presented in section 9.4.

Furthermore, potential impurities in the extracts were identified by the analysis and comparison between the spectra of the extract and the spectra of reference samples. For these samples the cocaine was dissolved in the extraction solvent. Their spectra were measured at different concentrations and compared with the spectra of the extracts to confirm the cocaine extraction. As a comparison specimen the pure solvent was analyzed, too, to investigate impurities in the solvent.

In addition, the extraction method was validated for street samples of cocaine. Street samples contain many adulterants and other impurities (cf. section 5.5). The samples were acquired from the Forensic Science Institute Zurich in powder form. Thus, the cocaine concentration in the liquid was prepared by weighing. The mixing and extraction process followed the second extraction method.

7.2 Water based samples

Cocaine and metabolites used for water-based samples were bought from Lipomed. The other products for the water-based samples were bought in regular shops, pharmacies and health food shops. Most of the samples were acquired in powder form. They were weighed (Mettler Toledo Classic AB 265-S) and diluted with demineralized water to acquire the desired concentrations. Some samples were pressed in pills, e.g. painkillers. For these samples the pills were mashed into powder form and then treated like described above.

Chapter 8

FTIR-ATR analysis

Most samples were analyzed with a Fourier Transform Infrared (FTIR) spectrometer[1] from Perkin Elmer (Paragon PC 1000) with a spectral resolution of 1 cm^{-1}. The samples of street cocaine and of the substances hydroxyzine, phenacetin, levamisole and creatine were analyzed with a Bruker Vertex 80 FTIR spectrometer (resolution $1\,cm^{-1}$). Attenuated total reflection (ATR) measurements were conducted by a matching ATR unit for the Perkin Elmer FTIR. It consists of 4 mirrors that guide the light into and out of the ZnSe crystal that is mounted in the top plate of the ATR unit. This top plate is exchangeable to support between 11 and 12 reflections inside the crystal.

The sample volume was 400 µl. All samples were measured at least 3 times[2]. The number of averaged scans that contributed to one spectrum[3] strongly depended on the solvent of the sample. Water based samples had at least 20 scans, whereas the interaction time with the ATR crystal was kept shorter for more aggressive sol-

[1] Part of this chapter was previously published in [12, 36–41].
[2] Each sample is placed 3 times on the ATR crystal to be measured.
[3] One measurement is one spectrum and consists of many scans.

vents like dichloroethane (only 5 scans for each sample). Spectra are displayed in the spectral range from 2000 to 1000 cm^{-1}.

8.1 Measurement noise

The measurement noise of the absorption depends on the wavelength for two reasons. First, the wavelength range between 2000 and 1350 cm^{-1} exhibits strong water vapor absorption. Therefore, variations in the concentration of the ambient water vapor influence the detection of cocaine[4] (cf. figure 8.1). The FWHM (full width of half maximum) of the water vapor of $< 0.2\,\mathrm{cm}^{-1}$ is smaller than the resolution of the FTIR spectrometer. However, the absorption is detectable since the displayed spectrum is a convolution between the absorption line and the instrument response function. An experimental compensation of the water vapor absorption is not fully possible since the reproducibility of the wavenumber is not accurate enough. Hence, an increase in the noise is observed for the spectral range between 2000 and 1350 cm^{-1} compared to wavelengths outside this range.

The second reason for the measurement uncertainty (noise) is inherent in the measurement method. The intensity of the source light is not constant in the spectral range. A lower intensity implies a higher noise. This increase in noise is detectable at the water vapor absorption lines and towards the lower wavelength range, where the intensity of the light source is decreased.

Figure 8.1 B displays the standard deviation of the light source spectrum for three consecutive measurement cycles (cf. table 8.1). Each spectrum depicted in (black, red, blue) is based on the standard deviation between the 3 cycles.

[4] i.e., strong interferences are caused by the water vapor absorptions.

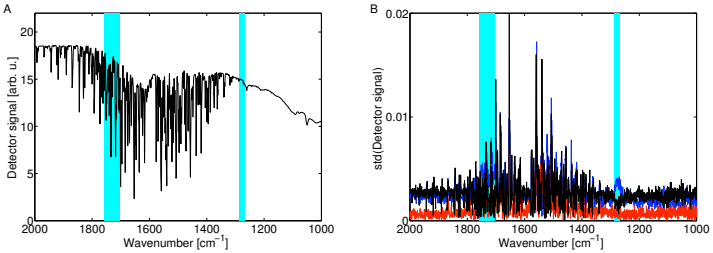


Figure 8.1: **Analysis of the measurement noise.** A: Spectrum of the light source of the FTIR-spectrometer is shown. The water vapor absorption is observed in narrow absorption lines between 2000 and 1350 cm^{-1}. B: Standard deviations of light source spectra measured for 3 different consecutive measurement cycles are shown three times (black, blue, red). The difference in the standard deviation is caused by stronger or weaker changes of the humidity during the three measurement cycles. Highlighted areas mark high cocaine absorption (cf. chapter 9).

8.2 Drying technique

The measurement of a homogenous liquid has the advantage that infrared measurements yield a quantitative result. Unfortunately, almost all solvents absorb in the infrared. Water for example has an absorption coefficient α varying between α=235 cm^{-1} (at the wavelength 1880 cm^{-1}) and α=2738 cm^{-1} (at 1630 cm^{-1} [21]) in the mid-infrared spectral range from 2000 to 1000 cm^{-1} (for more details see section 9.4.1). Saliva mostly consists of water, thus the LOD is increased for solutes like cocaine. However, when saliva is dried on top of the ATR crystal, the absorption of the solvent decreases and a lower LOD can be expected. A steady, controlled airflow was used to speed up the drying process. The drying of saliva on the ZnSe crystal takes about 30 min at ambient temperature and pres-

sure. Organic solvents are more volatile and can be dried in less than 7 min.

The increase in the signal has two reasons. First the concentration of the solute in the solvent rises during the drying process which results in a higher absorption. The second reason is found in the logarithmic decrease of the evanescent field outside the ZnSe crystal. The drying process decreases the spatial difference between the solute and the crystal surface which further enhances the signal.

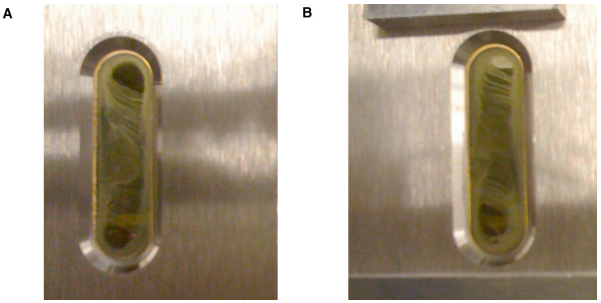


Figure 8.2: **Differences in drying patterns.** Picture A and B show two different drying pattern of saliva samples.

The disadvantage of the drying process is that the solute does not always form the same pattern (cf. figure 8.2). Hence the measurement uncertainty is increased. This variation is the reason why each sample is dried and measured at least 3 times and the reason why the measurements of the dried samples are only semi-quantitative. Despite this disadvantage, the limit of detection is significantly lower with the drying process than without. For this reason, drying was implemented in the measurement protocol (cf. table 8.1).

8.3 Experimental measurement technique

Different samples were analyzed in this thesis. One group of samples was only analyzed in its liquid phase. For these samples the spectra of the solvent was recorded in addition to the sample itself.

The second group of samples were samples that were measured in liquid and dried form. For these samples the measurement protocol according to table 8.1 was applied.

The first power spectrum of the light source was used as a basis for compensation for the spectrum of the liquid sample taking into account both spectral power variations of the light source and absorption changes of the ambient water vapor. The compensation was done by dividing the liquid sample spectrum by the light source spectrum (both are recoded in transmission). In the same way, the second spectrum of the light source was used for compensating the spectrum of the dried sample.

8.4 Data processing for cocaine extracts

The measured spectra of the extracts s_m were fitted with selected, previously measured absorbance spectra. The fit was calculated by Matlab and based on the linear combination s_{fit} (cf. equation 8.1 and figure 8.3). The parameters can be divided into two groups. The first group consists of a cocaine spectrum of a reference sample with a high signal-to-noise ratio. This spectrum can be used to evaluate the cocaine concentration of the extract with the help of a calibration based on reference samples with different cocaine concentrations ($A \cdot s_A$). The second group of spectra can compensate partly for impurities in the sample or changes in the experimental conditions (e.g., in humidity). Spectral characteristics of impurities of the sample, e.g., protein residuals from saliva ($E \cdot s_E$ and $F \cdot s_F$),

Table 8.1: **Protocol for dried samples.** To increase the signal-to-noise ratio this protocol was established for dried samples. (One full measurement cycle is depicted.)

Measurement of the spectrum of the light source
Spectral measurement of the solvent
Spectral measurement of the sample
Drying of the sample
Spectral measurement of the dried sample
Cleaning of the ATR crystal
Measurement of the spectrum of the light source

of contamination of the extraction solvent before the extraction, or changes in the experimental conditions ($D \cdot s_D$) were included in the spectral fit. In addition, a first order baseline fit ($-B - C\tilde{\nu}$) was included to account for scattered light[5]. Hence, s_m is approximated as:

$$s_m \approx s_{fit} = A \cdot s_A - B - C \cdot \tilde{\nu} + D \cdot s_D + E \cdot s_E + F \cdot s_F. \quad (8.1)$$

where $A,\ B,\ C,\ D,\ E,\ F$ indicate the fitting parameters and $\tilde{\nu}$ denotes the wavenumber. For fitting the spectra only the wavelength ranges of strong cocaine absorption were used (1800 to 1650 cm^{-1} and 1300 to 1200 cm^{-1})[6]. The parameter A scales the cocaine spectrum to the investigated spectrum and can therefore be correlated with the cocaine concentration in the extract (cf. equation 8.2). The quality of the sample and the stability of the experimental conditions, e.g., humidity changes of the ambient air, are indicated by the other parameters.

[5] scattered light was also observed by [164]

[6] Samples extracted according to the first extraction method were fitted to the range from 1820 to 1650 cm^{-1} and from 1350 to 1200 cm^{-1}.

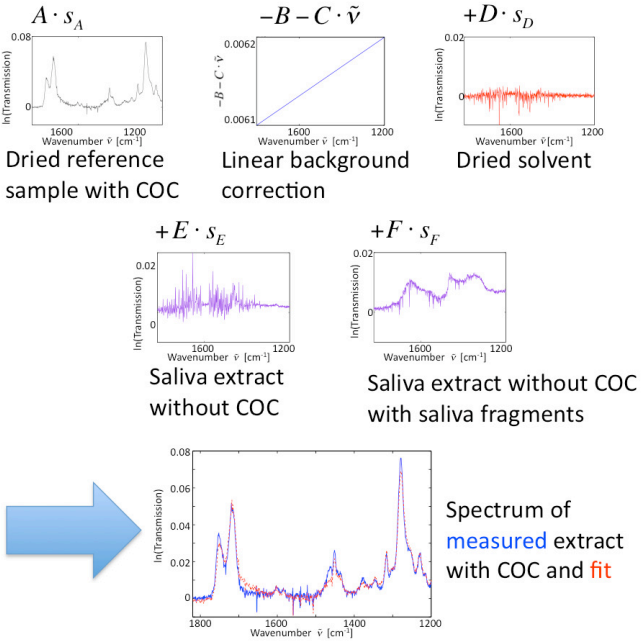


Figure 8.3: **Fitting method for the extracts.** The fitting parameter A can be correlated to the cocaine (COC) concentration, whereas B, C, D, E, F take impurities of both, the sample and the solvent as well as the temporal variations of the experimental conditions such as changes in humidity into account.

8.4.1 Efficiency of the cocaine extraction

In this section the extraction efficiency η is introduced. In contrast to the log K_{ow}-value, the extraction efficiency indicates directly how much of the initial cocaine concentration is extracted from saliva into the solvent. The extraction efficiency η is calculated for cocaine and adulterants in sections 9.4.2 and 9.5.1, respectively.

To calculate the cocaine content in the solvent a correlation between the cocaine concentration and the fitting parameter A (see Equation (8.1)) is established with the help of reference samples (solvent spiked with cocaine) of known concentration $c_{initial}$ yielding a linear relation:

$$A = m \cdot c_{initial} + k$$

where k indicates the intercept and m the slope of the linear relation. In inverting this linear relation the unknown concentration c_{cal} in an extract is calculated:

$$c_{cal} = \frac{A - k}{m}. \tag{8.2}$$

The extraction efficiency η is defined as the ratio between the concentration c_{cal} in the extract and the initial concentration:

$$\eta = c_{cal}/c_{initial}. \tag{8.3}$$

Chapter 9

Results of the FTIR-ATR analysis

In this chapter the spectra of cocaine and its major metabolites[1] are presented and possible interferences with other substances are analyzed. Thereafter, the results of the extraction are discussed[2].

9.1 Spectra of cocaine and metabolites

The infrared spectrum of cocaine depends on the state of cocaine, i.e. powder form, solution or suspension. Examples for this dependence are depicted in figure 9.1 and table 9.1.

The spectrum of cocaine is influenced by the optical absorption of the solvent. As an example, there is more than one order of magnitude difference in the infrared absorbance between water and tetrachloroethylene (TCE, cf. figure 9.1). The other major differ-

[1] more information about the metabolites can be found in section 5.4
[2] Part of this chapter was published in [12, 36–41]

Table 9.1: **Comparison of wavelength position of selected cocaine absorption peaks.** Reported positions of cocaine absorption peaks 1-6 (cf. figure 9.1 A for assignments) compared with experimental data in this thesis. The relative magnitude of the peaks differ from the shown spectrum in figure 9.1 but also vary between the sources. However, good agreement of the peak position with the literature is found. (Abbreviations: app.- approximate estimated peak position; nl- not listed; susp.- suspension in H_2O; tol- toluene; TCE- tetrachloroethylene; hep- n-heptane; n.sig.- represent no significant peaks)[12]

Form	Peak 1 [cm^{-1}]	Peak 2 [cm^{-1}]	Peak 3 [cm^{-1}]	Peak 4 [cm^{-1}]	Peak 5 [cm^{-1}]	Peak 6 [cm^{-1}]	Ref.
Powder	app. 1736	app. 1714	app. 1268	nl	app. 1107	nl	[165]
Powder	1734.5	1705.9	1272.6	1227.7	1107.5	1035.6	[166]
Powder	1733.7	1706.7	nl	nl	nl	nl	[155]
Powder	1729.9	app. 1710	app. 1278	app. 1230	1109.4	1027.5	[167]
Powder	1728-1725	1711-1706	app. 1263	app. 1232	app. 1110	app. 1020	[88]
Susp. in H_2O	1734	1706	1274	1227	1110	1037	this thesis/[12]
Solved in tol	1754	1718	1278	n.sig.	n.sig.	n.sig.	this thesis
Solved in TCE	1756	1720	1278	n.sig.	n.sig.	n.sig.	this thesis
Solved in hep	1756	1720	1278	n.sig.	n.sig.	n.sig.	this thesis

ence in these spectra is caused by the difference in solubility. While, cocaine base solves well in TCE, it is more challenging to solve it in water. The single powder grains have to be sonicated with the water before they can dissolve properly. The spectra depicted in figure 9.1 A were recorded from an non-sonicated sample. Therefore, this sample was rather a suspension than a solution. Consequently, the depicted spectra is a superposition of solid cocaine and cocaine dissolved in water. Since the solid particles in the suspension settle over time on the surface of the ATR crystal, where the evanescent field is strongest, their contribution to the spectrum is larger than in a solution (cf. figure 9.1B). Furthermore it is evident that there is a slight shift in the position of the absorbance peaks.

Literature data are compared to the results acquired during this thesis (cf. table 9.1). The reported peak position in literature and this thesis are in good agreement. However, the magnitude of the absorption peaks varies markedly between the different sources.

The absorbance is given in arbitrary units because the transmission measurements through the ATR crystal (cf. chapter 2) do not include any corrections for the pathlength of the evanescent field. The absorbance spectra of the metabolites of cocaine are depicted in figure 9.2. Their quantitative appearance differs due to different concentrations and different solubility characteristics. For example, benzoylecgonine dissolves much better in water than cocaethylene (COE). Despite these differences, qualitative information about the absorbance of the metabolites can be gained.

Spectral interference[3] for the detection of cocaine is caused by cocaethylene. However, this might rather be of advantage to detect people on a cocaine-induced "high", since cocaethylene is an

[3]Spectral interferences are encountered when one substance absorbs in the same spectral range like another one. This results in the detection of the superimposed absorbance, which might yield a wrong identification or quantification of the substance.

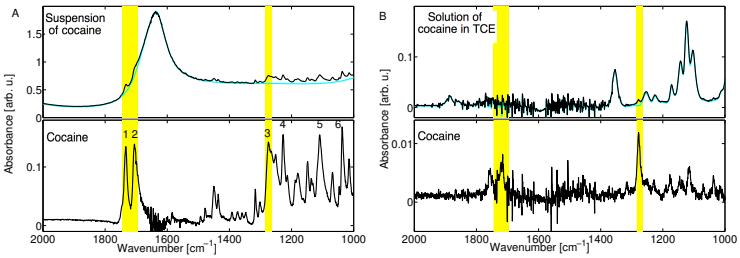


Figure 9.1: **Absorbance spectra of cocaine.** A: A suspension of cocaine ($1.26\,\mathrm{mg\,ml^{-1}}$) in water is depicted with the water absorbance in cyan. The spectrum on the bottom is showing the difference of the two spectra above. B: Solution of cocaine in tetrachloroethylene ($500\,\mu\mathrm{g\,ml^{-1}}$ COC in TCE). In the upper graph the spectrum of cocaine in TCE and TCE are depicted. The lower graph shows the difference of both spectra. The yellow areas mark high cocaine absorbance in water. The absorbance spectrum of cocaine evidently depends not only on its concentration but also on the solvent and its state (suspension or solution).

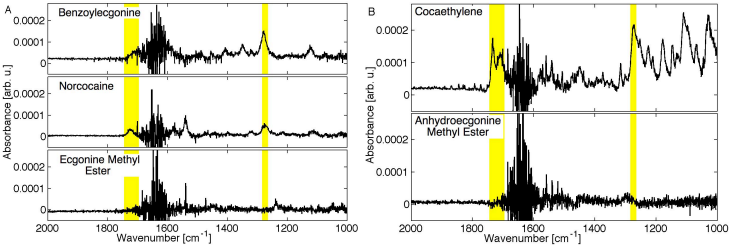


Figure 9.2: **Spectra of metabolites of cocaine.** A: Spectra of benzoylecgonione (BE, $1.7\,\mathrm{mg\,ml^{-1}}$), norcocaine (NCOC, $0.6\,\mathrm{mg\,ml^{-1}}$) and ecgonine methyl ester (EME, $> 0.3\,\mathrm{mg\,ml^{-1}}$) suspensions in water are depicted. B: Spectra of cocaethylene (COE, $1.7\,\mathrm{mg\,ml^{-1}}$) and anhydroecgonine ethyl ester (AME, $0.8\,\mathrm{mg\,ml^{-1}}$) suspensions in H_2O are displayed. The background absorption of water was subtracted from each spectrum. Highlighted areas are the spectral regions of high cocaine absorption (cf. figure 9.1 A) .

even more psychoactive drug than cocaine. Nevertheless, the COE concentration is significantly smaller during a "high" than the concentration of cocaine in saliva (cf. section 5.4) and therefore the influence on the detection of cocaine should be rather small.

9.2 Potentially interfering substances

Numerous substances were investigated with respect to a potential spectral interference with the cocaine spectrum. Substances common in everydays environment like soft or energy drinks were assessed as well as substances recommended to yield a false negative result on a drug test (cf. section 5.9). In addition many cutting agents were investigated since they are also occurring in legal contexts like baking powder or sugars. Finally, potential interference with common medicine was examined. A list of the investigated

substances is found in table 9.2.

Table 9.2: **List of the investigated substances that might cause spectral interferences with the cocaine spectrum.** The spectra can be found in the following denoted figures. (Abbreviations: ep- everyday product; ma- masking agent; ca- cutting agent; ad- adulterant; cm- common medicine; ED budget- Energie Drink Pr!x Garantie; $MgCO_3$- magnesium carbonate)

substance	ep	ma	ca	ad	cm	figure
Coca-Cola	x					9.3
Coca-Cola light	x					E.1 (appendix)
Coca-Cola zero	x					E.1 (appendix)
Assurgin (sweetener)	x					E.1 (appendix)
Vodka	x					9.5
Red Bull	x					9.3
Guarana	x					13.1
ED budget	x					9.3
Coffee	x					13.1
Milk powder	x					E.1 (appendix)
Egg white	x					E.1 (appendix)
Egg yolk	x					E.1 (appendix)
White vinegar	x	x				E.1 (appendix)
Red balsamic vinegar	x	x				E.1 (appendix)
Merdiol	x	x				9.4
Lysterine	x	x				9.4
Dentofit	x	x				9.4
Vitamin C	x				x	9.4
Birth control pill	x				x	9.4
Panadol					x	9.4
Aspirin					x	9.4
Dolormin					x	9.4
Manitol			x		x	9.5

to be continued

continued table 9.2

substance	ep	ma	ca	ad	cm	figure
Lactose	x		x			9.3
Glucose	x		x			9.3
Saccarose	x		x			9.3
Fructose	x		x			9.3
Maltose	x		x			9.3
Maizena (starch)	x		x			E.1 (appendix)
Baby powder	x		x			E.1 (appendix)
$MgCO_3$	x		x			E.1 (appendix)
Caffeine	x		x	x		9.5
Natron	x		x			9.5
Baking powder/soda	x		x			9.5
Benzocaine			x	x		9.6
Lidocaine			x	x		9.6
Hydroxyzin			x	x		9.6
Levamisol			x	x		9.6
Phenacetin			x	x		9.6
Creatine			x	x		9.6

The spectra of sugars are displayed in figure 9.3 A. Sugars are often used to dilute street cocaine samples (cf. section 5.5) since they are cheap and readily available. But of course they also appear in other context. Lactose is a disaccharide consisting of galactose and glucose. It naturally occurs in milk. Maltose[4], i.e. malt sugar, is a disaccharide, too. Saccharose also called sucrose is the traditional household sugar. It is a disaccharide consisting of glucose and fructose whereas glucose and fructose are monosaccharides. All these sugars are available in white powder form and have their major absorption between 1150 and 1000 cm^{-1}. In conclusion, the spectra of assessed sugars show no major interference with the absorption spectra of cocaine, neither in the spectral range around 1730 cm^{-1} nor around 1270 cm^{-1}.

[4]consists two joint glucose molecules

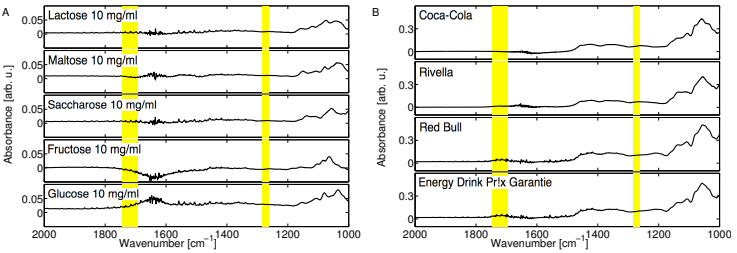


Figure 9.3: **Spectra of everyday products.** The background absorption of water was subtracted from each spectra. A: Spectra of common sugars dissolved in water at a concentration of $10\,\mathrm{mg\,ml^{-1}}$ (also used as diluents for street cocaine) are shown. Their major absorption is between 1150 and $1000\,\mathrm{cm^{-1}}$. B: Spectra of common soft and energy drinks containing sugar are depicted. Major absorption is observed in the sugar absorption range. Highlighted areas mark high cocaine absorption. The spectra of sugar and sugar containing soft drinks do not interfere with the cocaine spectra, neither around $1730\,\mathrm{cm^{-1}}$ nor around $1270\,\mathrm{cm^{-1}}$.

Common soft and energy drinks were analyzed since they are consumed via the oral cavity. The spectra of Coca-Cola, Rivella[5], Red Bull and Energy Drink Pr!x Garantie[6] are displayed in figure 9.3 B. Their spectra are dominated by the sugar absorption between 1150 and 1000 cm^{-1}. Their absorption is significantly higher around 1270 cm^{-1} than around the first two absorption peaks of cocaine in the spectral range around 1730 cm^{-1}.

Mouthwashes are often recommended to disguise the usage of drugs (cf. chapter 5.9). In this thesis common mouth washes were investigated. The spectra of the mouthwash brands Dentofit, Meridol and Listerine are depicted in figure 9.4 A. The spectrum of Listerine is dominated by the alcohol absorption between 1150 and 1000 cm^{-1}. Dentofit also strongly absorbs in this spectral range. All mouthwashes absorb around 1270 cm^{-1}, but almost no interferences were observed around 1730 cm^{-1} where the first two distinct cocaine absorption peaks are located.

Common medicine was investigated to analyze potential interferences with the cocaine spectrum. The following brands of painkillers were investigated: Dolormin (active substance ibuprofen 6.66 mg/ml), Panadol (active substance paracetamol 17 mg/ml) and Aspirin (active substance acetylsalicylic acid 6.6 mg/ml). The spectra are depicted in figure 9.4 B. The major absorbance was detected between 1100 and 1000 cm^{-1}. The birth control pill Yasmin was examined as well. It contains the active hormones Dospirenonum[7] (0.2 mg/ml) and Ethinylestradiolum[8] (0.002 mg/ml). Major absorbance was observed between 1100 and 1000 cm^{-1}. Vitamin C showed potential for spectral interference with cocaine, but the investigated concentration of 10 $mg\,ml^{-1}$ exceeds common concentration in saliva. Fur-

[5] a famous Swiss soft drink based on whey
[6] brand name of a low budget energy drink
[7] also known as drospirenone
[8] also known as ethinyl estradiol

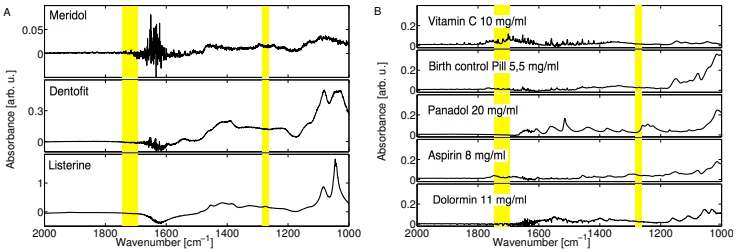


Figure 9.4: **Spectra of mouthwash and common medicine.** The background absorption of water was subtracted from each spectrum. A: Spectra of common mouthwashes are displayed. The major absorbance of the mouthwashes was found between 1200 and 1000 cm^{-1}. B: Spectra of common medicine like vitamin C, birth control pill and common pain killers (Panadol, Aspirin and Dolormin) are depicted. Only minor interferences are found at the first two cocaine absorption peaks around 1730 cm^{-1}. Highlighted areas mark high cocaine absorption.

thermore, its absorption peaks are differently shaped compared to the cocaine spectrum. Thus, a differentiation can be made between the two substances in measuring the cocaine spectrum at 2 different wavelengths.

The measured spectra of common cutting agents are shown in figure 9.5 A. Mannitol is used as legal medicine and absorbs mainly in the spectral range from 1100 to 1000 cm^{-1}. Caffeine is a very widely spread substance and has an absorbance peak at $\sim$ 1700 cm^{-1}. Therefore, a major interference is expected between the cocaine detection at the second cocaine peak (cf. figure 9.1) and the caffeine absorbance. However, the first cocaine absorbance peak is not affected by this interference. Natron[9] and baking powder are very important cutting agents since they are used to produce crack[10]. Their major absorption range does not interfere with the cocaine absorption around $\sim$ 1730 cm^{-1}.

Since the combination of the consume of the drug cocaine and alcohol is common (cf. section 5.3), the spectra of vodka and an ethanol-water mixture were analyzed (cf. figure 9.5 B). Major absorbance occurs in the spectral range between 1100 and 1000 cm^{-1}. Only minor interference is detectable for the spectral range around 1730 cm^{-1}.

Spectra of some of the most common adulterants are depicted in figure 9.6. While the former substances were analyzed when dissolved in water, these substances were dissolved in TCE (phenacetin, levamisole, hydroxyzine, creatine) or water (lidocaine, benzocaine) and then dried on the ATR crystal before recording the spectra. This procedure allows a higher signal-to-noise ratio. Unfortunately, some problems with the dissolving of the adulterants occurred since not all the substances dissolve very well. Therefore, the spectra can-

[9] also known as sodium carbonate decahydrate
[10] a widespread form of cocaine (cf. section 5.2.3)

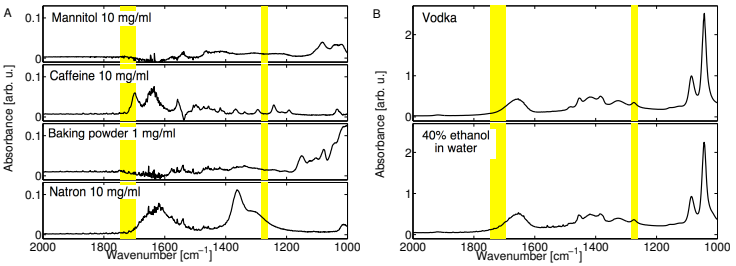


Figure 9.5: **Spectra of adulterants and alcohol.** The background absorption of water was subtracted from each spectrum. A: Spectra of mannitol, caffeine, baking powder and natron are displayed. B: Spectra of vodka and of a water-ethanol mixture are depicted. Highlighted areas mark high cocaine absorption. Only caffeine has major interferences with the second cocaine peak (cf. figure 9.1). However, no major spectral interference with the first peak at $\sim$1734 cm^{-1} is found.

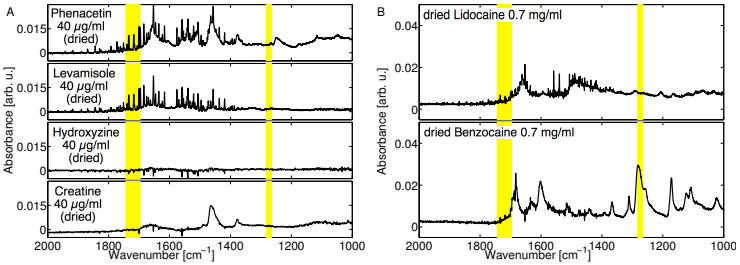


Figure 9.6: **Spectra of adulterants.** A: Spectra of adulterants dissolved in TCE and then dried on the ATR crystal are depicted (phenacetin, levamisole hydrochloride, hydroxyzine dihydrochloride and creatine). B: Spectra of adulterants dissolved in H_2O (lidocaine and benzocaine) and then dried on the ATR crystal are displayed. Highlighted areas mark high cocaine absorption. No major interference with the spectral interval around $1730\,\mathrm{cm}^{-1}$ are found.

not be taken quantitatively, but offer qualitative information about the spectrum of the substance. No major interference is recorded for the spectral range around $1730\,\mathrm{cm}^{-1}$.

The spectra of some additional substances can be found in the appendix E.

In conclusion, major spectral interferences were found for the spectral range between 1300 and $1000\,\mathrm{cm}^{-1}$ and only very minor interferences for the cocaine absorption at the first peak around $1730\,\mathrm{cm}^{-1}$.

9.3 Saliva

Saliva in six different conditions was spectrally investigated (cf. table 9.3). In addition, a spectral comparison of the absorbance of

the supernatant of healthy persons and test persons with aggressive periodonotits was made (results are in chapter 15).

Table 9.3: **Overview of the saliva trial.** Saliva spectra from different test persons in different situations were taken and compared to the saliva spectra of the fasting person. The situations are listed in the table with the figure number of the spectra.

circumstances	figure
saliva after red bull	9.7
saliva after the usage of mouthwash	9.7
saliva after poppyseed cake consumption	9.8
saliva after alcohol consume	9.8
saliva after smoking	9.9
saliva after coffee	13.1
supernatant of saliva form patients with Aggressive periodontitis	15.1

The saliva spectrum of a fasting person is compared to the saliva spectrum of a person who consumed two energy drinks. The change of the saliva composition and therefore of its spectrum is depicted in figure 9.7 A. The change is most obvious in the spectral range between 1400 and 1000 cm^{-1}. This coincides with the region of the highest absorption of the energy drinks (cf. figure 9.3 B). The change in the spectrum is evident for over an hour. Thereafter, the spectrum returns (within the error) to its initial signature. The change in absorbance at $\sim$1000 cm^{-1} is a measurement artifact. The noise is increased due to water vapor absorbance (cf. chapter 8).

A similar change is visible for the influence of the mouthwash Meridol on the spectrum of saliva. In contrast to the energy drink the spectrum returns after 30 min to its initial characteristic (cf. fig-

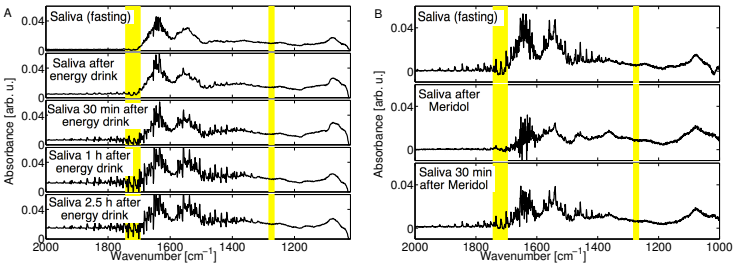


Figure 9.7: **Spectra of saliva before and after the consume of two energy drinks and the application of a mouthwash.** The background absorption of water was subtracted from the spectra. A: Changes in the spectrum of saliva before and after the consume of two energy drinks are displayed. B: Influences of the mouthwash Meridol on the saliva spectrum are depicted. Highlighted areas mark high cocaine absorption.

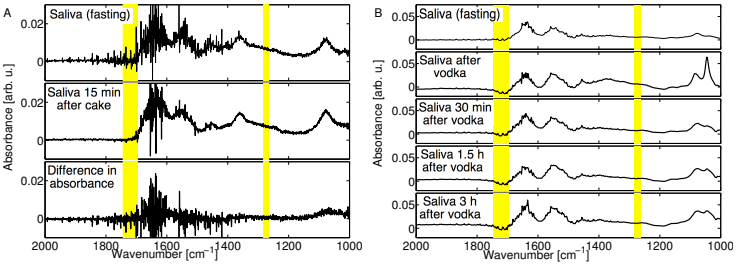


Figure 9.8: **Spectra of saliva before and after the consume of poppy seed cake and vodka.** The background absorption of water was subtracted from the spectra. A: The change of the saliva spectrum induced by the consume of poppy seed cake is displayed. B: The change in saliva spectra induced by alcohol consume is depicted. Highlighted areas mark high cocaine absorption.

ure 9.7 B).

Poppy seeds are known to raise the morphine levels in saliva [35] (cf. chapter 4). Therefore, the influence of the consume of poppy seed cake was also investigated. To which extend morphine levels rise is strongly dependent on the origin of the poppy seeds [168]. The consumed poppy seed cake in this study did not raise the morphine levels to a detectable level of absorption (cf. figure 9.8 A). A difference in the saliva spectrum could only be observed in the spectral range from 1100 to 1000 cm^{-1}.

The alcohol study showed a clear ethanol absorption signal in saliva. A high ethanol absorption was observed in the spectrum of saliva collected directly after the alcohol consumption (cf. figure 9.8 B; ethanol absorption in figure 9.5). Its magnitude decreases within the first 30 min after the consume. Afterwards a slight increase in the alcohol content of the saliva can be seen as the blood alcohol level rises. This decrease is expected since the remaining contamination of the alcohol is diluted with time. The specific alcohol signature is still clearly observable in the sample taken 3 h after the alcohol consume, when the study was ended. This development was expected since the test person had a calculated blood alcohol content of 0.12 % (in % by volume; calculated on the basis of [169]). Other studies suggest to take saliva instead of blood for quantitative analysis of alcohol content, a correlation between these data has been shown [170].

Smoking induces many changes in salivary flow due to its irritating effect [24] and introduces substances like nicotine into the oral cavity [31]. Therefore, the saliva of a fasting person was compared to the saliva of a person who smoked a cigarette (cf. figure 9.9 A). The observed differences in the spectra do not interfere with the absorption ranges of cocaine.

The saliva spectrum of one test person on different days and the

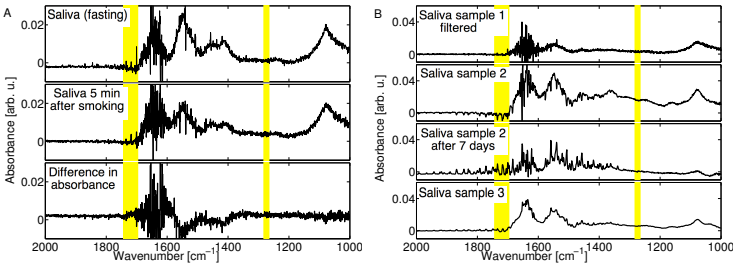


Figure 9.9: **Spectra of saliva before and after smoking; analysis of the spectral change in saliva with time.** The background absorption of water was subtracted from the spectra. A: Spectra of saliva of a person before and after the smoking of a cigarette are displayed. B: Spectra of saliva of a fasting person on different days (sample 1,2,3) and once filtered saliva of the same person (sample 1) are depicted. In addition, the spectral changes for storage at 4 °C were analyzed. Highlighted areas mark high cocaine absorption.

influence of the 0.2 µm syringe filters on the saliva spectrum can be seen in figure 9.9 B. A decrease in absorption in the spectral range from 1700 to 1500 cm^{-1} was detected between the spectra of the filtered and unfiltered samples. This change is most likely caused by filtering out many proteins. A similar spectral change is also detectable when comparing the spectrum of saliva sample 2 directly after the acquisition and 7 days later. A significant decrease in the absorbance is observed most likely caused by the decay of the body fluid during the storage at 4 °C.

The influence of coffee on the saliva spectrum is investigated in chapter 13 in figure 13.1 and the influence of periodontitis on the saliva spectrum is investigated in chapter 15. In summary, no major interferences with the cocaine absorption in the spectral range around 1730 cm^{-1} were detected.

9.4 Cocaine extracted from saliva

In this section the limitations of the detection of cocaine in saliva are explored. In addition, the results of the extraction technique are discussed and their dependence on important parameters like the pH are evaluated.

9.4.1 Saliva and cocaine

In real-life situations cocaine concentrations in saliva range from 0 to 500 µg ml^{-1}. A reasonable lower limit of detection range would be between 700 and 100 ng ml^{-1} (cf. chapter 5).

A comparison between the infrared spectra of pure saliva and saliva spiked with 85 µg ml^{-1} cocaine is depicted in figure 9.10 A. A difference between the two spectra is not detectable. A concentration of cocaine or its metabolites below a few 100 µg ml^{-1} is almost impossible to detect in water or in saliva due to the high water ab-

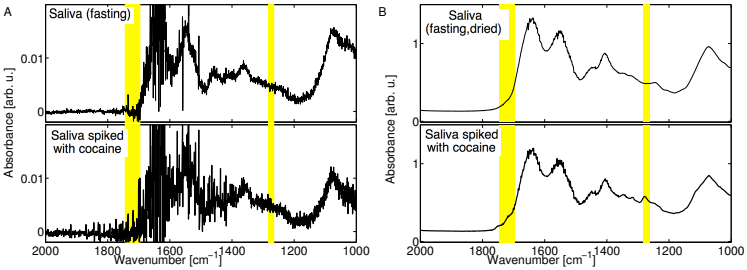


Figure 9.10: **Spectra of saliva spiked with cocaine.** A: Spectra of pure saliva and of saliva spiked with cocaine are displayed. The water background was subtracted from the spectra. B: Spectra of dried saliva before spiking and of dried saliva spiked with a concentration of 85 µg ml^{-1} cocaine are depicted.

sorption (cf. section 9.1).

The absorption coefficient α (cf. equation 2.15) of cocaine is proportional to the concentration of the substance. Therefore, the concentration can be calculated from the absorption coefficient α derived from cocaine measurements at higher concentrations. Cocaine at a concentration of 0.1 µg ml^{-1} has a calculated absorption coefficient $\alpha_{0.1}$ at $\sim$1756 cm^{-1} of[11]:

$$\alpha_{0.1} = 0.0012 \pm 0.0004\,\mathrm{cm}^{-1}.$$

In comparison, water has an absorption coefficient α_{H_2O} of $\sim 720 \pm 20\,\mathrm{cm}^{-1}$ at 1756 cm^{-1} and an optical path length l of 9.4 µm for 11 reflections on the ATR crystal.

Hence, the change in transmission ΔT caused by 0.1 µg ml^{-1}

[11]The absorption coefficient was calculated from 3 different cocaine concentration measurements and scaled to the concentration of 0.1 µg ml^{-1}

cocaine is:

$$\Delta T = \mathrm{e}^{-(\alpha_{\mathrm{H_2O}} \cdot l)} - \mathrm{e}^{-(\alpha_{\mathrm{H_2O}} + \alpha_{0.1}) \cdot l} = 5.7 \cdot 10^{-7}$$

for a path length l of 9.4 µm at 1756 cm^{-1} on top of a transmission of $T = \mathrm{e}^{-(\alpha_{\mathrm{H_2O}} \cdot l)} = 0.5$ caused by water itself (n from [21]). The standard deviation of the transmission of the FTIR is ~ 0.005 for three measurements in one measurement cycle (cf. figure 8.1). Thus, to detect a change ΔT in this order of magnitude the signal resolution of the FTIR is not sufficient.

9.4.2 Calculation of the extraction efficiency of cocaine

In this thesis a one-step extraction process was developed utilizing tetrachloroethylene (TCE) to extract cocaine from saliva. The details of the extraction process are found in section 7.1. In chapter 3, the chemical knowledge needed to estimate the result of the extraction was introduced. In this section, the extraction coefficient η (cf. equation 8.3) for cocaine is estimated for a system similar to the TCE-saliva system investigated in this thesis.

The pK_a value and the distribution coefficient K_{app} (cf. section 3.2 and 3.3) are needed to calculate the theoretical concentration of a solute in the involved solvents. Since no information about the distribution coefficient of the TCE-saliva system was found in the literature, the system was approximated with the 1-octanol-water system. To approximate saliva with water is legitimate since saliva consists of >90 % of water (cf. chapter 4). The approximation of 1-octanol with TCE is not ideal but acceptable because of the very low solubility of TCE in water and the very low polarity of TCE [171]. In section 3.3 the relevance of a difference in the polarity for the partition coefficient was discussed. Tetrachloroethylene is even less polar than 1-octanol and should therefore extract large organic molecules like cocaine ($\mathrm{C_{17}H_{21}NO_4}$) very well.

In the literature, experimental (measured) data for the K_{ow}/pK_a parameters and theoretical (mostly numerical calculated) data can be found. In this work the experimental data are used because they are more accurate [23]:

$$\begin{aligned} \log K_{ow}(\text{cocaine}) &= 3.01 \quad [172] \\ pK_a(\text{cocaine}) &= 8.6 \quad [173] \end{aligned}$$

When cocaine ($C_{17}H_{21}NO_4$) is dissolved in water, the following equilibrium is established:

$$C_{17}H_{21}NO_4 + H_3O^+ \rightleftharpoons C_{17}H_{22}NO_4^+ + H_2O$$

$$C_{17}H_{22}NO_4^+ + HO^- \rightleftharpoons C_{17}H_{21}NO_4 + H_2O$$

Hence, $C_{17}H_{22}NO_4^+$ denotes a Brønsted acid and $C_{17}H_{21}NO_4$ a Brønsted base (cf. section 3.2). To push the equilibrium toward the uncharged (or neutral) form of cocaine, the pH is raised to a value of 9, above the pK_a value of cocaine (as described in chapter 7.1 and explained in section 3.2). To estimate the extraction efficiency for cocaine from water with 1-octanol at a pH of 9 we use equation 3.11:

$$\begin{aligned} \log_{10} K_{app} &= \log_{10} K_{ow} - \log_{10}\left(1 + 10^{pH - pK_a}\right) \\ \log_{10} K_{app} &= 3.01 - \log_{10}(1 + 10^{0.4}) \\ \log_{10} K_{app} &\approx 2.464 \end{aligned}$$

With equation 3.8 the concentration in octanol $c_o(C_{17}H_{21}NO_4)$ can be calculated:

$$c_o(C_{17}H_{21}NO_4) = K_{app} \cdot \left(c_w(C_{17}H_{21}NO_4) + c_w(C_{17}H_{22}NO_4^+)\right)$$

Consequently, an initial concentration of, e.g., $50\,\mu g\,ml^{-1}$ of cocaine dissolved in water would result in the following concentrations at equlibrium:

- $49.83\,\mu g\,ml^{-1}$ (99.7 %) cocaine dissolved in 1-octanol and

- $0.17\,\mu g\,ml^{-1}$ (0.3 %) cocaine dissolved in water.

With the help of equation 3.6 we can calculate how many charged and neutral cocaine molecules remain in water:

$$\log_{10}\left(\frac{c_w(C_{17}H_{22}NO_4^+)}{c_w(C_{17}H_{21}NO_4)}\right) = pK_a - pH = -0.4$$
$$c_w(C_{17}H_{22}NO_4^+) = c_w(C_{17}H_{21}NO_4)\cdot 10^{-0.4}$$

If we also take into account that:
$c_w(C_{17}H_{22}NO_4^+) + c_w(C_{17}H_{21}NO_4) = 0.17\,\mu g\,ml^{-1}$
we can calculate the concentration:

$$c_w(C_{17}H_{22}NO_4^+) = (0.17\,\mu g\,ml^{-1} - c_w(C_{17}H_{22}NO_4^+))\cdot 10^{-0.4}$$
$$c_w(C_{17}H_{22}NO_4^+) = (0.17\,\mu g\,ml^{-1}/(10^{0.4}+1)) \approx 0.048\ (\sim 28.2\,\%)$$
$$\Rightarrow c_w(C_{17}H_{21}NO_4) = 0.122\,\mu g\,ml^{-1}(\sim 71.8\,\%)$$

Hence, of the remaining $0.17\,\mu g\,ml^{-1}$ cocaine in water, 71.8 % of the cocaine will be in the uncharged (neutral form) and 28.2 % will be in the charged form dissolved in water.

Overall, the data for the saliva-TCE system for the solute cocaine should be similar for the above mentioned reasons. Therefore, an extraction efficiency η close to 99.7 % can be expected.

9.4.3 The choice of the extraction solvent

The criteria for the choice of the solvent have been introduced in section 7.1.1 and the following solvents were investigated for the extraction of cocaine from saliva: 1-octanol, iso-octane, n-heptane, dibutyl ether, toluene, dichoroethane and tetrachloroethylene. Selected solvents were judged in their performance to extract cocaine based on the following criteria:

- Is the solvent capable to extract cocaine? The solvent phase after the extraction was analyzed for traces of cocaine.

- Is a measurable concentration of cocaine left in the saliva? The saliva phase was analyzed for traces of cocaine[12].
- Do the positions of the absorption peaks of cocaine shift? As mentioned earlier in section 9.1 the position of the absorption peaks depends on the solvent. Therefore, the shift of the position of the peaks had to be analyzed.
- How easy is the handling in view of the micro fluidic application? Some of the mixtures showed the formation of stable droplets within the other liquid which changed their fluidic properties and hindered the self-separation of the fluids. For some mixtures the addition of isopropanol to the mixture would resolve part of the droplets or hinder their formation.
- How challenging is the background subtraction? Background subtraction of simple solutions is not difficult. First the spectrum of the solvent is recorded followed by the spectrum of the solution. The difference in absorbance then shows the spectrum of the solute, i.e. of cocaine in this case. Solutions consisting of more than one solute or more then one solvent are much more challenging in terms of background subtraction. This problem was encountered when adding isopropanol[13] to the mixture to resolve stable droplets or when some components of the saliva transferred into the solvent phase.
- Is the solvent compatible with the material used for the microfluidic channels?

These criteria are analyzed in table 9.4. The measurements showed that dibutyl ether, 1-octanol and iso-octane formed stable droplets, which were not resolved by adding isopropanol to the mixture. N-heptane also had a certain tendency to form stable droplets,

[12]The first extraction method was used to find the best-suited solvent (cf. section 7.1.2).

[13]The partition coefficient for the saliva-solvent system for isopropanol was not known.

Table 9.4: **Choice of the solvent.** The table shows the results of the extraction experiments with different solvents. It displays whether cocaine could be found in the solvent phase, at which wavenumber the position of the cocaine absorption peaks was, whether the background could be subtracted, whether the fluid formed stable droplets and whether there were traces of cocaine found in the saliva phase of the sample. (Abbreviations: $\tilde{\nu}$- wavenumber; n.m.- not measurable; traces?- most likely traces of cocaine remain in the saliva phase after the extraction)

Solvent	extracts cocaine?	$\tilde{\nu}$ of COC peaks [cm^{-1}]	Background subtraction	stable droplets?	COC in saliva
Dibutylether	yes	1758 & 1722	challenging	many	n.m.
Dichloroethane	yes	1746 & 1716	possible	few	traces?
1-Octanol	?	If:∼1758 & 1723	very challenging	many	traces?
Toluene	yes	1756 & 1720	possible	few	n.m.
Tetrachloroethylene	yes	1756 & 1720	possible	small & few	n.m.
N-heptane	yes	1756 & ∼1721	possible	tiny & many	n.m.
N-heptane with isop.	yes	1761 & ∼1724	possible	no	n.m.
Iso-octane	yes	1762 & 1724	challenging	many	traces?

but the formation could partly be hindered by adding isopropanol. Dichloroethylene was not compatible with the material used for the microfluidic channels [163]. The three remaining solvents were tetrachloroethylene, toluene and n-heptane. All three solvents can extract cocaine base as well as cocaine hydrochloride from saliva. In addition, these solvents were tested for their extraction ability regarding the metabolites of cocaine.

9.4.4 Extraction of the metabolites

Toluene, n-heptane and tetrachloroethylene were investigated towards their ability to extract the major metabolites of cocaine: benzoylecgonine (BE), norcocaine (NCOC), ecgonine methyl ester (EME), anhydro ecgonine methyl ester (AME) and cocaethylene (COE; see section 5.4).

Extracting metabolites has two sides. On the one side, some of the spectra of the metabolites interfere with the cocaine spectrum (cf. figure 9.2), therefore they might falsify the quantitative measurement of cocaine in saliva. On the other side, the occurrence of cocaine metabolites in saliva indicates the administration of cocaine. Person-to-person variances in the concentration of cocaine and metabolites in saliva are very high (cf. section 5.4). Furthermore, the concentrations vary with time. The analysis of cocaine and the metabolites therefore appears more promising than the analysis of only one substance. Hence, if the metabolites contribute to the absorbance this might falsify the cocaine concentration but might also aid the overall risk assessment, e.g. COE (cf. section 5.3). Overall, the advantages of extracting the metabolites of cocaine with the cocaine outweigh the disadvantages.

In figure 9.11 the results of the extraction of COE and NCOC are depicted. Saliva was spiked with NCOC and COE and then mixed with either toluene, TCE or n-heptane. The spectra of the solvent phase demonstrate that TCE is the most successful extrac-

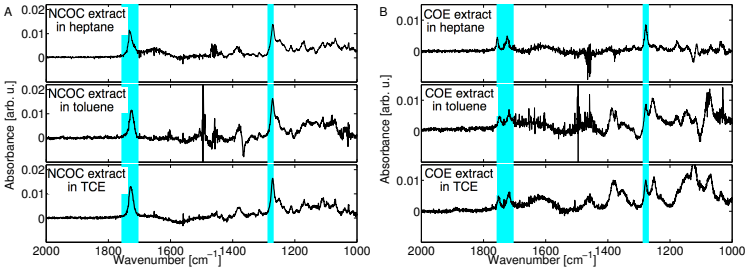


Figure 9.11: **Extraction of metabolites from saliva.** N-heptane, toluene and tetrachloroethylene (TCE) were used to extract norcocaine (NCOC, $0.835\,\mathrm{mg\,ml^{-1}}$) and cocaethylene (COE, $0.51\,\mathrm{mg\,ml^{-1}}$) from saliva. The difference in spectra of saliva spiked with cocaine and saliva are compared to the extract from saliva. The highlighted area displays the area of strong absorption of cocaine when solved in TCE, toluene or heptane.

tion solvent yielding the highest absorption of the solute. Similar results were obtained for the other 3 main metabolites. Therefore, the following extractions were exclusively conducted with TCE.

9.4.5 The improvement gained by TCE extraction

This section introduces the major advantages gained by the extraction when comparing the absorbance signal of cocaine in saliva with the signal in tetrachloroethylene (TCE) after the extraction.

As it is challenging to detect absorptions of cocaine in liquid water based samples at concentrations below a few $100\,\mathrm{\mu g\,ml^{-1}}$ (cf. section 9.4.1). The saliva samples spiked with cocaine were dried on top of the ATR crystal before recording the spectra for the second time (cf. section 8.2). The absorbance of the dried saliva

around $1730\,\mathrm{cm}^{-1}$ is at least 5 times higher than the additional absorbance caused by spiking the saliva with cocaine. In addition, the absorbance in the spectral range of the first two cocaine peaks is changing strongly. Therefore those peaks are not easy to identify. The peak around $1270\,\mathrm{cm}^{-1}$ is easier to identify but it is less unique due to spectral interferences by many substances (cf. section 9.2). Therefore, the easiest way to determine whether or not the sample contains cocaine is to compare it with a spectrum of unspiked saliva. The difference between spectra of dried spiked and dried pure saliva samples are depicted in figure 9.12. The high noise level and negative absorbance is caused by differences in the drying patterns which leads to variations between the saliva spectra. The cocaine signature in the spectrum of the saliva sample spiked with $8.5\,\mu\mathrm{g\,ml}^{-1}$ of cocaine is hardly observable. Figure 9.12 B shows the dried extracts of the same samples. The cocaine signature in both samples is clearly detectable. The signal-to-noise ratio is significantly improved and it is not necessary to have an unspiked sample for comparison. This corresponds to the real case when dealing with people under the influence of drugs.

In section 9.4.1 the change in transmission induced by a concentration of $0.1\,\mu\mathrm{g\,ml}^{-1}$ cocaine in water was calculated to $5.7 \cdot 10^{-7}$. Now, the change in transmission of a cocaine concentration of $0.1\,\mu\mathrm{g\,ml}^{-1}$ in TCE is calculated. TCE has a refractive index $n = 1.5054$ [174] and its absorption coefficient α_{TCE} has been determined as $7\,\mathrm{cm}^{-1}$ at the wavelength $1756\,\mathrm{cm}^{-1}$. Therefore the total optical path calculates to $12.7\,\mu\mathrm{m}$. Hence, a change in transmission caused by a cocaine concentration of $0.1\,\mu\mathrm{g\,ml}^{-1}$ in TCE would lead to a change in the transmission of:

$$\Delta T = \mathrm{e}^{-(\alpha_{\mathrm{TCE}} \cdot l)} - \mathrm{e}^{-(\alpha_{\mathrm{TCE}} + \alpha_{0.1}) \cdot l} = 1.5 \cdot 10^{-6}$$

on a background signal of $T = \mathrm{e}^{-(\alpha_{\mathrm{TCE}} \cdot l)} = 0.99$ from pure TCE (n=1.5054 [174]). Although this difference is larger than the difference calculated for water, it is not within the amplitude resolution

of the FTIR spectrometer (cf. figure 8.1). Due to this mismatch in resolution all samples were dried on top of the ATR crystal to enhance the absorbance signal (for the drying method see section 8.2).

9.4.6 Results of the first extraction method

The first group of samples was prepared and the extraction process was performed according to the first extraction method (cf. section 7.1.2). The saliva was acquired from 4 volunteers (3 male and 1 female). The age of the volunteers was between 28 and 42 years. Twelve extractions were performed which resulted in 36 measurements. In addition, 74 reference measurements were acquired for the calibration. The results are depicted in figure 9.13. A correlation coefficient of $r = 0.99$ was found between fitting parameter of the reference measurements and their initial concentration. The calibration for the calculated concentration was based on these data.

The fitting and calculating of the concentration was done according to section 8.4. A mean extraction efficiency[14] η of $102 \pm 47\,\%$ was found. The Lilliefors test applied to this data set cannot contradict the hypothesis that these data points are from the same Gaussian distribution. Hence, this distribution indicates a random and not a systematic error. Nevertheless, an uncertainty of $47\,\%$ is very close to the person-to-person variations (cf. section 5.4).

The high uncertainty might originate from variations in the balance. The scale was not ideally set up and exposed to vibrations and pressure changes in the room, which results in variations of the mean extraction efficiencies as large as $20\,\%$. In addition, variations in the pH of the saliva were observed (pH range between 7 and 8). As a consequence the dependence on the pH was investigated in more detail.

[14] for definition see section 8.4.1

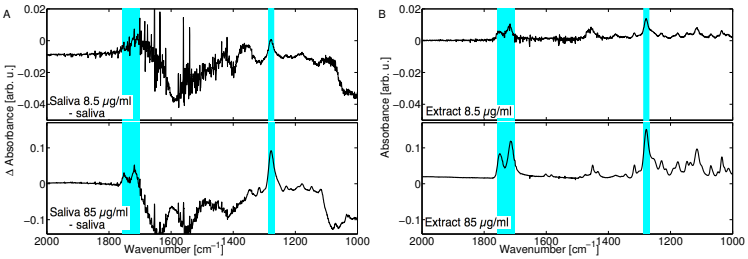


Figure 9.12: **Improvement by the extraction.** Comparison between differences in spectra of dried saliva spiked with cocaine and saliva (A) and dried extracts from saliva are displayed (B). Highlighted areas display areas of strong absorption of cocaine.

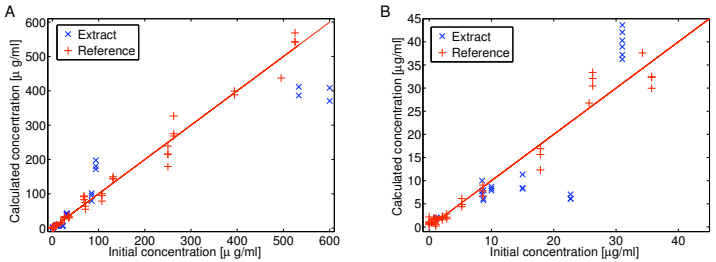


Figure 9.13: **Calculated concentration versus initial concentration without buffer.** The extraction of the spectra was done according to the procedure explained in figure 7.1 and the fitting according to equation 8.2 and figure 8.3 ($r = 0.99$). Plot B is a magnification of plot A. The calculated concentration for the reference measurements (+) and for the extracts (x) are plotted vs. the initial concentration of the samples.

9.4.7 Influence of pH

The extraction efficiency depends on the pH of the saliva (cf. chapter 3). Variation in the saliva pH increases the measurement uncertainty. Therefore, an optimum pH of saliva is defined for high signal-to-noise in the spectra and minimized measurement uncertainty. The pH of saliva was varied by adding a concentrated buffer (Titrisol, cf. section 7.1.2).

Two experiments were conducted. Each experiment was based on the same saliva sample to minimize potential errors in the cocaine concentration of the sample. The first experiment compared the absorbance of cocaine in the extract retrieved from unbuffered saliva (pH 7) to the one retrieved from saliva buffered to pH 11 and 13 (cf. figure 9.14 A). Theoretically, a higher pH promotes the uncharged state of cocaine and should therefore improve the extraction of cocaine in saliva. However, the absorbance of cocaine in the extract of pH 13 was much lower than of pH 11. This reduction might be caused by chemical reactions in the saliva supported by the drastic pH change. In addition, a change in the partition coefficient is possible.

In a second experiment pH values of saliva spiked with cocaine close to the pK_a- value of cocaine ($pK_a = 8.6$ [173]) were tested and compared to unbuffered ones at pH 7 (cf. figure 9.14 B). The cocaine absorbance in the extract retrieved from pH 9 was the highest. In addition, a pH of 9 is a good compromise between rising above the pK_a value (with the majority of the cocaine molecules in the neutral/uncharged state) and keeping the change in the pH as small as possible.

9.4.8 Results on the second extraction method

Based on the results of the first extraction method two changes were implemented. The pH of the saliva spiked with cocaine was stabi-

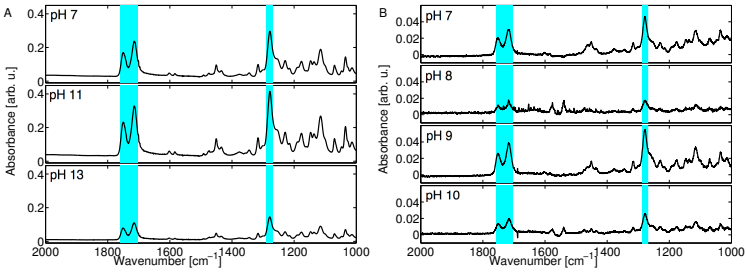


Figure 9.14: **Influence of the pH on the extraction.** Saliva was spiked with cocaine and buffered to different pH values to analyze the influence of the pH on the absorbance of cocaine in the extract. A: Spectra of the dried TCE phase after the extraction from saliva spiked with cocaine ($\sim$94 µg ml^{-1}) and buffered to pH 11 and 13 compared to the unchanged spiked saliva. B: Spectra of the dried TCE phase after the extraction from saliva spiked with cocaine (29 µg ml^{-1}) and buffered to a pH of 8, 9 and 10 compared to the unchanged spiked saliva are depicted. Highlighted areas mark high cocaine absorption (for cocaine dissolved in TCE and dried on the crystal).

lized to 9 and the cocaine was no longer weighed, but rather determined by pipetting and drying calibrated cocaine solution in glass tubes (cf. section 7.1.2, figure 7.3).

Precision of the second, improved extraction method

The investigations were conducted with six test persons (2 female, 4 male) with an age from 25 to 64 years. The precision of the extraction was not only examined with saliva from different test persons but also with saliva collected in different situations. The influence of collecting saliva after smoking or eating poppy seed[15] cake on the extraction method were tested. In addition, the extract of supernatant of saliva spiked with cocaine and retrieved from patients with generalized aggressive periodontitis (G-AgP, cf. part IV) was compared to the extract from spike supernatant of healthy volunteers.

In total 29 extraction were analyzed (each measured 3 times). The second extraction method proved invariant for all the tested parameters. For the calibration 18 reference samples (each measured 3 times) were analyzed (for fitting and calibration see section 7.1 and 8.4). The results are displayed in figure 9.15. The calibration of the concentration was calculated based on a linear regression[16] of the fitting data of the reference samples yielding a square root of the coefficient of determination of $r = 0.99$. Deviations between calculated and initial cocaine concentration are most likely not caused by variations in the extraction but rather by the random inhomogeneous drying of the sample on the ATR crystal (cf. section 8.2). This hypothesis is supported by two facts. First, the deviations

[15] Poppy seed cake is ideal for this test since it does not only contain poppy seeds but also sugars, milk, proteins and fruit.

[16] The linear regression is used as calibration between the fitting parameter A (cf. equation 8.2) and the cocaine concentration in TCE.

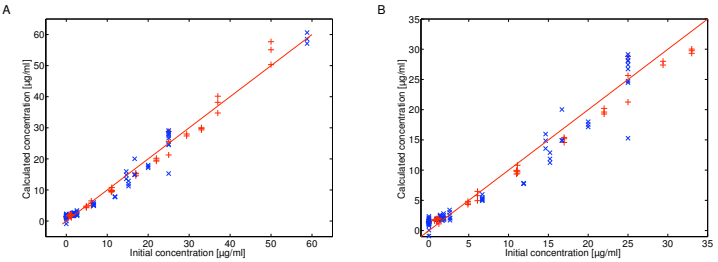


Figure 9.15: **Results of the second improved extraction method.** The calculated concentration (cf. Equation 8.3) in the extract is plotted versus the initial concentration in saliva. A: The graph shows an overview over the extracts' (x) and reference samples' (+) data. B: The graph shows a magnification of graph A.

of the data for the reference samples[17] are similar to the deviation of the values of the extracts. Second, the extract drying should be a random process. Thus if the deviation is caused by the drying pattern the distribution of the calculated concentrations for one concentration interval should follow a Gaussian distribution. Therefore, the Lilliefors-test [175] was applied on several concentration intervals. The results of this test could not contradict that the values are from a Gaussian distribution. For example 6 measurements with the initial concentration of $\sim 1.4\,\mu g\,ml^{-1}$ passed the Lilliefors-test. In comparison to the first extraction method and unstabilized pH (cf. figure 9.13) the second extraction method and a stabilized pH of 9 considerably improved the variability of the extraction (cf. figure 9.15).

[17] Deviation from the ideal line in figure 9.15 and therefore error in the estimation of the concentration.

Efficiency of extraction

The second extraction method yields an extraction efficiency of $\eta \approx 100\,\%$. The uncertainty of $\sim 30\,\%$ is significantly lower than the uncertainty for the first extraction method and lower than the person-to-person variation in the concentration of cocaine in saliva [64]. This uncertainty is therefore sufficient to detect the cocaine concentration in saliva of cocaine consuming people. The error most likely originates from the difference in the drying patterns. This hypothesis is supported by the result of the Lilliefors-test applied to all values for η. As theoretical model the extraction efficiency of the octanol-water system was calculated to $> 99\,\%$ at a pH of 9 (cf. section 9.4.2). Hence, the results suggest, that a reproducible one-step extraction method with semi-quantitative results is suitable for the detection of cocaine in saliva.

Limit of detection

The limit of detection (LOD) was estimated from the standard deviation of blank extracts and multiplied by 2. This procedure yields a limit of detection of $1.3\,\mu g\,ml^{-1}$ $(2\ \sigma)$ of cocaine in saliva. This LOD was also confirmed by a one-sided t-test. The closest measured cocaine concentration to the limit of detection was $1.4\,\mu g\,ml^{-1}$. The results of this extraction were compared to the LOD in an one-sided t-test. The result suggests a significant difference from the previously assessed LOD. Comparing the results for the extracts with an initial concentration of $1.4\,\mu g\,ml^{-1}$ with the LOD of $1.3\,\mu g\,ml^{-1}$ for cocaine in saliva this t-test yielded a significant difference.

This LOD can be further improved by preconcentration. On the one side a preconcentration after the extraction is possible[18]. For this option usually the extract is fully evaporated and the residue is dissolved again with less solvent. On the other hand it is possible to use less solvent from the beginning. An experiment was conducted

[18] often done for other extraction methods [157, 158]

using 3.4 times more saliva spiked with $0.37\,\mu g\,ml^{-1}$ cocaine than TCE for the extraction. The result was not significantly different from an extraction conducted with a concentration of $1.2\,\mu g\,ml^{-1}$ concentration and a solvent to saliva ratio of 1:1. In conclusion the limit of detection can be lowered by preconcentration to the regime of a few $100\,ng\,ml^{-1}$ which is the average concentration found in the saliva of a person during a "high" (cf. chapter 5).

9.4.9 Conclusion

Different categories of tests should be evaluated by different criteria (cf. section 5.7 and figure 5.3). Some key criteria for these tests are compared between new and established common tests in table 9.5. The currently common on-site test like immunoassays (e.g., OraLab® and Dräger DrugTest®) and common laboratory tests (like GC/LC-MS) have significant lower LODs for cocaine in saliva than the method developed in this thesis. However, the introduced method possesses an LOD that allows to distinguish between saliva from a person on a cocaine induced "high" and a control sample.

The IR spectroscopy method presented here uses reasonable sample volumes and the success rate[19] is significantly higher than for immunoassays (cf. table 9.5). In addition, the required measurement time is shorter than for GC/LC-MS analysis and equal to on-site tests.

The method offers semi-quantitative results in terms of precision, which is sufficient (cf. section 5.4) and can be taught to non-professionals within a day. Selectivity is sufficient for a quick semi-quantitative test, too.

In addition, the test offers great potential for miniaturization

[19] probability to acquire a result when the test is taken

Table 9.5: **Key characteristics of common detections methods and optical alternatives for cocaine or metabolites in body fluids.** Darker shading highlights less preferable characteristics. (Abbreviations: LOD- limit of detection; time- acquisition and measurement time; training- degree or duration of the training necessary to execute the method; y/n- binary result (positive/negative); Volume- sample volume; sample prep.- sample preparation; success- probability to gain a result if the test is executed; COC- cocaine; BE- benzoylecgonine; OSE- one-step extraction; SPE- solid phase extraction; nm- not mentioned)[36]

method	LOD	time	training	result	target	Volume	sample prep.	success	reference
Varian OraLab®	20 ng/ml	10 - 15 min	short training	y/n	COC in saliva	nm	non	75%	[109]
Dräger DrugTest®	5 ng/ml	17 min	short training	y/n	COC in saliva	nm	Dräger protocol	90%	[109]
LC/MS	~1 ng/ml	>37 min	professional	conc	COC in saliva	100 µl	SPE	100%	[109]
GC/MS	~1 ng/ml	>51 min	professional	conc	COC in saliva	1 ml	SPE	nm	[74]
fluorescence immunoassay	50 ng/ml	> 66 min	professional	conc	BE in urine	75 µl	dilution	nm	[138]
Fluorescence w. aptermeric sens.	121 ng/ml	> 120 min	professional	conc	COC in serum	10 µl	dilution	nm	[140]
SERS w. metal doped sol gels	<250 µg/ml	<10 min	professional	conc	COC in saliva	~300 µl	sol gel extract	nm	[148]
UV Spec	1.25 µg/ml	> 15 min	professional	conc	BE in urine	10 ml	liq.-liq. extract plus hydrolysis	nm	[154]
FTIR Spec.	<1.3 µg/ml	14 min	short training	conc	COC in saliva	400 µl	OSE	100%	this thesis

since its compatibility with microfluidics was tested by [163] and the FTIR spectrometer can be replaced with QCLs emitting at the cocaine absorbance peak around 1750 cm^{-1} and at a reference wavelength (cf. chapter 11).

9.5 Street cocaine analysis

In contrast to pure cocaine as discussed above, so called street cocaine often contains various adulterants (cf. section 5.5). In this section the extraction efficiency of the adulterants is estimated. In addition, street cocaine samples from the Forensic Science Institute Zurich are spectrally analyzed[20].

9.5.1 Calculation of extraction efficiency of adulterants

The extraction efficiency of selected adulterants found in the street samples are estimated. The calculations are based on the on chemistry explained in chapter 3 and are analogue to the calculations made to estimate the extraction efficiency of cocaine in section 9.4.2. The displayed values are calculated for the 1-octanol-water system (cf. section 9.4.2) and uses equation 3.11 as well as equation 3.8.

The pK_a and pK_{ow} data used for the calculation of the extraction efficiency (solute concentration in 1-octanol divided by the initial solute concentration in water) are listed for the selected adulterants of cocaine in table 9.6. In addition, the water solubility of the solute is listed. Since the solute is always solved in water/saliva first, the concentration in 1-octanol is limited as well by the solute solubility (i.e. maximum possible concentration in water). The maximum concentration was calculated together with the extraction efficiency for the equilibria given in table 9.7.

[20] Part of this chapter was published in [36, 41]

Table 9.6: **Selected chemical parameters for adulterants.** The listed adulterants were found in the street samples investigated in this thesis. The necessary parameters to calculate the extraction of the adulterants from H_2O with 1-octanol are listed with the CAS number of the substance. (Abbreviations: pK_{ow}- is the negative logarithm of the partition coefficient; pK_a- the negative logarithm of the acidic constant; sol.- indicates the solubility of the substance in H_2O for the given temperature) All entries that are not cited otherwise, are retrieved from [176].

Substance	CAS	pK_{ow}	pK_a	Sol. in H_2O	
Benzocaine	94-09-7	1.89 [172]	2.51	$1.310\,\mathrm{mg\,ml^{-1}}$	at 30 °C
Procaine	59-46-1	2.14 [172]	8.05	$9.45\,\mathrm{mg\,ml^{-1}}$	at 30 °C
Lidocaine	137-58-6	2.44 [172]	8.01	$4.1\,\mathrm{mg\,ml^{-1}}$	at 30 °C
Ibuprofen	15687-27-1	4.13 [172]	4.91	$0.021\,\mathrm{mg\,ml^{-1}}$	at 25 °C
Caffeine	58-08-2	-0.07 [177]	10.4	$21.600\,\mathrm{mg\,ml^{-1}}$	at 25 °C
Phenacetin	62-44-2	1.58 [177]	2.2 [178]	$0.766\,\mathrm{mg\,ml^{-1}}$	at 25 °C
Levamisole	14769-73-4	1.84 [179]	8[180]	$210\,\mathrm{mg\,ml^{-1}}$	
Creatine	57-00-1	-0.2	3.4	$13.3\,\mathrm{mg\,ml^{-1}}$	at 18 °C
Mannitol	69-65-8	-3.10	13.5	$216\,\mathrm{mg\,ml^{-1}}$	at 25 °C
Hydroxyzine	68-88-2	2.7	1.8-2.1 [178]	$< 700\,\mathrm{mg\,ml^{-1}}$	

Table 9.7: **Predicted extraction of selected adulterants from water into 1-octanol for the indicated equilibrium.** The data are calculated for a pH of 9 (as used for the extraction procedure in this thesis, cf. section 7.1). The predicted extraction efficiency (extr. eff.) is noted in %. In addition, the maximum concentration of the substance in 1-octanol is given, calculated on the basis of the solubility in H_2O (cf. table 9.6) and the extr. eff. noted in this table (max. conc. in 1-octanol).

Substance	Equilibrium			Extr. eff. [%]	Max. conc. in 1-octanol [mg/ml]
Benzocaine	$H_2O + C_9H_{12}NO_2^+ +$	$\rightleftharpoons$	$H_3O^+ + C_9H_{11}NO_2$	0.00251	$3.3 \cdot 10^{-5}$
Procaine	$H_2O + C_{13}H_{21}N_2O_2^+$	$\rightleftharpoons$	$H_3O^+ + C_{13}H_{20}N_2O_2$	93.3	8.8
Lidocaine	$H_2O + C_{14}H_{23}N_2O^+$	$\rightleftharpoons$	$H_3O^+ + C_{14}H_{22}N_2O$	96.2	3.9
Ibuprofen	$H_2O + C_{13}H_{18}O_2$	$\rightleftharpoons$	$H_3O^+ + C_{13}H_{17}O_2^-$	52.3	0.011
Caffeine	$H_2O + C_8H_{11}N_4O_2^+$	$\rightleftharpoons$	$H_3O^+ + C_8H_{10}N_4O_2$	45	9.7
Phenacetin	$H_2O + C_{10}H_{14}NO_2^+$	$\rightleftharpoons$	$H_3O^+ + C_{10}H_{13}NO_2$	97.4	0.75
Levamisole	$H_2O + C_{11}H_{13}N_2S^+$	$\rightleftharpoons$	$H_3O^+ + C_{11}H_{12}N_2S$	86.3	180
Creatine	$H_2O + C_4H_9N_3O_2$	$\rightleftharpoons$	$H_3O^+ + C_4H_8N_3O_2^-$	0.000158	$2.0 \cdot 10^{-5}$
Mannitol	$H_2O + C_6H_{14}O_6$	$\rightleftharpoons$	$H_3O^+ + C_6H_{13}O_6^-$	0.0794	0.17
Hydroxyzine	$H_2O + C_{21}H_{28}ClN_2O_2^+$	$\rightleftharpoons$	$H_3O^+ + C_{21}H_{27}ClN_2O_2$	0.005	< 0.35

Overall, the extraction efficiency for benzocaine, creatine, mannitol and hydroxyzin is very bad ($< 0.1\,\%$), whereas procaine, lidocaine, ibuprofen, caffeine, phenactein and levamisole have good extraction efficiencies (cf. table 9.7). Nevertheless, it should be taken into account that these data are only a rough estimation since no literature pK_a or pK_{ow} data for the saliva-TCE system is known. Furthermore, equation 3.8 is only accurate when the pH value of the solution is close to the pK_a data of the solute which is not fulfilled for all calculations.

9.5.2 Measurements of Street cocaine

In this section the spectra of street cocaine dissolved in tetrachloroethylene (TCE) and the spectra of extracts from saliva samples spiked with street cocaine were analyzed.

The street cocaine samples were examined from the Forensic Science Institute Zurich. Six different samples were acquired with various adulterants and different cocaine concentrations between 5 % to 74 % (cf. table 9.8). Their spectra were analyzed according to the drying method which includes dissolving the powder in TCE and drying it on the ATR crystal (cf. figure 9.16). Compared to a spectrum of pure cocaine, the measured spectra are characterized by additional absorption lines. All spectra of the street cocaine samples were scaled to the same cocaine concentration and are compared in the following paragraph.

The spectrum of street cocaine sample 1 exhibits an absorption peak around $\sim 1658\,\mathrm{cm}^{-1}$ most likely caused by phenacetin (cf. figure 9.6). In the spectrum of the second street cocaine sample the absorption between 1770 and $1680\,\mathrm{cm}^{-1}$ is significantly higher compared to the other spectra in this wavelength range. This increase in absorption is most likely caused by the superposition of the absorbance of cocaine and caffeine. Whereas, the absorption peak around $1658\,\mathrm{cm}^{-1}$ most likely results from a combination of

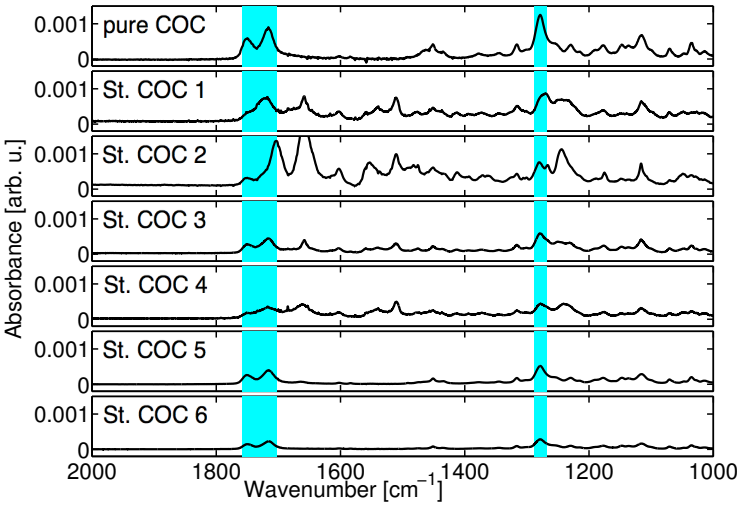


Figure 9.16: **Spectra of street cocaine (St.COC).** The street cocaine samples were dissolved in TCE and dried on the ATR crystal. All spectra are scaled to the same cocaine concentration (based on the dissolved mass and table 9.8). Differences might be caused by uncertainties of the weighing process and by problems with the dissolving of the samples. Highlighted areas mark high cocaine absorption. The graph at the top is showing a spectrum of pure cocaine.

Table 9.8: **Overview of the investigated street samples.** Samples were analyzed with GC-MS by the Forensic Science Institute Zurich.

sample no.	COC conc. [%]	found cutting agents according to GC-MS
1	5	phenacetin, glucose, lactose
2	21	phenacetin, creatine, caffeine, lidocaine, levamisole, lactose
3	26	phenacetin, levamisole
4	51	phenacetin, caffeine, mannitol, hydroxyzine
5	54	mannitol
6	74	comparatively high amount of benzoylecgonine

the absorbance of lidocaine and phenacetin in this spectral range. Spectral interference between the spectra of creatine and other adulterants impedes a distinguishable absorption peak of creatine around $1670\,\mathrm{cm}^{-1}$, but the small change around $1378\,\mathrm{cm}^{-1}$ could be caused by creatine. The spectra of the third and forth street samples show spectral absorption features of phenacetin around $1658\,\mathrm{cm}^{-1}$. In the spectrum of the forth street sample the absorption in the interval between 1730 and $1680\,\mathrm{cm}^{-1}$ is higher compared to the other spectra which is most likely caused by the caffeine absorption. No significant absorbance from diluents and adulterants was observed in the spectra of street cocaine samples 5 and 6.

Dissolving the street cocaine samples in TCE proved to be rather challenging despite a prolonged ultrasonic bath treatment. Therefore, no quantitative analysis was conducted with the spectra. But the results permit a qualitative fit of the cocaine spectrum to the spectrum of the street sample. Hence, an analysis with a different solvent might enable a quantitative analysis of the spectrum (cf. figure 9.17). Furthermore, the dissolving process might be improved by

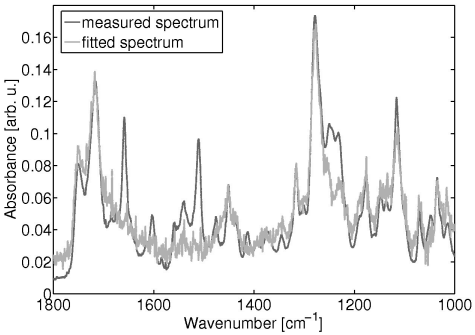


Figure 9.17: **Spectrum of street cocaine sample 3 fitted with a COC spectrum.** The additional absorption in the street cocaine spectrum is most likely caused by cutting agents. The higher noise level in the fitted spectrum compared to the street cocaine spectrum is caused by the large difference in concentration between the two spectra [41].

grinding the street cocaine powder before dissolving. Larger grains have a small surface to volume ratio, thus they dissolve slower than small grains.

Furthermore, the street cocaine samples dissolved in saliva and extracted by TCE were investigated by FTIR-ATR spectroscopy (cf. figure 9.18). The measured spectra of the extracts are compared with the spectrum of pure cocaine. As a result, the spectra of street cocaine extracted from saliva resemble the cocaine spectrum much more than the spectra of the street cocaine samples directly dissolved in TCE (cf. figure 9.16). In conclusion, solving the street cocaine in saliva and extracting it with TCE reduces the number of cutting agents in the samples. For example less caffeine and phenacetin absorption is observed in the spectra, although the extraction efficiency based on a similar model[21] was calculated to 45 % for caffeine and 97.4 % for phenacetin (cf. table 9.7).

Clear absorption lines of adulterants were detected only in the spectra of the extracts from street cocaine samples 2 and 3. The absorption line in the spectrum of sample 2 around $1660\,\mathrm{cm}^{-1}$ is most likely caused by lidocaine (cf. figure 9.6). Since no signature of phenacetin is detected in the spectra of the extracts from the street cocaine samples 1, 3 and 4. Thus, phenacetin seems only poorly extracted from saliva. The absorption at $1600\,\mathrm{cm}^{-1}$ in the spectrum of extract of sample 3 is most likely caused by levamisole. Only low cocaine absorption was observed in the spectrum which might have two reasons. First, it could be due to an uncertainty in the sample preparation. Second, levamisole might change either the solubility of cocaine or the partition coefficient. This could be investigated by generating an artificial mixture of levamisole and cocaine and repeating the extraction. Nevertheless, for better and quantitative analysis of the street cocaine samples the accuracy of weighing the

[21] The TCE-saliva system was approximated by the octanol-water system, since the partition coefficient for the TCE-saliva system is unknown.

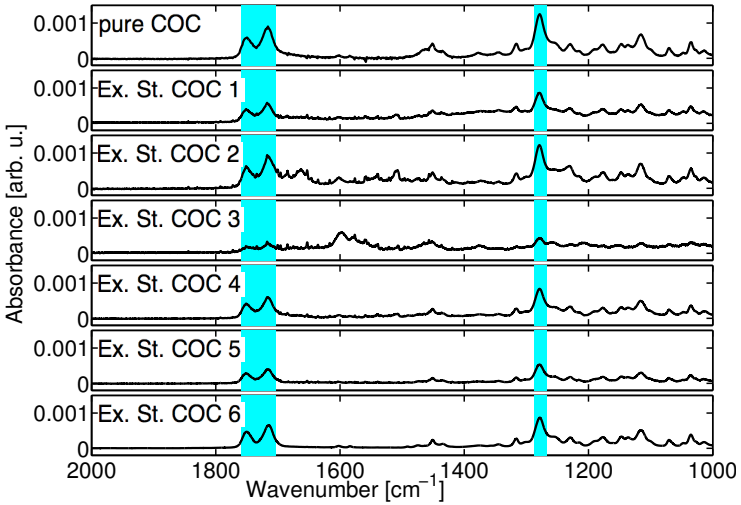


Figure 9.18: **Spectra of extracts from saliva spiked with street cocaine.** Saliva sample were spiked with street cocaine and extracted with TCE. The samples were dried on an ATR crystal and measured with an FTIR spectrometer. The spectra are scaled to the same concentration (based on the dissolved mass and table 9.8). Highlighted areas mark high cocaine absorption.

samples has to be improved.

Furthermore, the levamisol absorption is only detected in the spectrum of the extracts from saliva (sample 3), but not in the spectrum of the street cocaine sample 3 solved in TCE (cf. figure 9.19). This fact is in good agreement with the spectrum of levamisole (cf. figure 9.6). Levamisole was bought in its hydrochloride form and dissolves therefore very well in water or saliva but less in TCE. The difference in the solubility is the reason why the spectrum of pure levamisole is almost not detectable but the spectrum of the extract is well characterized.

In conclusion, the extraction method for cocaine was validated with street cocaine. The extraction method reduces the number of cutting agents since only cutting agents with a high solubility in saliva and a high extraction efficiency for the TCE-saliva system are extracted. Therefore, the extraction method can be considered to introduce a selective step with respect to the spectral analysis. Future studies might investigate the influence of levamisole on the extraction process in more detail.

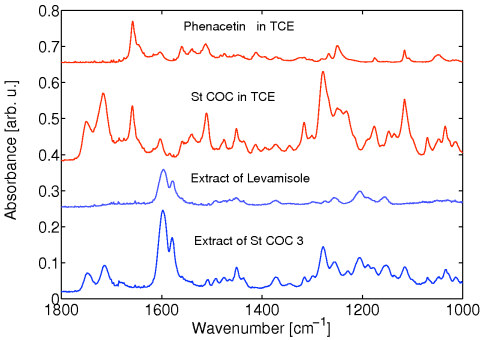


Figure 9.19: **Spectra of street cocaine sample 3 in comparison with spectra of various adulterants** (cf. table 9.8). The spectrum of the extract of street sample 3 is compared to the spectrum of the street sample 3 dissolved in TCE. In addition the spectrum of phenacetin dissolved in TCE and dried on the ATR crystal and the spectrum of levamisole extracted from saliva and dried on the ATR crystal are shown. Spectra are stacked and scaled for better comparison [41].

Chapter 10

Quantum cascade laser set-up

As discussed in section 2.3 a quantum cascade laser (QCL) has been employed as a compact light source in addition to the FTIR spectrometer. The QCL has been combined with an ATR unit (cf. section 10.1) and transmission measurements (cf. section 10.2). The design and fabrication of the home-built transmission cell is also presented[1].

The set-up for the measurements with the QCL is depicted in figure 10.1. The distributed feedback (DFB) QCL (cf. chapter 2.3.1) is tunable by temperature from 1746 cm^{-1} to 1756 cm^{-1} [40] and has a cw-power[2] output of a few mW. To correlate the temperature with the wavelength the absorption of water vapor was simulated with the Hitran data base [181] and measured with the QCL (cf. figure 10.2). Hence, the temperature $\mathfrak{T}$ is correlated with the wavelength $\tilde{\nu}$ by the

[1] Parts of this chapter have been published previously [38, 40–42]
[2] cw- continuous wave

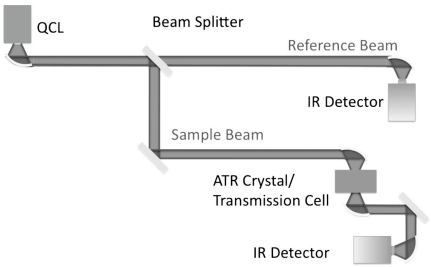


Figure 10.1: **Scheme of the QCL set-up.** The beam is split by a CaF_2 beam splitter into reference and sample beam. The sample beam can be guided either through the transmission cell or the ATR crystal [38].

following relation:

$$\tilde{\nu} = -0.1437\,\mathrm{cm}^{-1}\,^{\circ}\mathrm{C}^{-1} \cdot \mathfrak{T} + 1749.2\,\mathrm{cm}^{-1} \tag{10.1}$$

where the wavelength $\tilde{\nu}$ is given in cm^{-1} and the temperature $\mathfrak{T}$ in °C.

If not indicated otherwise, the laser was set to a wavelength of $\sim$1750 cm^{-1} ($\sim$5.714 µm). The previous FTIR studies implied that the first strong cocaine absorption peak, occurring around 1756 cm^{-1} in organic solvents (with a FWHM[3] of $> 10\,\mathrm{cm}^{-1}$), has the least interference with other substances potentially appearing in saliva (cf. chapter 9), i.e. this wavelength exhibits a strong cocaine absorption. Nevertheless, the QCL wavelength was not set exactly to 1756 cm^{-1} since both the laser noise and the water vapor absorption are lower at 1750 cm^{-1}.

[3]full width of half maximum

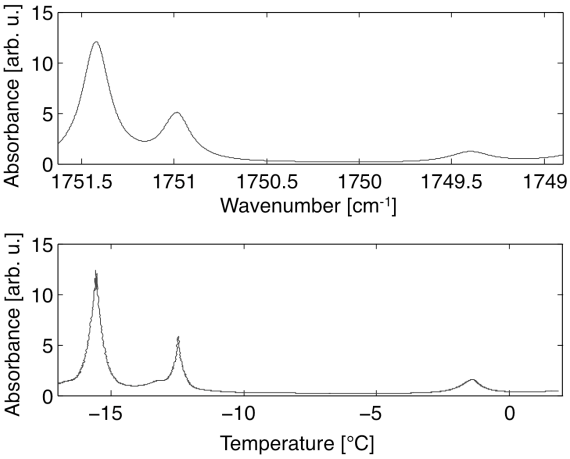


Figure 10.2: **Absorbance of the water vapor./ Tuning range of the QCL.** To correlate the temperature of the QCL with the wavelength the water vapor absorption was measured with the QCL (bottom, spectrum is scaled) and simulated with Hitran data (top) [40, 181].

The QCL is current modulated with a sine-wave generator at a frequency of $\sim$ 1 MHz. The laser beam is collimated with an off-axis parabolic gold mirror (Edmund Optics). After the collimation the laser beam is split by a coated CaF_2 beam splitter (Thorlabs) into the sample beam (SB) and the reference beam (RB). The RB is recorded with an infrared photovoltaic detector (VIGO, MIPDC-5, PVI-4TE-6) to compensate for laser power fluctuations. The SB passes either through the attenuated total reflection (ATR) unit or the transmission cell and is then recorded by an identical infrared detector.

After the recording of the modulated signal, the data is Fourier transformed and passed through a band pass filter around the modulated frequency to minimize the noise. In addition, corrections can be made for the laser power fluctuations to further minimize the noise. The set-up still has potential for improvement in terms of stability and therefore it is important to determine the source of the sudden changes in the signal. Sudden changes caused by the laser would be visible in the SB and RB and therefore cancel out in the ratio SB/RB (cf. section 10.2).

10.1 ATR measurements

The ATR unit (Perkin Elmer) is described in chapter 8. The ATR top plate with 11 reflections in the ZnSe crystal[4] was used for this set-up. The sample volume was 400 µl and samples were dried on top of the ATR crystal. The drying method is described in section 8.2. The QCL was operated in cw mode.

[4]In contrast to the 12 reflection top-plate of the ATR unit, the 11-reflections plate has a built-in trough which is better suited for liquids.

10.2 Transmission measurements & cell design

For the transmission measurements the QCL was operated in pulsed mode. Pulses were ~ 300 ns long and repeated with ~ 1 MHz. The transmission cell was designed and built in-house and consisted of two NaCl windows (24.4 x 50.8 x 5 mm, Korth Kristalle GmbH), a structured Norland Optical Adhesive 81 (NOA 81) layer and teflon tubes (cf. figure 10.3).

NOA 81 was chosen as material to fabricate microfluidic channels since it can resist to tetrachloroethylene (TCE) and other extraction solvents [182] employed in this thesis for several hours. The channel mask[5] was fabricated from a polydimethylsiloxane (PDMS, Silicone Elastomer, DC 184, Dow Corning Sylgard) layer, with a height of 1 mm. The layer was set upon the NaCl window, where the cavities were filled with liquid NOA 81. NOA in its liquid form contains many solvents which can form air holes in a NOA structure if the adhesive is cured too quickly. Therefore a time span of at least 1 h passed before further processing to allow most of the solvents to evaporate. Afterwards, the second NaCl window was set on top of the PDMS layer and the NOA was cured under UV light for 5 min (LOT-Oriel 450W Hg UV-Source). The NaCl window creates a planar surface during the curing and small air reservoirs in the middle of the structures. After removing the top NaCl window, the PDMS layer can be removed and the small reservoirs between the channels can be filled with NOA. In addition, the teflon tubes are placed onto the lower NaCl window and kept on the spot with NOA. As a last step the NaCl window is placed again on top of the NOA layer and cured under UV-light.

The margins of the cell are covered with epoxy (Devcon 2 Ton Epoxy) to ensure a leak-proof sealing. With this manufacturing pro-

[5] The PDMS layer was cut into the "negative" of the channel structure.

cess channels with different widths can be produced and investigated on a single NaCl window. For the measurements only one channel was used. It was filled and emptied with glass micro syringes (Innovative Labor Systeme). The channel was $\sim 1\,\text{mm}$ thick, $\sim 2\,\text{mm}$ wide and $\sim 5\,\text{mm}$ long (cf. figure 10.3 C). The laser enters perpendicular to the NaCl window and is focused at the middle of the cell.

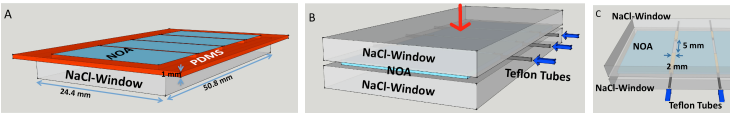


Figure 10.3: **Scheme of the transmission cell.** A: NaCl window with the PDMS frame and NOA-fillings is shown. B: The transmission cell after the second curing process with all connecting tubes is displayed. Laser light (red arrow) passes through the cell perpendicular to the NaCl-window. C: The complete cell is depicted [38].

10.2.1 Transmission cell measurement protocol

The transmission cell was filled with a glass syringe containing either TCE or TCE spiked with cocaine. While filling and emptying the cell special care has to be taken, not to move the cell by accident since these movements can dramatically influence the signal recorded by the detector placed on the opposite side of the transmission cell (cf. figure 10.1). Such changes can be identified by changes in the SB and in the ratio of the SB to the RB, but no changes in the RB would be detected. Movements of the transmission cell hinder comparisons between the signals. Consequently, the intensity of the light was measured before, during and after the filling of the cell. Large differences in the signal before and after the filling indicate

changes of the set-up. Therefore these data-points were excluded from comparisons.

Chapter 11

QCL analysis

In this chapter the results of the analysis with the quantum cascade laser (QCL) are summarized (set-up in chapter 10)[1]. First, the results gained with the QCL and the attenuated total reflection (ATR) unit are described. Then the results acquired with the QCL and a transmission cell, which was built in-house, are presented.

11.1 QCL-ATR measurements

Like for the FTIR-ATR measurements (cf. chapter 8), the ATR unit allows for preconcentration of the sample by drying them on top of the ZnSe crystal during measurements (cf. chapter 8). Tetrachloroethylene (TCE) samples spiked with cocaine were analyzed to investigate the limit of detection (LOD) of this set-up. Samples require a volume of 1.2 ml (3 times 400 µl) and were prepared with a cocaine concentration in the range from 3 to 38 $\mu g\,ml^{-1}$. For all three measurements of each sample the absorbance was calculated by the negative logarithm of the ratio of the transmission through the ATR-crystal (signal with sample divided by signal without sample;

[1] A part of this chapter was previously published in [38, 40–42]

cf. figure 11.1). The transmission signal was normalized with the laser power from the reference beam. As a result, the absorbance is linearly proportional to the cocaine concentration with a linear correlation coefficient of $r^2 = 0.99$. The limit of detection for the best measurement was $\sim 3\,\mu g\,ml^{-1}$. The variation in the absorbance is most likely caused by different drying patterns on the ATR crystal (cf. section 8.2).

11.2 QCL transmission measurements

Samples of TCE spiked with cocaine were measured in the transmission cell and compared to the transmittance of pure TCE as reference. Samples with the cocaine concentration of 0.1, 1, 10 and $100\,\mu g\,ml^{-1}$ in TCE were examined. The transmittance of the cell before the filling, filled and after the filling of the sample was recorded. Movements of the transmission cell were determined by comparing subsequent measurements of the empty cell[2]. Subsequent measurements without movements are compared to each other (cf. highlighted areas in figure 11.2).

In order to account for power fluctuations of the QCL, the transmittance of the TCE samples spiked with cocaine are normalized with the reference beam. As a result, difference between the samples with 0.1 and $1\,\mu g\,ml^{-1}$ cocaine concentration in TCE did not exceed the noise fluctuations. Higher cocaine concentrations can be distinguished between each other yielding a limit of detection $< 10\,\mu g\,ml^{-1}$ for a sample volume of $100\,\mu l$.

[2]A movement of the cell is determined by a significant change in the transmittance of the cell in between two measurements of the empty cell.

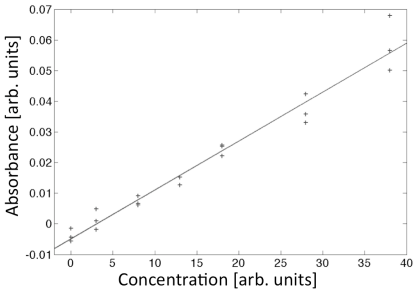


Figure 11.1: **Absorbance of reference samples measured with the QCL for various cocaine concentrations.** A linear relation between concentration and absorbance is observed ($r^2 = 0.99$) [38].

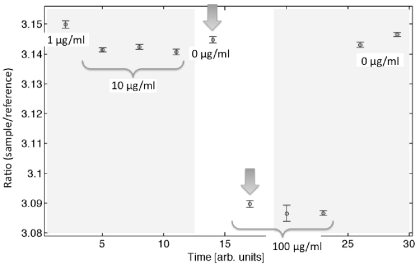


Figure 11.2: **Transmittance measurements with the QCL.** Highlighted data blocks indicate comparable data regimes. Arrows indicate a recorded movement of the transmission cell. The difference in transmission between the TCE samples with a cocaine concentration of 1 and 10 $\mu g\,ml^{-1}$ is detected significantly [38].

11.3 Conclusion of the QCL measurements

The implementation of the transmission cell into the set-up was successful. The cell could be connected to a microfluidic set-up. It could be used as a flow cell for the TCE samples. The limit of detection is $< 10\,\mu g\,ml^{-1}$ for a sample volume of $100\,\mu l$. In addition, the ATR-QCL set-up was successfully tested with a measured limit of detection of $3\,\mu g\,ml^{-1}$ for a sample volume of $400\,\mu l$.

The difference in LOD for both QCL measure methods might originate in the preconcentration of the sample during the drying process for ATR measurements. Furthermore, the absorption peak slightly shifts towards lower wavenumbers (cf. figure 11.3). In addition, the optical path length is very different in both approaches. The ATR unit is characterized by a path length of several µm whereas the transmission cell by a path length of $\sim 1\,mm$. Both approaches enter the concentration regime of real-life applications[3], but need further improvement to reach a feasible limit of detection in the $\sim 100\,ng\,ml^{-1}$ regime (cf. chapter 5).

[3]The cocaine concentration in saliva can get as high as $500\,\mu g\,ml^{-1}$ for a single dose of 40 mg cocaine [64].

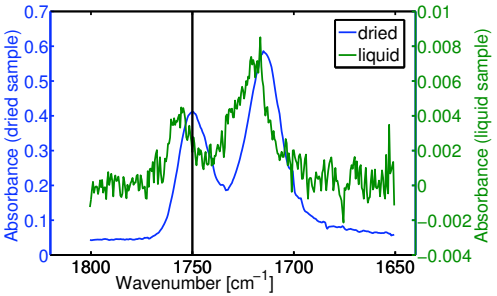


Figure 11.3: **Shift of the cocaine absorption.** The spectrum of TCE samples spiked with cocaine shift towards lower wavenumbers by drying on the ATR crystal. The vertical line marks the QCL emission at 1750 cm^{-1}

Part III

Caffeine

Chapter 12

Caffeine: Introduction & method

In the following, the relevance of caffeine measurements is explained followed by a short presentation of some experimental techniques to determine caffeine concentrations[1]. Thereafter, the sample preparation used for the caffeine samples is described.

Caffeine interacts the adenosine receptors in the brain. Therefore, it might have an influence on the following diseases: Alzheimer's, Parkinson's, Huntigton's disease, Epilepsy, migraine and Schizophrenia [183]. In many cases the connection between the disease and caffeine is not quite clear. It is argued, for example, that people with moderate caffeine consume might reduce the risk for Parkinson's or Alzheimer's disease [3].

Studies investigating these connections usually require test persons that abstain from caffeine-containing beverages. Instead a controlled dose of caffeine or placebo is administered for analysis. Such

[1] Parts of this chapter was pulished in [40]

a study protocol is challenged by the problem that coffee and tea are very social drinks in society, which makes abstaining rather difficult. In order to overcome this problem and allow for reliable study protocols it is suggested to monitor the caffeine intake of the test persons during the studies instead of abstaining. Such a protocol would allow for monitoring of caffeine consume in real life. An easy to handle hand-held device for convenient measurements of the caffeine concentration in the consumed beverage is required for such an analysis.

In this thesis, we present both a simple extraction method that could be miniaturized on a microfluidic chip based on (NOA 81, for criteria for the extraction see section 7.1.1 and 9.4.3) and the analysis of the extracts with an FTIR-spectrometer.

12.1 Effects of caffeine

The consumption of caffeine-containing beverages in moderate doses (< 6 cups per day) can lead to fewer cognitive failures, less depressive symptoms and lower the risk of suicide [184]. Other studies associated moderate caffeine intake with increased vigilance and motor activity. In addition, caffeine decreases the need to sleep and produces sensations of well-being and energy [185].

However, caffeine consumed in high doses can lead to discomfort, including anxiety, nervousness and insomnia [185]. In addition, it can stimulate psychotic and maniac symptoms [184].

12.2 Caffeine content

There are different caffeine sources, but the most common are probably tea, guarana and coffee. The caffeine concentration of coffee was found to be 190 to 890 $\mu g\,ml^{-1}$ and for black tea 130 to

500 µg ml^{-1}[186]. The caffeine content in beverages depends strongly on the coffee brand and brewing technique. In table 12.1 the caffeine content is shown in mass percent of the tea leave, coffee bean or guarana berry.

12.3 Extraction of caffeine

Often caffeine is extracted from, e.g., coffee beans or guarana berries, with one of the following solvents or a mixture of them: water, supercritical CO_2, ethanol, chloroform, acetone, methylene chlorides, trichloromethane, methanol, and toluene [187, 190–195]. Solvents used for caffeine extraction should provide a high solubility and a high extraction efficiency for caffeine.

Many extraction methods are based on additional chemical substances like NaOH or ammonia. Others use bulky laboratory equipment for the extraction like centrifuges and vacuum pumps [190, 194, 195], which are not convenient for miniaturization.

Several techniques are used for the analysis of caffeine. For example UV spectroscopy was combined with a dichloromethane extraction [193]. HPLC combined with multiple extractions was used

Table 12.1: **Caffeine content in different products.** Concentrations are given in mass percent [40, 187–189].

product	concentration
coffee	0.44 to 4.35
white tea	3.4 to 5.7
black tea	2.0 to 5.4
guarana	4.1 to 4.4

to analyze caffeine in teas [196]. Raman Spectroscopy was used to analyze the caffeine content in cacao [197].

FTIR- spectroscopy was utilized to detect caffeine in extracts from grained coffee beans with trichloromethane and ammonia [195], from black tea leaves [198], soft drinks [199], and from coffee (drink) [200].

The first two extraction procedures [195, 198] use ammonia which posseses a strong pungent odor, that might lead to complications in a hand held device. The latter study used chloroform for an extraction, which is not feasible with the micro fluidic material NOA targeted in this study.

Therefore, a new extraction technique was explored with liquids that are feasible with the material NOA 81.

12.4 Method: Caffeine extraction

In this thesis caffeine was examined in 4 liquids: beverages containing caffeine, saliva after the consume of caffeine containing beverages, saliva spiked with caffeine and reference samples.

The saliva acquisition is described in chapter 6. The extraction method is similar to the first extraction method of cocaine described in section 7.1. The saliva samples spiked with caffeine were prepared in filtering the saliva, spiking it with caffeine weighed beforehand, and mixing it with the extraction solvent. The analyzed beverages were mixed with the extraction solvent and then spectrally investigated.

For the caffeine extraction the following solvents were tested: 1-octanol, iso-octane, n-heptane, dibutyl ether, toluene, dichloroethane, tetrachloroethylene, diethyl ether and dichloromethane. The sol-

vents were chosen based on the criteria listed in section 7.1.1.

The measurements were calibrated with the help of reference samples. These reference samples consisted of the extraction solvent spiked with caffeine, directly.

Chapter 13

Infrared spectroscopy on caffeine

In this chapter the results of the caffeine analysis are presented[1]. Four types of liquids were analyzed: caffeine-containing beverages, caffeine-spiked saliva[2], saliva after the consume of caffeine-containing beverages and reference samples (toluene spiked with caffeine).

13.1 Caffeine in beverages and saliva

Like for cocaine, the detection of caffeine in beverages or saliva is very challenging due to the high background absorption of water (cf. section 9.4.1). No significant characteristics of caffeine absorption were found, neither in the analyzed caffeine-containing beverages (cf. figure 13.1, 9.3 and E.1) nor in saliva after the consume of these beverages (cf. figure 13.1 and 9.7).

[1] Parts of this chapter were published previously in [40].
[2] For details of the saliva acquisition see chapter 6.

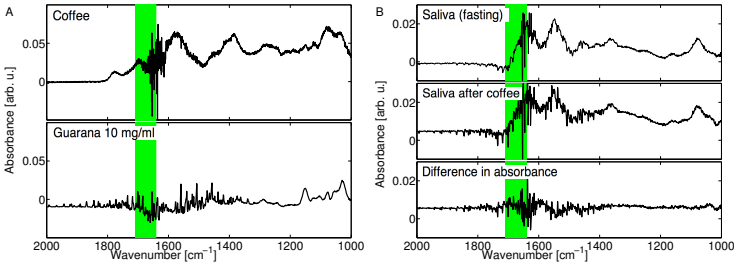


Figure 13.1: **Spectra of caffeine-containing products and saliva after coffee consume.** A: Spectra of a suspension of guarana and coffee are displayed. B: Spectra of saliva collected from a fasting person, after coffee consume and the difference in the spectra are depicted. The background absorption of water is subtracted for all displayed spectra. The highlighted area marks high caffeine absorption, no distinguishable caffeine absorption peak is detectable in both figures A and B.

As a consequence an extraction method for caffeine from saliva and from caffeine-containing beverages was developed that could be miniaturized on a microfluidic chip. The criteria relevant for the choice of a solvent have been discussed in section 7.1. The following solvents were tested on saliva samples spiked with caffeine: 1-octanol, iso-octane, n-heptane, diethyl ether, dibutyl ether, toluene, dichloromethane, dichloroethane and tetrachloroethylene (TCE).

The spiked saliva and solvents were mixed and spectrally analyzed after their self-separation (cf. first extraction method, section 7.1). As a result, diethyl ether was excluded from further extraction studies due to its high volatility. An intermediate phase of stable droplets, i.e. droplets of the solvent in saliva (similar like in figure 7.2), is formed for the following solvents: dichloromethane, dichloroethan and 1-ocantol. A low absorbance of caffeine in the extract was recorded for iso-octan, dibutyl ether and n-heptane (cf. figure 13.2). The most suitable solvents for the extraction were toluene and TCE. The absorbance of caffeine in the extract toluene (cf. figure 13.2) was higher than for the extract with TCE. The difference in absorbance is most likely caused by a higher concentration of caffeine in the solvent. In conclusion, toluene is the most suitable solvent to extract caffeine from solutions.

The caffeine in coffee was extracted with toluene. The spectra of coffee before and after the extraction are compared to the absorption of the extract (cf. figure 13.2). The caffeine is well detectable in the toluene phase of the extraction. The new absorbance peaks in the spectrum of coffee are due to small contaminations with toluene of the sample, since toluene absorbs strongly at $1495\,\mathrm{cm}^{-1}$.

The measured absorbance of caffeine depends on the state. Caffeine dissolved in toluene has a strong absorption peak around $\sim 1710\,\mathrm{cm}^{-1}$ and at $\sim 1688\,\mathrm{cm}^{-1}$, whereas dried caffeine[3] is char-

[3]The drying technique is explained in section 8.2

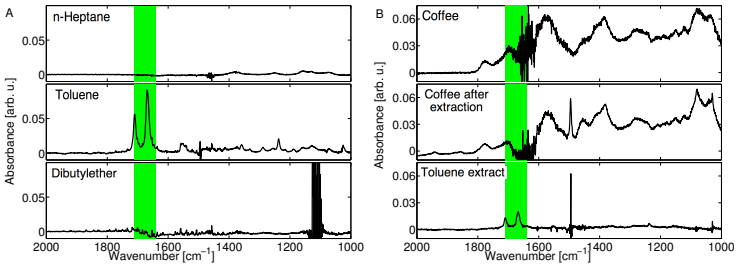


Figure 13.2: **Spectra of extracts from saliva spiked with caffeine, coffee before and after extraction and the extract of coffee.** A: Spectra of the extracts form saliva spiked with caffeine are shown. No significant absorbance of caffeine in n-heptane of dibutyl ether. The caffeine absorption in toluene is clearly detectable. All solvent backgrounds were subtracted. B: Spectra of coffee, before and after the extraction with toluene. Absorption lines of toluene are observed in the spectra after extraction. The background absorption of water was subtracted for all coffee spectra. The spectrum of the toluene phase after the extraction is shown at the bottom, after the subtraction of the toluene spectrum. The highlighted area marks high caffeine absorption, caffeine peaks are clearly visible.

acterized by a maximum in absorbance at $\sim 1701\,\mathrm{cm}^{-1}$ and at $\sim 1655\,\mathrm{cm}^{-1}$ (cf. figure 13.3).

Caffeine was also extracted from saliva from a test-person who consumed two espressos just before sampling (cf. figure 13.3). Due to difficulties with the sample volume, the resulting extract was less than 400 µl and therefore, a quantitative analysis could not be performed in this case. However, the caffeine absorbance peaks can clearly be observed in the extract.

13.2 Quantitative measurements

Calibration measurements for caffeine concentrations in toluene were conducted with reference samples[4] (cf. figure 13.4). Samples were investigated in dissolved and dried form. The spectra of the reference samples with different concentrations of caffeine were fitted with one previously measured caffeine spectrum with a high signal-to-noise ratio. A linear regression analysis was performed to correlate the fitting parameter to the caffeine concentration (cf. figure 13.4). Unlike the calculation for the cocaine concentration described in detail in chapter 8 only the spectra of caffeine and of the solvent were used for fitting in this case.

The fitting parameter of the reference samples in their liquid form yield a correlation coefficient of $r^2 = 0.9994$. The fitting parameter for the spectrum of the dried samples is not linear for the whole concentration regime. This deviation from linearity is most likely caused by saturation in absorbance. Therefore, only the fitting parameters calculated for spectra retrieved from concentrations $\leq 500\,\mu\mathrm{g\,ml}^{-1}$ were correlated with the concentration. The correlation coefficient yields $r^2 = 0.994$.

[4] Reference samples consist of caffeine dissolved in toluene at various concentrations.

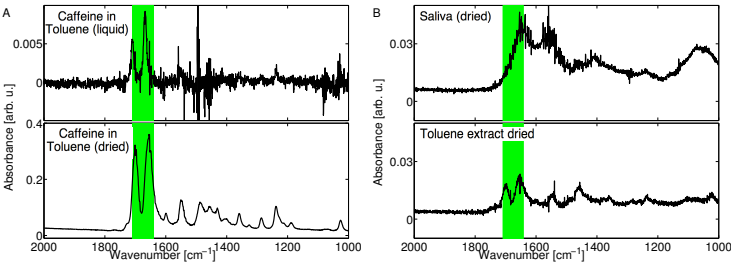


Figure 13.3: **Spectra of caffeine in toluene.** A: The caffeine concentration in both spectra was 250 µg ml^{-1}. The drying process shifts the caffeine absorption peak towards lower wavenumbers. B: Spectra of saliva and extract after coffee consume. The highlighted area marks high caffeine absorption.

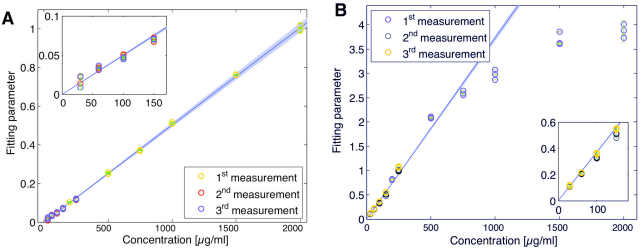


Figure 13.4: **Calibration with reference samples for caffeine.** A: The fitting parameter for the spectra of caffeine samples dissolved in toluene correlate linearly to the caffeine concentrations from 30 µg ml^{-1} to 2000 µg ml^{-1}. B: The fitting parameters for spectra of caffeine dissolved in toluene and dried on the ATR crystal are plotted vs. the caffeine concentration of the solution. Linearity can be found up to a concentration of 500 µg ml^{-1} of caffeine in toluene. The circles denote single measurements, the average slope is depicted in blue with standard deviation and the error bars are shown in green. Each measurement was repeated three times [40].

Extracts from coffee, guarana, white and black tea were investigated. The concentration of the extracted caffeine was determined but the solution was not pH stabilized (cf. table 13.1). The calculated concentration of the powder or the leaves is significantly lower than the literature data for two reasons (cf. table 12.1). First, the data in literature were directly extracted from the powder, bean or leave, in contrast to the extraction from the beverage. Therefore, the caffeine content was reduced twice by producing the beverage and by extraction of the caffeine from the beverage. The second reason might be caused by the charged state of caffeine. Caffeine has a pK_a-value of 10.4 (cf. table 9.6) and the analyzed beverages were characterized by pH-values between 5 and 6. Therefore most of the caffeine was in its charged state, which impedes an extraction into a nonpolar solvent like toluene (cf. chapter 3).

13.3 Conclusion

In conclusion a successful one-step extraction for caffeine was developed. Toluene is the most suitable solvent for caffeine extraction among all tested solvents. A successful extraction of caffeine was demonstrated from saliva spiked with it, saliva after the consume of coffee and of caffeine-containing beverages.

For future examinations the pH should be stabilized, e.g., with a concentrated buffer. The analysis should be conducted towards the ideal pH. In addition, the reproducibility of the method needs to be investigated further to calculate the limit of detection and uncertainty of the extraction. Overall, the extraction results are very promising and have the potential to be miniaturized on a microfluidic chip. A hand-held device could be manufactured combining an infrared detector and a quantum cascade laser emitting at one of the caffeine absorption peaks. Such a device would aid future studies on the correlation between Alzheimer's, Parkinson's, Huntigton's

Table 13.1: **Quantitative analysis of extracts from caffeine-containing beverages.** (Abbreviations: est. dried reference- estimation of the concentration of the extract from spectra of dried reference samples; est. liquid reference- estimation of the concentration of the extract form spectra of liquid reference samples; weight- total leave or powder weight; Volume- Volume of the beverage; min. caff.- minimal caffeine content of the powder or leave; [1]- according to Nescafé® Dolce Gusto®) [40].

substance	coffee	guaranna	white tea	black tea
est. dried reference [$\mu g\,ml^{-1}$]	192 ± 7	87 ± 2	29 ± 1	66 ± 1
est. liquid reference [$\mu g\,ml^{-1}$]	205 ± 50	69 ± 14	20 ± 8	71 ± 8
weight [g]	10[1]	3.039	1.078	1.493
volume [ml]	180	200	200	200
min. caff. [mass %]	0.36	0.45	0.37	0.88
pH of the beverage	5	5-6	6	5-6

disease, Epilepsy, migraine and Schizophrenia and caffeine consume (cf. chapter 12).

Part IV

Periodontitis trial

Chapter 14

Periodontitis: Introduction & method

In this chapter the periodontitis and its connection to porphyromonas gingivalis bacteria is introduced[1]. In addition, the analysed samples are described.

14.1 Porphyromonas gingivalis bacteria and periodontitis

Periodontitis might occur as a combination of gingival inflammation and a pathological detachment of collagen fibers from the cementum. In addition, it is characterized by an apical migration of the junctional epithelium, i.e. formation of periodontal pockets [202] (cf. figure 14.1). Usually an increased occurrence of bacteria is found in the periodontitis site, i.e. on and in the ginival epithelium and on the tooth. The most common bacterium on these inflamed sites is

[1] A part of this chapter was previously published in [201].

porphyromonas ginivalis [203].

Periodontitis in patients is diagnosed by certain signs and symptoms like gingivitis, bleeding on probing (BOP), pocket probing depth (PPD), clinical attachment loss (CAL) as well as radiographically assessed alveolar bone loss [202]. These techniques only investigate the effects of periodontitis and offer only limited insight in the future development of the disease, e.g. whether the patient is at high risk for rapid periodontal disease progression, or not.

Periodontitis is a common disease. In Oslo a study with 35-year old people determined in 1973 that 54 % of all investigated individuals had suffered bone loss. The number decreased to 24 % in 2003 most likely due to an improved oral hygiene [4]. Nevertheless, a significant part of the population is still affected by this disease today.

Periodontitis is not restricted to the oral cavity alone, since it is also associated with increased risk of coronary heart disease. A meta-study concluded that prevalence and incidence of coronary heart disease are significantly increased in periodontitis patients [5].

The oral health is often evaluated by probing the gingival crevicular fluid (GCF). Gingival crevicular fluid is a secretion that is collected at the site where the gum line meets the teeth. The infrared spectrum of the GCF is influenced by periodontitis and gingivitis. Certain spectral ranges were identified which allow for differentiation of people with and without periodontitis [204].

Saliva has proven to be an easily accessible reservoir as an indicator of periodontal infections, as well as for general health [205, 206]. Differences in the saliva of patients with periodontitis and control were found by real-time polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA) [207]. Hence, the question arises, whether the IR-spectra of saliva allow differentiating between periodontitis patients and a control group.

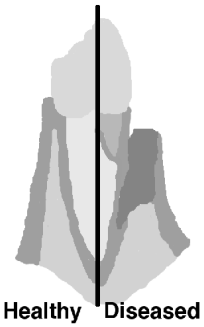


Figure 14.1: **Periodontal pocket.** Comparison between a healthy and a diseased tooth showing a periodontal pocket.

This research question is examined in this thesis by comparing the IR-spectra of saliva from patients with generalized aggressive Periodontitis (G-AgP) with saliva from a control group. The primary features of G-AgP are rapid attachment loss and bone destruction in systemically healthy patients. The disease is usually found in patients, which are under the age of 30. A study investigating the general periodontal health of Israeli Army personal (18 to 30 years) observed the disease in 2 % of them [208]. Even though, the prevalence of this type of disease is lower than periodontitis, an early diagnosis would provide an enhanced understanding of its progression.

14.2 Samples

Saliva samples used for the analysis of aggressive periodontitis were acquired by the Institute of Oral Biology at the University of Zurich.

There, saliva samples are centrifuged at 10000 g for 15 min at 5 °C. After the centrifugation the saliva is separated into supernatants and cell pellets. These samples were frozen at −80 °C. In this thesis the supernatant is analyzed with infrared spectroscopy (cf. chapter 15 and for more details concerning sampling and the involved test persons see [201]).

Chapter 15

Results of the G-AgP trial

In this chapter the results of the generalized aggressive periodontitis (G-AgP) trial are presented[1]. Two types of samples were investigated: saliva spiked with porphyromonas gingivalis bacteria and supernatant[2] of saliva from G-AgP patients and a control group. The samples were acquired from the Institute of Oral Biology (University of Zurich).

15.1 Supernatant of saliva from G-AgP patients

The spectra of supernatants retrieved from saliva of either G-AgP patients or healthy volunteers were compared. The samples were

[1] A part of this chapter was previously published in [201].

[2] When saliva is centrifuged it divides into supernatant and cell pellets. In this thesis only the supernatant of saliva was investigated.

dried on an ATR crystal and measured with a FTIR- spectrometer (cf. section 8.2).

Ten test persons participated in this study (5 G-AgP patients and 5 healthy volunteers). Each test person provided one sample and each sample was measured 3 times. The resulting spectra were then divided into the two groups (G-AgP and control) and averaged. No significant difference between both groups can be observed in the spectra of their liquid samples (cf. figure 15.1).

Differences in the spectra of the dried samples from both groups (G-AgP and control) are observed in the spectral range from 1230 to 1180 cm^{-1} and around 910 cm^{-1} (cf. figure 15.2). As a first step the difference in absorbance ΔA was calculated for the absorbance at 1206 cm^{-1} and at 1196 cm^{-1} (cf. figure 15.3) as:

$$\Delta A = A(1206\,\mathrm{cm}^{-1}) - A(1196\,\mathrm{cm}^{-1}) \tag{15.1}$$

where $A(1206\,\mathrm{cm}^{-1})$ indicates the absorbance at 1206 cm^{-1} and $A(1196\,\mathrm{cm}^{-1})$ the absorbance at 1196 cm^{-1}. This calculation takes the shift in the offset of absorbance between different samples into account and emphasizes the difference in the absorption pattern between the two groups.

The two data sets (of ΔA) between both groups were evaluated with the analysis of variance (ANOVA) method[3] after checking for normal distribution by the Lilliefors analysis. Each group consisted of 15 data points (5 samples, each measured 3 times). The ANOVA analysis concludes that the probability for both groups to be from the same normal distribution with the same mean is 0.002. Hence, the difference in both groups is most likely significant.

Furthermore, a principal component analysis (PCA)[4] of the absorbance data in the spectral interval from 1230 to 1180 cm^{-1} was

[3]Matlab: anova1, Statistics_Toolbox, Matlab V7.2 (R2009b)
[4]Matlab: princomp, Statistics_Toolbox, Matlab V7.2 (R2009b)

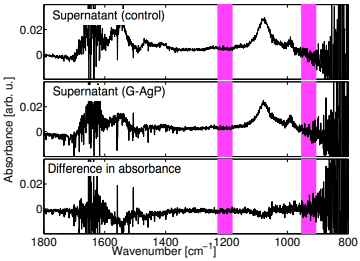


Figure 15.1: **Spectra of supernatant of saliva.** The spectra of supernatant of saliva from patients with G-AgP and from healthy volunteers (control) are depicted together with their difference. The background absorption of water was subtracted from each spectrum. Highlighted areas mark a difference found in dried samples (cf. figure 15.2). No significant differences can be observed in the spectra of liquid samples.

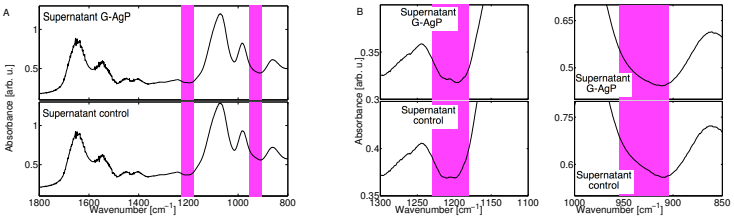


Figure 15.2: **Spectra of dried supernatant of saliva.** A: The spectra of supernatants for both groups (G-AgP and control) are shown. B: A magnification of the two most interesting areas (1230 to 1180 cm^{-1} and around 910 cm^{-1}) is given. Highlighted areas mark main differences in the absorption between the spectra of the supernatant of the two groups.

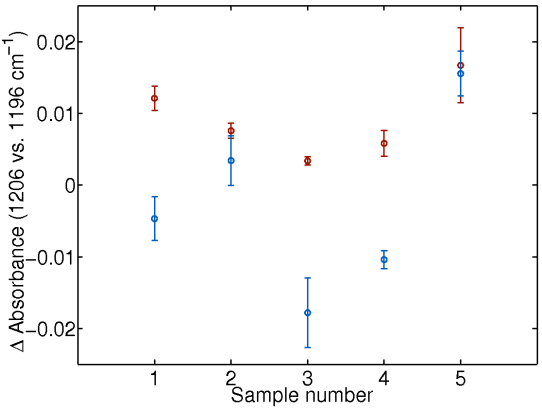


Figure 15.3: **Difference between the absorbance of the investigated supernatant at** $1206\,\mathrm{cm}^{-1}$ **and at** $1196\,\mathrm{cm}^{-1}$**.** The red circles display the difference in absorbance acquired from the G-AgP patients. The blue circles represent the difference in absorbance of the control group. The standard deviation is plotted with error bars. A difference in ΔA between the two groups is detectable except for sample 5 of the control group [201].

applied. An analysis of the second and third principal component allows a differentiation of the data (cf. figure 15.4). The only exception is again sample 5 of the control group. One of the corresponding data points of sample 5, score outside the control group margin, but the average is still differentiable from the G-AgP group.

Other tests on these samples were conducted at the Institute of Oral Biology. These results confirmed that sample 5 was an outlier. For example, the total protein concentration in saliva was measured. The protein concentration of sample 5 was 3.3 times higher than the protein concentration of all the other samples from healthy volunteers.

15.2 Spectral analysis of porphyromonas gingivalis

The concentration of porphyromonas gingivalis bacteria in saliva of periodontitis patients is higher than in saliva from healthy test persons [203]. Therefore, the question arose, whether the concentration of porphyromonas gingivalis can be directly detected in saliva samples with infrared spectroscopy.

The porphyromonas gingivalis bacteria were acquired from the Institute of Oral Biology in a sodium chloride (NaCl) solution (0.9 M) at a concentration of 10^9 bacteria ml^{-1}. Saliva samples were taken from one volunteer, filtered with 0.2 µm sterile syringe filters (Sarsted) and then spiked with bacteria at various concentration (10^5, 10^6, 10^7, 10^8 bacteria ml^{-1}). Each saliva sample was adjusted for the same amount of NaCl solution and was compared to saliva spiked with the same volume of NaCl solution.

The samples were analyzed after drying them on top of the attenuated total reflectance (ATR) unit with the FTIR spectrometer

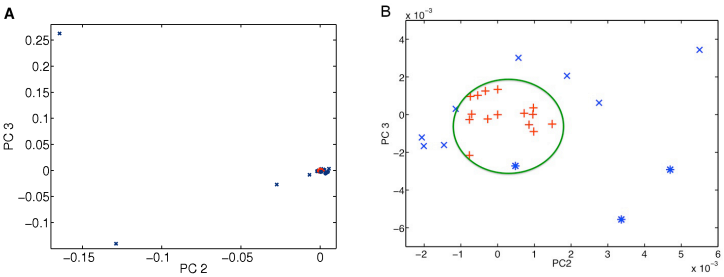


Figure 15.4: **PCA analysis of the absorbance spectra assessed from the analyzed supernatant.** The samples were dried and the measured spectra were analyzed with PCA. The second principal component (PC2) is plotted vs. the third principal component (PC3). Plot B shows a magnification of Plot A. The plot shows the separation of the data acquired for the G-AgP (+) patients from the control (x) with the exception of sample 5 (*). The circled area emphasizes the difference between the data points.

(cf. section 8.2). Each sample was measured 3 times. The averaged spectra were compared by subtracting the saliva spectrum from the spectra spiked with bacteria (cf. figure 15.5). No significant correlation between the spectra recorded from saliva spiked with bacteria and the bacteria concentration could be established. Most likely the spectrum of the bacteria is too similar to the saliva spectrum to show any significant difference.

15.3 Conclusion

In conclusion, a successful differentiation of the two groups (G-AgP and control), is possible as shown by calculating the difference in absorbance for two fix wavlengths ($1196\,\mathrm{cm}^{-1}$ and $1206\,\mathrm{cm}^{-1}$). The results are confirmed by the ANOVA method. In addition, a successful separation of the two groups is also demonstrated by a principal component analysis (PCA). However, no significant difference is found between the saliva spectra and the saliva spectra spiked with porphyromonas gingivalis.

Further investigations might include a larger sample set to determine whether sample 5 was an exception or part of the normal parameter variation. FTIR analysis appears as a promising tool for the analysis of saliva samples. The analyzed narrow spectral range that yields significant differences lies within the tuning range of quantum cascade lasers. This fact allows a miniaturization of the set-up into a hand-held device suitable for chair-side tests.

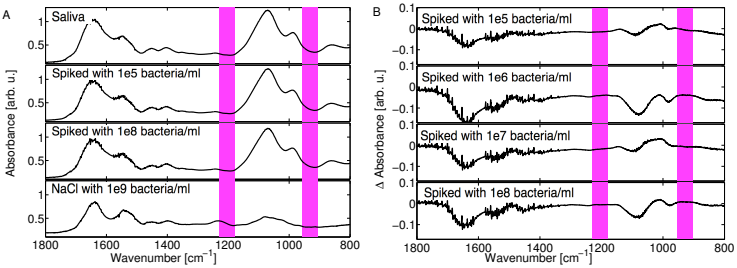


Figure 15.5: **Spectrum of porphyromonas gingivalis and spectra of saliva spiked with the bacteria.** A: Spectra of saliva spiked with various concentration of the bacteria porphyromonas gingivalis (0, 10^5, 10^8 bacteria/ml) is shown together with the spectrum of the bacterium in NaCl solution at a concentration of 10^9 bacteria ml^{-1}. B: Difference in absorbance between spectra of saliva and saliva spiked with bacteria. Highlighted areas mark the difference found in the spectra of the G-AgP patients compared to control. No significant differences between the spectra of saliva and saliva spiked with bacteria are found.

Part V

Conclusion, outlook and supplemental material

Chapter 16

Conclusion and outlook

In this thesis infrared spectroscopy was used to investigate its potential in three rather different forensic and medical areas with the goal to develope a sensor for detecting minute amounts of selected substances in human saliva. Since saliva is more easily accesible than blood or urine it represents the preferred matrix material which enables field tests. The three topics studied in detail are:

- Detection of drugs like cocaine
- Detection of caffeine both caffeine-containing beverages and saliva,
- Spectral differentiation between saliva from patients with generalized aggressive periodontitis and saliva from healthy people.

A Fourier transform infrared (FTIR) spectrometer was used through out the studies in addition to a quantum cascade laser setup which finally allows miniaturization.
For the first time −to our knowledge− cocaine was successfully

measured in saliva with infrared spectroscopy. Since infrared spectroscopy allows for a miniaturized hand-held device that could provide (semi-)quantitative results on-site and therefore allow risk assessment for the police or ambulance staff. The spectrum of cocaine, its metabolites and saliva was thoroughly investigated (cf. chapter 9). In addition, many products that might interfere with cocaine detection were analyzed. These investigations yielded the spectral window ideal for cocaine detection with minimal interferences form other substances ($\sim 1750\,\mathrm{cm}^{-1}$). A one-step extraction method was developed and evaluated to lower the limit of detection (LOD) significantly to $\sim 1\,\mu\mathrm{g\,ml}^{-1}$. Further preconcentration yields an even lower limit of detection in the concentration range of a few $100\,\mathrm{ng\,ml}^{-1}$, needed to identify a person on a cocaine induced "high" (cf. section 5.6). Furthermore, the extraction method was validated with street cocaine. The measurements with a quantum cascade laser (QCL) emitting at $\sim 1750\,\mathrm{cm}^{-1}$ yield a higher LOD (between 3 and $<10\,\mu\mathrm{g\,ml}^{-1}$ depending on the method) than the investigations with the FTIR spectrometer, but the sample volume was reduced by a factor 4 to 100 µl for the transmission analysis (cf. chapter 11). This can be essential for field tests and an improved stabilization of the set-up could further lower the LOD. Overall, the method is very promising and further stabilization of the set-up should yield an even lower LOD.

The detection of caffeine in beverages could help to investigate the correlation between caffeine and diseases like Alzheimer's or Parkinson's disease. A successful one-step extraction method was established based on the solvent toluene. Saliva samples spiked with caffeine were analyzed together with saliva after the consume of espresso (cf. chapter 13). Both analyses yield a successful detection of caffeine. In addition, the caffeine content in the toluene extract retrieved from caffeine-containing beverages was determined. Future investigations require a stabilized pH value of the collected saliva and beverages in order to achieve a higher signal-to-noise ratio and thus a lower LOD. Overall, the method shows great potential

for miniaturization since the absorbance could be measured with a QCL emitting at one of the major caffeine absorption wavelengths.

Finally, spectra of supernatant of saliva from patients with generalized aggressive periodontitis (G-AgP) were compared to a control group utilizing infrared spectroscopy. For the first time −to our knowledge− the differentiation between the two groups was demonstrated. The future development of a quick chair-side test could yield a better understanding of the disease and an improved risk assessment for the patient (cf. chapter 14). The spectra were differentiated by both a simple difference in absorbance at two wavelengths (1206 and 1196 cm^{-1}) and by a principal component analysis of the spectral range from 1230 to 1180 cm^{-1} (cf. chapter 15). The narrow spectral range is suitable for the application of a QCL. However, these results need to be confirmed with larger groups of patients and volunteers in a broad clinical study.

In summary, the successful outcome of the studies in three areas represents a cruical step towards QCL-based hand-held devices to be applied in the field by the police or in the doctors' offices.

Appendix A

Information for participants

Information sheet for participants
in the saliva study of the Nano-Tera project *IrSens*

1 Introduction

In the Nano-Tera project *IrSens* (Integrated sensing platform for gases and liquids in the near and mid-infrared range), we try to build a portable system for detection of small amounts of certain molecules in gases and liquids, by using techniques like optical and mass spectrometry.
As a test application, we want to detect cocaine traces in human saliva, and for this goal, we need to understand the variations in physical and chemical properties of saliva among different people.

The flow of saliva and therefore the composition is changing with many factors, even with details like the body posture and smoking. Smokers have a higher salivary flow partly due to the irritating effect of tobacco. In addition the inhaled nicotine changes the composition of the saliva. Medicine taken regularly can alter the salivary flow and change its composition. Physical exercise can change the components of saliva especially regarding the hormone, lactate, protein and electrolyte levels[1].
Stimulated salivary flow differs from unstimulated flow in the concentration of the protein, sodium, calcium, chloride and bicarbonate levels. In addition, the pH of the saliva is changing and there is less inorganic phosphate and magnesium in stimulated saliva than in unstimulated. Salivary flow can be stimulated by chewing. Chewing something with citric acid does stimulate the salivary flow even more. This interaction of your body with gums and candies is why you will be asked about your regular consumption of these products[1]
The effect of age on salivary components is still under investigation which is why we ask you to state yours.[1]
Caffeine and alcohol have a very characteristic absorption in the infrared, which is why we ask you to state your consume on the day of the sampling.
Pre-studies have shown that mouthwashes have a significant influence on the infrared spectra, which is the reason for the questions about your oral hygiene.

For all these reasons, we need to work with saliva samples from people having different age, sex, diet, consumption of legal drugs, constitution and health status, and the samples must be taken in different situations (time of the day, season, before or after eating, brushing teeth, chewing any type of food, sleeping, physical exercises, etc).
Of these samples we will measure physical properties like density, viscosity, optical absorption, content of solid particles, and chemical properties like pH-value (acidity), content of drugs (alcohol, nicotine, painkillers, stimulants, etc) and the products the human body will make of them.

Certain samples might be spiked with cocaine or similar substances after the initial measurement, in which case they might be kept for longer than just the time needed for the initial measurement. However, in any case the saliva samples will be disposed of (via the normal sewage system or a dedicated drug collection and disposal facility) before the end of the project in the year 2013.

The results will be linked to the information you have to give in the application form, but they will be kept confidential. Results might be published only in aggregate form, so that you cannot be identified.
These data will help us to know with what variations our system will have to cope, and to reduce the amount of errors it will make during measurement.

The study questionnaire will enable us to differentiate some of the changes in physical and chemical properties of saliva. As a consequence, it is important that you fill in the questionnaire honestly and completely. We would like to thank you for your help.

[1] P. Del VignadeAlmeida et al.: „Saliva Composition and Functions: A comprehensive review", The journal of Contemporary Dental Practice 9(3), 2008

2 Study details

2.1 Research procedure (methods)

Saliva will be taken from you by various physical methods (spitting into a glass tube, wiping with cotton pads, etc.), and measured for chemical and physical properties, to reveal presence of drugs and other components of saliva.

2.2 Schedule

The collection of saliva as described above will take up to some minutes per method. In certain cases, additional saliva samples will be taken at a later time (up to some months later), if you individually agree.

2.3 Conditions to be met for participation

You have to answer the participants questionnaire honestly and completely, and you must be willing to have some milliliters of your saliva taken from you by one or several methods. You must not be addicted to illegal drugs. You must be of legal age, *i.e.*, at least 18 years old and mature.

2.4 Advantages/disadvantages, risks

You will run no risks, as the taking of the saliva is done with harmless swipe, capillary or spitting techniques.

2.5 Source of funding of this project

Swiss National Science Foundation (SNSF) Project number: 20NAN1_123589

2.6 Compensation/Reimbursement

You will receive neither compensation nor reimbursement.

2.7 Right of withdrawal

As a participant, you have the right to withdraw from the study at any time without needing to specify any reasons nor facing negative consequences.

2.8 Data protection

Every participant will receive an identification number, which is only known to the participant and the responsible scientist. The obtained data will be stored safely and reported in an anonymous form. The assignment between participant and participant number will not be stored electronically. Only the responsible investigators or the members of the ethical committee have access to the original data under strict confidentiality.

2.9 Insurance coverage

Possible damages to your health, which are directly related to the study and are demonstrably the fault of ETH Zurich, are covered by the general liability insurance of ETH Zurich (insurance policy no. 100.001 of the Swiss Mobiliar insurance company).

However, beyond the aforementioned, the health insurance and the accident insurance (e.g. for the way to or back from the study location) is in the responsibility of the participant.

2.10 Contact persons

Yargo Bonetti <yargo@phys.ethz.ch>, Telefon +41-4463-32998.

Project responsible: Professor Jérôme Faist <jfaist@phys.ethz.ch>, Telefon +41-4463-37280.

Appendix B

Consent form

Consent Form for participants in the saliva study of the Nano-Tera project IrSens

⇒ ***Please read this form carefully.***
⇒ ***Please ask the investigator or the contact person if you have any questions.***

Study title: ***Saliva study of the Nano-Tera project IrSens***

Study location: ETH Zürich/ EPFL Neuchâtel

Principal Investigator's Name and First Name: *Faist, Jérôme*

Participant's Name and First Name: . . . . . .

Declaration:

⇒ I participate in this study on a voluntary basis and can withdraw from the study at any time without giving reasons and without any negative consequences.
⇒ I have been informed orally and in writing about the aims and the procedures of the study, the advantages and disadvantages as well as potential risks.
⇒ I have read the written information for the volunteers. My questions related to the study participation have been answered satisfactorily. I have been given a copy of the information for the volunteers and the consent form.
⇒ I was given sufficient time to make a decision about participating in the study.
⇒ With my signature I certify that I fulfill the requirements for the study participation mentioned in the information for the volunteers.
⇒ I have been informed that possible damages to my health which are directly related to the study and are demonstrably the fault of ETH Zurich, are covered by the general liability insurance of ETH Zurich (insurance policy no. 100.001 of the Swiss Mobiliar insurance company). However, beyond the before mentioned, my health- and/or accident insurance (e.g. for the way to or back from the study location) will apply.
⇒ I agree that the responsible investigators and/or the members of the ethical committee have access to the original data under strict confidentiality.
⇒ I am aware that during the study I have to comply with the requirements and limitations described in the information for the volunteers. In my own health interest the investigators can, without mutual consent, exclude me from the study.
⇒ I will inform the investigators about my medical treatments and medication (prescribed by medical doctors or self-purchased).
⇒ If in the course of the study, a clinical finding occurs which could lead to diagnosis, treatment or prevention of existing or future diseases
☐ I want to be informed.
☐ I do not want to be informed.
(Tick appropriate.)

Location, date . . . . . Signature volunteer . . . . . .

Location, date . . . . . Signature investigator . . . . . .

Appendix C

Questionaire for participants

Questionnaire for participants
in the saliva study of the Nano-Tera project *IrSens*

Participant number:

How old are you?

Are you: male or female

Did you take any medicine yesterday or today? Including birth control pill, pain killers (e.g. aspirin), medication for common colds? If yes, please specify:

Do you have a special medical condition like diabetes? If yes, please specify:

Do you use nutritional or dietary supplement like Vitamin C? If yes, please specify.

Do you smoke? If yes: How much yesterday and today? When was the last time you smoked?

Do you consume caffeine-containing beverages (coffee, energy drinks, coke)? If yes: What do you consume? How much did you drink yesterday and today? When did you drink it?

Did you drink alcohol-containing beverages yesterday or today? If yes: What was it? How much did you drink? When did you drink it?

Did you eat anything yesterday or today? If yes: What was it? When did you eat it?

Did you drink or eat anything within the last hour that you did not state yet?

When was the last time you used mouthwash or brushed your teeth?

When was the last time you ate a candy, bonbon or a chewing gum?

Are you hungry at the moment? Please state in numbers from 1 to 10, where 1 is not hungry at all and 10 is close to starving.

Did you use lipstick or lip balm yesterday or today? When did you use it for the last time?

Did you do physical exercises within the last two hours? What and when did you do?

Do you currently feel sick? If yes, please specify.

How would you describe your saliva today? Do you have a particular dry/wet mouth today?

Appendix D

Authorization to work with drugs

Kantonale Heilmittelkontrolle Zürich

Regionale Fachstelle der Ost- und Zentralschweiz
Haldenbachstrasse 12 8006 Zürich Tel. 044-255 32 00 Fax 044-255 44 37 E-Mail heilmittelkontrolle-zh@usz.ch

Bewilligung für Betäubungsmittel, Institut
(Neuerteilung)

Aufgrund des Gesuches vom 5. Februar 2010 wird

v e r f ü g t :

1. Der ETH Zürich, Institut für Quantenelektronik/Laserspektr. u. Analytik, Schafmattstrasse 16, 8093 Zürich, wird die Bewilligung erteilt, Betäubungsmittel nach Massgabe des Eigenbedarfs des Institutes zu wissenschaftlichen Zwecken zu beziehen, zu lagern und zu verwenden.
2. Verantwortliche Person ist: Prof. Dr. Markus Sigrist, geb. 6. September 1948, Physiker, von Rafz ZH.
3. Diese Bewilligung gilt bis zum 31. Dezember 2014. Sie kann jederzeit eingeschränkt, mit Auflagen versehen oder aufgehoben werden, sofern die geltenden Anforderungen nicht erfüllt werden.
4. Jeder Wechsel der verantwortlichen Person ist der Kantonalen Heilmittelkontrolle unverzüglich zu melden.
5. Die Kosten von Fr. 250 werden der Bewilligungsinhaberin auferlegt und sind innert 30 Tagen gemäss beiliegender Rechnung zu bezahlen.
6. Gegen diese Verfügung kann innert 30 Tagen, von der Mitteilung an gerechnet, beim Verwaltungsgericht des Kantons Zürich schriftlich Beschwerde eingereicht werden. Die Beschwerdeschrift muss einen Antrag und dessen Begründung enthalten. Der angefochtene Entscheid ist beizulegen oder genau zu bezeichnen. Die angerufenen Beweismittel sind genau zu bezeichnen und soweit möglich beizulegen.
7. Mitteilung an die Bewilligungsinhaberin, an die verantwortliche Person und an Swissmedic, Bern.

Zürich, 11. Februar 2010
er

Kantonale Heilmittelkontrolle Zürich
Regionale Fachstelle der Ost- und Zentralschweiz

i. V.

Dr. W. J. Pletscher, Kantonsapotheker

Appendix E

Interfering substances

This appendix serves as a continuation of the investigation of spectral interferences with the cocaine spectra (cf. section 9.2)[1].

No major interference from the soft drinks coca-cola-light or coca-cola zero is detectable for the first cocaine absorbance peak (cf. figure E.1). The same conclusion holds for the sweetener Assurgin. The sweetening compounds are Sodium cyclamate (3.2 mg/ml E 952) and saccharin (0.8 mg/ml, E 954).

Baby powder, $MgCO_3$ and starch are all white powders that are ideal for diluting cocaine. Their major absorbance is between 1200 and 1000 cm^{-1} and 1500 and 1400 cm^{-1}, respectively.

Vinegar, eggs and milk powder are additionally investigated as representative every day products. Even though egg white and yolk, absorb in the range of interest, the spectrum is significantly different. It should be possible to differentiate the spectra with one wavelength as a control around 1770 cm^{-1}. Similar conclusions can be made for

[1] Part of this appendix has been published in [12, 37]

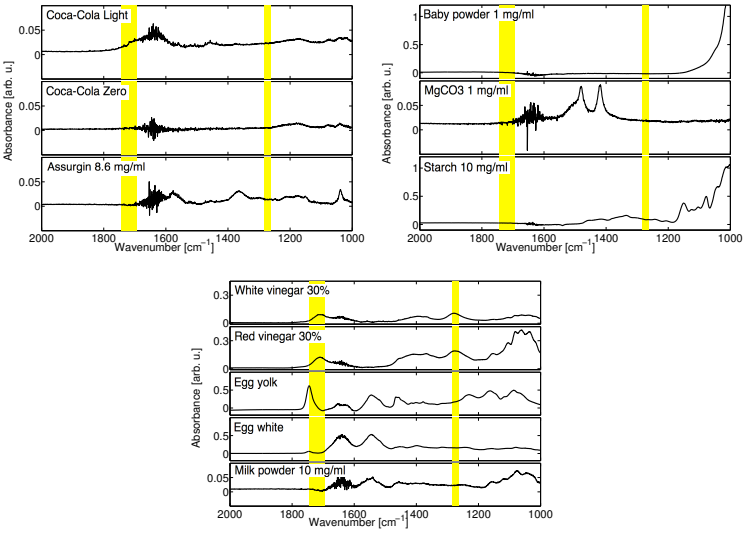


Figure E.1: **Spectra of diluents and everyday products.** The background absorption of water was subtracted. Highlighted areas mark high cocaine absorption.

the absorbance of vinegar (cf. figure E.1). Dissolved milk powder does not interfere with the region of interest.

Bibliography

[1] Bundesamt für Statistik (BFS). 11.6.1.1 strassenverkehrsunfälle mutmasslicher einfluss von alkohol, drogen oder medikamenten (su-b-11.03.05-svu-e34uv).

[2] nano tera.ch. Irsens: Integrated sensing platform for gases and liquids in the near and mid-infrared range.

[3] Alexandre de Mendonça and Rodrigo A. Cunha. Therapeutic opportunities for caffeine in alzheimer's disease and other neurodegenerative disorders. *Journal of Alzheimer's Disease*, 20:S1–S2, 2010.

[4] Anders Hugoson and Ola Norderyd. Has the prevalence of periodontitis changed during the last 30 years? *Journal of Clinical Periodontology*, 35:338–345, 2008.

[5] Amol Ashok Bahekar, Sarabjeet Singh, Sandeep Saha, Janos Molnar, and Rohit Arora. The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: A meta-analysis. *American Heart Journal*, 154(5):830–837, 2007.

[6] R. A. Spragg. *Encyclopedia of Spectroscopy and Spectrometry*, volume 2. Academic Press, London, San Diego, 2000.

[7] P. G. L. Andrus and R.D. Stickland. Cancer grading by fourier transform infrared spectroscopy. *Biospectroscopy*, 4: 37–46, 1998.

[8] H. Fabian, L. P. Choo, G. I. Szendrei, M. Jackson, W. C. Halliday, L. Otvos, and H. H. Mantsch. Infrared spectroscopic characterization of alzheimer plaques. *Applied Spectroscopy*, 47:1513–1518, 1993.

[9] Z. Ge, K.-Z. Liu, X. Xiang, Q. Yang, J. Hui, E. Kohlenber, and M.G. Sowa. Assessment of local hemodynamics in periodontal inflammation using optical spectroscopy. *J Periodontol.*, 82: 1161–1168, 2011.

[10] R. E. Baier and P. O. Glantz. Characterization of oral invivo films formed on different types of solid-surfaces. *Acta Odontologica Scandinavica*, 36:289–301, 1978.

[11] Christian P. Schultz, M. Khalique Ahmed, Colin Dawes, and Henry H. Mantsch. Thiocyanate levels in human saliva: Quantitation by fourier transform infrared spectroscopy. *Analytical Biochemistry*, 240:7–12, 1996.

[12] Kerstin M.-C. Hans, Susanne Müller, and Markus W. Sigrist. Infrared attenuated total reflection (ir-atr) spectroscopy for detecting drugs in human saliva. *Drug Testing and Analysis*, 4:420–429, 2012.

[13] Milan Milosevic. *Internal Reflection and ATR Spectroscopy*. John Wiley and Sons, Hoboken, New Jersey, USA, 2012.

[14] J. Faist. *Quantum cascade lasers*. Oxford University Press, Oxford, 1st edition edition, 2013.

[15] F. K. Kneubühl and M.W. Sigrist. *Laser*, volume 7. Vieweg und Teubner, GWV Fachverlage, Wiesbaden, 2008.

[16] *Phocone AG - Laser Technology.* http://www.phocone.com/en/, visited August 2013.

[17] J. Faist, F. Capasso, D. L. Sivco, C. Sirtori, A. L. Hutchinson, and A. Y. Cho. Quantum cascade laser. *Science*, 264:553, 1994.

[18] M. Gianella and M.W. Sigrist. Improved algorithm for quantitative analyses of infrared spectra of multicomponent gas mixtures with unkown compositions. *Applied Spectroscopy*, 63: 338–343, 2009.

[19] L. van der Sneppen, F. Ariese, C. Gooijer, and W. Ubachs. Liquid-phase and evanescent-wave cavity ring-down spectroscopy in analytical chemistry. *Annual Review of Analytical Chemistry*, 2:13–35, 2009.

[20] V. Ya. Popov and V. V. Lavrent'ev. Role of depth of penetration in atr spectroscopy. *Zhurnal Prikladnoi Spektroskopii*, 32 (2):336–342, Ferbruary 1980.

[21] Harry D. Downing and Dudley Williams. Optical constants of water in the infrared. *Journal of Geophysical Research*, 80 (12):1656–1661, 1975.

[22] Charles E. Mortimer and Ulrich Müller. *Chemie.* Gerorg Thieme Verlag KG, Stuttgart, Germany, 10th edition edition, 2010.

[23] J. Sangster. *Octanol-Water Partition Coefficient: Fundamentals and Physical Chemistry*, volume 2 of *Wiley Series in Solution Chemistry.* John Wiley and Sons, Chichester, West Sussex, England, 1997.

[24] Patricia Del Vigna de Almeida, Ana Maria Trindade Grégio, Maria Ângela Naval Machado, Antônio Adilson Soares de

Lima, and Luciana Reis Azevedo. Saliva composition and functions: A comprehensive review. *The Journal of Contemporary Dental Practice*, 9(3):072–080, 2008.

[25] S. A. Khaustova, M. U. Shkurbikov, E. S. Grebenyuk, V. G. Artyushenko, and A. G. Tonevitsky. Assessment of biochemical characteristics of the saliva using transform mid-infrared spectroscopy. *Bulletin of Experimantal Biology and Medicine*, 148(5):841–844, 2009.

[26] J. O. Tenovuo. *Human Saliva: Clinical Chemistry and Microbiology*, volume 2. CRC Press, Boca Raton, Florida, 1989.

[27] K.R. Atkinson, K. R. Lo, S. R. Payne, J. S. Mitchell, and J. R. Ingram. Rapid saliva processing technique for near real-time analysis of salivary steroids and protein. *Journal for Clinical Laboratory Analysis*, 22:395–402, 2008.

[28] Léon C.P.M. Schenkels, Enno C.I. Veerman, and Arie V. Nieuw Amerongen. Biochemical composition of human saliva in relation to other mucosal fluids. *Critical reviews in Oral Biology and Medicine*, 6(2):161–175, 1995.

[29] Dirk W. Lachenmeier, Szidönia Gumbel-Mako, Eva-Maria Sohnius, Andrea Keck-Wilhelm, Evamaria Kratz, and Gerd Mildau. Salivary acetaldehyde increase due to alcohol-containing mouthwash use: A risk factor for oral cancer. *International Journal of Cancer*, 125:730–735, 2009.

[30] J. van Haute and D. B. Green. Relationship between the concentration of bacteria in saliva and the colonization of teeth in humans. *Infection and Immunity*, 9(4):624–630, 1974.

[31] Vincenzo Teneggi, Lisa Squassante, Laura Iavarone, Stefano Milleri, Alan Bye, and Roberto Gomeni. Correlation and predictive prefomances of saliva and plasma nicotine concentration on tobacco withdrawl-induced craving. *Journal of Clinical Pharmacology*, 54:407–414, 2002.

[32] Kory J. Schuh and Ronald R. Griffiths. Caffeine reinforcement: the role of withdrawal. *Psychopharmacology*, 130:320–326, 1997.

[33] Carolyn Brice and Andrew Smith. Caffeine levels in saliva: associations with psychosocial factors and behavioural effects. *Human Psychopharmacology: Clinical and Experimental*, 16: 507–521, 2001.

[34] Sue P. Humphrey and Russel T. Williamson. A review of saliva: Normal composition and function. *The Journal of Prosthetic Dentistry*, 85(2):162, 2001.

[35] T. P. Rohrig. The determination of morphine in urine and oral fluid following ingestion of poppy seeds. *Journal of Analytical Toxicology*, 27:449–452, 2003.

[36] Kerstin M.-C. Hans, Matthias Müller, Tigran Petrosyan, and Markus W. Sigrist. Infrared detection of cocaine and street cocaine in saliva with a one-step extraction. *Anal. Methods*, 6: 666–673, 2014.

[37] Kerstin M.-C. Hans, Michele Gianella, and Markus W. Sigrist. Sensing cocaine in saliva with attenuated total reflection infrared (atr-ir) spectroscopy combined with a one-step extraction method. *Proc. of SPIE*, 8229:822919–1/7, 2012.

[38] Kerstin M.-C. Hans, Matthias Müller, Michele Gianella, Ph. Wagli, and Markus W. Sigrist. Sensing cocaine in saliva with infrared laser spectroscopy. *Proc. of SPIE*, 8591:85910F–1/7, 2013.

[39] Susanne Müller. Ir untersuchungen an flüssigkeiten mit atr-spektroskopie. Semesterarbeit, Laser Spectroscopy and Sensing Laboratory, ETH Zürich, 2010.

[40] Mathias Müller. Infrared spectroscopy on drugs in liquids. Master's thesis, ETH Zürich, July 2012.

[41] Tigran Petrosyan. Analysis of street cocaine with infrared spectroscopy. Master's thesis, ETH Zürich, July 2013.

[42] Sebastian Lienert. Remote control and characterization of an atr qcl setup. Master's thesis, ETH Zürich, January 2012.

[43] David Nutt, Leslie A King, William Saulsbury, and Colin Blakemore. Development of a rational scale to assess the harm of drugs of potential misuse. *The Lancet*, 369(9566):1047 – 1053, 2007.

[44] Michael A. Bozarth and Roy A. Wise. Toxicity associated with long-term intravenous heroin and cocaine self-administration in the rat. *Journal of the American Medical Association*, 254 (1):81–83, July 1985.

[45] K.L.L. Movig, M.P.M. Mathijssen, P.H.A. Nagel, T. van Egmondd, J.J. de Gier, H.G.M. Leufkens, and A.C.G. Egberts. Psychoactive substance use and the risk of motor vehicle accidents. *Accident Analysis and Prevention*, 36:631–636, 2004.

[46] Hallvard Gjerde, Asbjørg S. Christophersen, Per T. Normann, and Jørg Mørland. Toxicological investigations of drivers killed in road traffic accidents in norway during 2006–2008. *Forensic Science International*, 212(1–3):102–109, 2011.

[47] Maria-Cristina Senna, Marc Augsburger, Beat Aebi, Thomas A. Briellmann, Nicolas Donzé, Jean-Luc Dubugnon, Peter X. Iten, Christian Staub, Walter Sturm, and Karl Sutter. First nationwide study on driving under the influence of drugs in switzerland. *Forensic Science International journal*, 198:11–16, 2010.

[48] Wolfgang Schmidbauer and Jürgen vom Scheidt. *Handbuch der Rauschdrogen*. Fischer, 1998.

[49] Sebastian Scheerer and Irmgard Vogt. *Drogen und Drogen Politik: Ein Handbuch*. Campus, 1989.

[50] Ron Chepesiuk. *The war on Drugs: An International Encyclopedia*. ABC-CLIO, Inc., Santa Barbara, California, 1999.

[51] Richard Rudgley. *Lexikon der psychoaktiven Substanzen: Ein kulthistorischer Trip*. Econ und List Taschenbuch Verlag, 1999.

[52] Gary L. Fisher and Nancy A. Roget. *Encyclopedia of Substance Abuse Prevention, Treatment and Recovery Vol 1*. Sage, 2009.

[53] Edward J. Cone and William W. Weddington, Jr. Prolonged occurrence of cocaine in human saliva and urine after chronic use. *Journal of Analytical Toxicology*, 13:65–68, 1989.

[54] Scott MacDonald, R. Mann, M. Chipman, B. Pakula, P. Erickson, A. Hathway, and P. MacIntyre. Driving behavior under the influence of cannais or cocaine. *Traffic Injury Prevention*, 9(3):190–194, 2008.

[55] Fiona J. Couper and Barry K. Logan. Drugs and human preformance fact sheets. *National Highway Traffic Safety Administration, Washington*, 2004.

[56] Othmar Bäbler. Kontrollierter konsum von kokain. Studienarbeit, 2. Studienarbeit an der Hochschule für Angewandte Psychologie, Zürich, October 2002.

[57] Richard W. Foltin, Marian W. Fischman, Particia A. Pippen, and Thomas H. Kelly. Behavioral effects of cocaine alone and in combination with ethanol or marijuana in humans. *Drug and Alcohol Dependence*, 32:93–106, 1993.

[58] Cindy Achat-Mendes, Glenn R. Valdez, Donna M. Platt, James K. Rowlett, and Roger D. Spealman. Intravenous self-administration of etonitazene alone and combined with cocaine in rhesus monkeys: comparison with heroin and antagonism by naltrexone and naloxonazine. *Psychopharmacology*, 204: 489–498, 2009.

[59] R. W. Foltin and M. W. Fischman. The cardiovascular and subjective effects of intravenous cocaine and morphine combinations in humans. *The Journal of pharmacology and experimental therapeutics*, 261(2):623–632, 1992.

[60] Edward J. Cone and Sandra L. Menchen. Stability of cocaine in saliva. *Clinical Chemistry*, 34(7):1508, 1988.

[61] Rebecca A. Jufer, Sharon L. Walsh, and Edward J. Cone. Cocaine and metabolite concentrations in plasma during repeated oral administration: Development of a human laboratory model of chronic cocaine use. *Journal of Analytical Toxicology*, 22:435–444, 1998.

[62] Rebecca A. Jufer, Abraham Wstadik, Sharon L. Walsh, Berry S. Levine, and Edward J. Cone. Elimination of cocaine and metabolites in plasma, saliva, and urine following repeated oral administration to human volunteers. *Journal of Analytical Toxicology*, 24(Okt):467–477, 2000.

[63] Rebecca Jufer, Sharon L. Walsh, Edward J. Cone, and Angela Sampson-Cone. Effect of repeated cocaine administration on detection times in oral fluid and urine. *Journal of Analytical Toxicology*, 30(7):458–462, 2006.

[64] Amanda J. Jenkins, Jonathan M. Oyler, and Edward J. Cone. Comparison of heroin and cocaine concentrations in saliva with concentrations in blood and plasma. *Journal of Analytical Toxicology*, 19:359–374, 1995.

[65] Alan C. Spanbauer, David E. Moody, Rodger L. Foltz, and Sharon L. Walsh. A gas chromatographic-positive ion chemical ionization mass spectrometric method for determination of cocaine, benzoylecgonine, ecgonine, ecgonine methyl ester, and norcocaine in plasma: Detection of norcocaine in plasma after oral administration of cocaine. *Journal of Analytical Toxicology*, 24:453–455, 2000.

[66] Eric T. Moolchan, Edward J. Cone, Abraham Wstadik, Marilyn A. Huestis, and Kenzie L. Preston. Cocaine and metabolite elimination patterns in chronic cocaine users during cessation: Plasma and saliva analysis. *Journal of Analytical Toxicology*, 24(7):458–466, 2000.

[67] Erin A. Kolbrich, Insook Kim, Allan J. Barnes, Eric T. Moolchan, Lisa Wilson, Gail A. Cooper, Claire Reid, Dene Baldwin, Chris W. Hand, and Marilyn A. Huestis. Cozart rapiscan oral fluid testing system: An evaluation of sensitivity, specificity and efficiency for cocaine detection compared with elisa and gc-ms following controlled cocaine administration. *Journal of Analytical Toxicology*, 27(7):407–411, 2003.

[68] Erin A. Kolbrich, Allan J. Barnes, David A. Gorelick, Susan J. Boyd, Edward J. Cone, and Marilyn A. Huestis. Major and minor metabolites of cocaine in human plasma following controlled subcutaneous cocaine administration. *Journal of Analytical Toxicology*, 30(Okt):501–510, 2006.

[69] Edward J. Cone, Mary Hillsgrove, and William D. Darwin. Simultaneous measurements of cocaine, cocaethylene, their metabolites and "crack" pyrolysis products by gas chromatography-mass spectrometry. *Clinical Chemistry*, 40 (7):1299–1305, 1994.

[70] Buddah D. Paul, Shairose Lalani, Thomas Bosy, Aaron J. Jacobs, and Marilyn A. Huestis. Concentration profiles of cocaine, pyrolytic methyl ecgonidine and thirteen metabolites in human blood and urine: determination by gas chromatography-mass spectrometry. *Biomedical Chromatography*, 19:677–688, 2005.

[71] Anita Holmgren, Per Holmgren, Fredrik C. Kugelberg, A. Wayne Jones, and Johan Ahlner. Predominance of illict drugs and poly-drug use among drug-impaired drivers in sweden. *Traffic Injury Prevention*, 8(4):361–367, 2007.

[72] Riet Dams, Robin E. Choo, Willy E. Lambert, Hendree Jones, and Marilyn A. Huestis. Oral fluid as an alternative matrix to monitor opiate and cocaine use in substance-abuse treatment patients. *Drug and Alcohol Dependence*, 87(2-3):258–267, 2007.

[73] Diaa M. Shakleya, Riet Dams, Robin E. Choo, Hendree Jones, and Marilyn A. Huestis. Simultaneous liquid chromatography–mass spectrometry quantification of urinary opiates, cocaine, and metabolites in opiate-dependent pregnant women in methadone-maintenance treatment. *Journal of Analytical Toxicology*, 34(Jan/Feb):17–25, 2010.

[74] Wen-Ling Wang, William D. Darwin, and Edward J. Cone. Simultaneous assay of cocaine, heroin and metabolites in hair, plasma, saliva and urine by gas chromatography- mass spectrometry. *Journal of Chromatography B*, 660:279–290, 1994.

[75] Wayne C. Duer, Daniel J Spitz, and Shallyn McFarland. Relationships between concentrations of cocaine and its hydrolysates in peripheral blood, heart blood, vitreous humor and urine. *Journal of Forensic Sciences*, 51(2):421–425, 2006.

[76] Eric T. Shimomura, Gwendolyn D. Hodge, and Buddah D. Paul. Examination of postmortem fluids and tissues for the presence of methylecgonidine, ecgonidine, cocaine and benzoylecgonine using solid-phase extraction and gas chromatography-mass spectrometry. *Clinical Chemistry*, 47(6):1040–1047, 2001.

[77] Sci Finder. http://www.cas.org/products/sfacad/index.html. https://scifinder.cas.org.

[78] Pub Chem. Pubchem. http://pubchem.ncbi.nlm.nih.gov/.

[79] Edward J. Cone, Karen Kumor, Loren K. Thompson, and Michael Scherer. Correlation of saliva cocaine levels with

plasma levels and with pharmacologic effects after intravenous cocaine adminstration in human subjects. *Journal of Analytical Toxicology*, 12:200–206, 1988.

[80] Thomas Kraemer and Liane D. Paul. Bioanalytical procedures for determination of drugs of abuse in blood. *Analytical and Bioanalytical Chemistry*, 388(7):1415–1435, 2007.

[81] Frederick P. Smith and David A. Kidwell. Cocaine in hair, saliva, skin swabs, and urine of cocaine users' children. *Forensic Science International*, 83(3):179–189, 1996.

[82] C. C. Okeke, J.E. Wynn, and K.S. Patrick. Simultaneous analysis of cocaine, benzoylecgonine, methylecognine, and ecognine in plasma using an exchange resin and gas chromatography-mass spectrometry. *Chromatographia*, 38(1/2):52–56, 1994.

[83] P. Cámpora, A. M. Bermejo, M. J. Tabernero, and P. Fernández. Quantitation of cocaine and its major metabolites in human saliva using gas chromatography- positive chemical ionization-mass spectrometry (gc-pci-ms). *Journal of Analytical Toxicology*, 27:270–274, 2003.

[84] Emmanuelle Cognard, Stephane Bouchonnet, and Christian Staub. Validation of a gas chromatography-ion trap tandem mass spectroscopy for stimultaneous analyze of cocaine and its metabolites in saliva. *Journal of Pharmaceutical and Biomedical Analysis*, 41(3):925–934, 2006.

[85] John F. Casale. Detection of pseudoecgonine and differentiation from ecgonine in illict cocaine. *Forensic Science International*, 47:277–287, 1990.

[86] Davis C. Perry. Heroin and cocaine adulteration. *Clinical Toxicology*, 8(2):239–243, 1975.

[87] Gregorio Barrio, Paloma Saavedra, Luis de la Fuente, Luis Royuela, and the Spanish Group for the Study of the Purity of

Seized Drugs. Purity of cocaine seized in spain, 1985–1993: variations by weight, province and year of seizure. *Forensic Science International*, 85(1):15–28, 1997.

[88] Miguel López-Artíguez, Ana Cameán, and Manuel Repetto. Unequivocal identification of several common adulterants and diluents in street samples of cocaine by infrared spectroscopy. *Journal of Forensic Sciences*, 40(4):602–613, 1995.

[89] L. A. King. Drug content of powders and other illict preparations in the uk. *Forensic Science International*, 85(2):135–147, 1997.

[90] Nadia Fucci and Nadia De Giovanni. Adulterants encountered in the illict cocaine market. *Forensic Science International*, 95:247–252, 1998.

[91] Naissa Prévide Bernardo, Maria Elisa Pereira Bastos Siqueira, Maria José Nunes de Paiva, and Patrícia Penido Maia. Caffeine and other adulterants in seizures of street cocaine in brazil. *International Journal of Drug Policy*, 14:331–334, 2003.

[92] Tibor M. Brunt, Sander Rigter, Jani Hoek, Neeltje Vogels, Peter van Dijk, and Raymond J. M. Niesink. An analysis of cocaine powder in the netherlands: content and health hazards due to adulterants. *Addiction*, 104:798–805, 2009.

[93] Isabelle Evrard, Stéphane Legleye, and Agnès Cadet-Taïrou. Composition, purity and perceived quality of street cocaine in france. *International Journal of Drug Policy*, 21:399–406, 2010.

[94] Esam M. A. Ali, Howell G. M. Edwards, Michael D. Hargreaves, and Ian J. Scowen. Raman spectroscopic investigation of cocaine hydrochloride on human nail in a forensic context. *Analytical and Bioanalytical Chemistry*, 390(4):1159–1166, 2008.

[95] Insook Kim, Allan J. Barnes, Raf Schepers, Eric T. Moolchan, Lisa Wilson, Gail Cooper, Claire Reid, Chris Hand, and Marilyn A. Huestis. Sensitivity and specifity of the cozart microplate eia cocaine oral fluid at proposed screening and confirmation cutoffs. *Clinical Chemistry*, 49(9):1498–1503, 2003.

[96] Alan Wayne Jones. Driving under the influence of drugs in sweden with zero concentration limits in blood for controlled substances. *Traffic Injury Prevention*, 6(4):317–322, 2005.

[97] Horst Schulze (Project coordinator). Prevalence of alcohol and other psychoactive substances in drivers in general traffic part ii: Country reports. Technical report, DRUID-Driving under the Influence of Drugs, Alcohol and Medicines, Project No. TREN-05-FP6TR-S07.61320- 518404-DRUID, July 2011.

[98] David L. Blank and David A. Kidwell. Decontamination procedures for drugs of abuse in hair: are they sufficient? *Forensic Science International*, 70(1):13–38, 1995.

[99] Manuela Pellegrini, Adriana Casá, Emilia Marchei, Roberta Pacifici, Ruth Mayné, Vanessa Barbero, Oscar Gracia-Algar, and Simona Pichini. Development and validation of gas chromatography-mass spectroscopy assay for opiates and cocaine in human teeth. *Journal of Pharmaceutical and Biomedical Analysis*, 40:662–668, 2006.

[100] Bertrand R. Brunet, Allan J. Barnes, Karl B. Scheidweiler, Patrik Mura, and Marilyn A. Huestis. Development and validation of a solid-phase extraction gas chromatography-mass spectrometry method for the simultaneous quantification of methadone, heroin, cocaine and metabolites in sweat. *Analytical and Bioanalytical Chemistry*, 392:115–127, 2008.

[101] Rafael de la Torre, Jordi Ortuño, M. Luisa González, Magí Farré, Jordi Camí, and Jordi Segura. Determination of cocaine and its metabolites in human urine by gas chromotography/mass spectrometry after simultaneous use of cocaine and

ethanol. *Journal of Pharmaceutical and Biomedical Analysis*, 13(3):305–312, 1995.

[102] Anabel S. Fandiño, Stefan W. Toennes, and Gernold F. Kauert. Studies on in vitro degradation of anhydroecgonine methyl ester (methylecgonidine) in human plasma. *Journal of Analytical Toxicology*, 26:1–4, 2002.

[103] Marilyn A. Huestis, W. David Darwin, Eric Shimomura, Shairose A. Lalani, Daniel V. Trinidad, Amanda J. Jerkins, Edward J. Cone, Aaron J. Jacobs, Michael L. Smith, and Buddah D. Paul. Cocaine and metabolites urinary excretion after controlled smoked administration. *Journal of Analytical Toxicology*, 31:462–468, October 2007.

[104] P. López, A. M. Bermejo, M. J. Tabernero, P. Fernández, and I. álvarez. Determination of cocaine and heroin with their respective metabolites in meconium by gas chromatography-mass spectroscopy. *Journal of Analytical Toxicology*, 27:464–471, February 2007.

[105] Kazuo Matsubara, Masato Kagawa, and Yuko Fukui. In vivo and in vitro studies on cocaine metabolism: Ecgonine methyl ester as a major metabolite of cocaine. *Forensic Science International*, 26:169–180, 1984.

[106] Alan L. Myers, Heather E. Williams, James C. Kraner, and Patrik S. Callery. Identification of anhydroecgonine ethyl ester in the urine of a drug overdose victim. *Journal of Forensic Sciences*, 50(6):1–5, 2005.

[107] P. Mura, C. Chatelain, V. Dumestre, J. M. Gaulier, M. H. Ghysel, C. Lacroix, M.F. Kergueris, M. Lhermitte, M. Moulsma, G. Pépin, F. Vinvent, and P. Kintz. Use of drugs of abuse in less than 30-year-old drivers killed in a road crash in france: A spectacular increase for cannabis, cocaine

and amphetamines. *Forensic Science International*, 160(2-3): 168–172, 2006.

[108] Patrik J. Devine, Nabil A. Anis, Jeremy Wright, Sung Kim, Amira T. Eldefrawi, and Mohyee E. Eldefrawi. A fiber-optic cocaine biosensor. *Analytical Biochemistry*, 227(1):216–224, 1995.

[109] Marta Concheiro, Ana de Castro, óscar Quintela, Angelines Cruz, and Manuel López-Rivadulla. Confirmation by lc-ms of drugs in oral fluid obtained from roadside testing. *Forensic Science International*, 170(2-3):156–162, 2007.

[110] Marta Concheiro, Ana de Castro, óscar Quintela, Angelines Cruz, and Manuel López-Rivadulla. Determination of illict and medicinal drugs and their metabolites in oral fluid and preserved oral fluid by liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, 391: 2329–2338, 2008.

[111] Maria Huerta-Fontela, Maria Teresa Galceran, and Francesc Ventura. Ultraperformance liquid chromatography - tandem mass spectrometry analysis of stimulatory drugs of abuse in wastewater and surface water. *Analytical Chemistry*, 79:3821–3829, 2007.

[112] Eshwar Jagerdeo, Madeline A. Montgomery, Marc A. LeBeau, and Matrin Sibum. An automated spe/lc/ms/ms method for the analysis of cocaine and metabolites in whole blood. *Journal of Chromatography B*, 874:15–20, 2008.

[113] B. Kasprzyk-Hordern, R. M. Dinsdale, and A.J. Guwy. Multi-residue method for the determination of basic/neutral pharmaceuticals and illicit drugs in surface water by solid-phase extraction and ultra performance liquid chromatography–positive electrospray ionisation tandem mass spectrometry. *Journal of Chromatography A*, 1161:132–145, 2007.

[114] Cristina Postigo, Maria J. Lopez de Alda, and Damià Barceló. Fully automated determination in the low nanogram per liter level of different classes of drugs of abuse in sewage water by on-line solid-phase extraction-liquid chromatography-electrospray-tandem mass spectrometry. *Analytical Chemistry*, 80:3126–3134, 2008.

[115] Paul P. Robant, Louis J. Reda, and Kevin L. Klette. Complete automation of solid-pase extraction with subsequent liquid chromatography-tandem mass spectrometry for the quantification of benzoylecgonine, m-hydroxybenzoylecgonine, p-hydroxybenzoylecgonine and norbenzoylecgonine in urine - application to a high-throughput urine analysis labortory. *Journal of Analytical Toxicology*, 32:577–585, 2008.

[116] P. Fernández, L. Morales, C. Vázquez, A. M. Bermejo, and M. J. Tabernero. Hplc-dad determination of opioids, cocaine and their metabolites in plasma. *Forensic Science International*, 161(1):31–35, 2006.

[117] J. Michael Walsh, Ron Flegel, Dennis J. Crouch, Leo Cangianelli, and Jakub Baudys. An evaluation of rapid point-of-collection oral fluid drug-testing devices. *Journal of Analytical Toxicology*, 27(7):429–439, 2003.

[118] Sarah M.R. Wille, Nele Samyn, Maria del Mar Ramírez-Fernández, and Gert De Boeck. Evaluation of on-site oral fluid screening using drugwipe-5+®, rapidstat® and drug test 5000® for the detection of drugs of abuse in drivers. *Forensic Science International*, 198:2–6, 2010.

[119] Raphael C. Wong and Harley Y. Tse, editors. *Drugs of Abuse: Body fluid Testing*, volume Chapter 10; author Franz Aberl and Robert VanDine of *Forensic Science and Medicine*. Humana Press, 2005.

[120] N. Samyn and C. van Haeren. On-site testing of saliva and sweat with drugwipe and determination of concentrations of drugs of abuse in saliva, plasma and urine of suspected users. *International Journal of Legal Medicine*, 113:150–154, 2000.

[121] Franz Aberl, Johannes Boneberger, Ralf-Peter Berg, and Rudolf Zimmermann. Traces of illegal drugs on body surfaces - indicator for consuption or dealing? *Proceedings of SPIE-The International Society of Optical Engineering*, 2932(Human Detection and positive Identification: Methods and Technologies):16–26, 1997.

[122] Dennis J. Crouch, J. Michael Walsh, Ron Flegel, Leo Cangianelli, Jakub Baudys, and Randy Atkins. An evaluation of selected oaral fluid point of collection drug-testing devices. *Journal of Analytical Toxicology*, 29(4):244–248, 2005.

[123] Anna Pehrsson, Teemu Gunnar, Charlotta Engblom, Heikki Seppä, Ahlam Jama, and Pirjo Lillsunde. Roadside oral fluid testing: Comparison of the results of drugwipe 5 and drugwipe benzodiazepines on-site tests with laboratory confirmation results of oral fluid and whole blood. *Forensic Science International*, 175:140–148, 2008.

[124] Nele Samyn, Gert De Boeck, and Alain G. Verstraete. The use of oral fluid and sweat wipes for the detection of drugs of abuse in drivers. *Journal of Forensic Sciences*, 47(6):1380/1–8, 2002.

[125] Stefan W. Toennes, Gerold F. Kauert, Stefan Steinmeyer, and Manfred R. Moeller. Driving under the influence of drugs - evaluation of analytical data of drugs in oral fluid, serum and urine, and correlation with impairment symptons. *Forensic Science International*, 152:149–155, 2005.

[126] T. Speedy, D. Baldwin, G. Jowett, M. Gallina, and A. Jehanli. Development and validation of the cozart dds oral fluid

collection device. *Forensic Science International*, 170:117–120, 2007.

[127] Cozart. Technical specification: Cozart rapiscan drug test. *http://www.cozartgroup.com/download/C7116-COZART-RAPIDSCAN-2DPOCTS.pdf*, 2009.

[128] Gail A. Cooper, Lisa Wilson, Claire Reid, Lianne Main, and Chris Hand. Evaluation of the cozart rapiscan drug test system for opiates and cocaine in oral fluid. *Forensic Science International*, 150:239–243, 2005.

[129] Nadia De Giovanni, Nadia Fucci, Mercekko Chiarotti, and Salvatore Scarlata. Cozart rapiscan system: our experience with saliva test. *Journal of Chromatography B*, 773:1–6, 2002.

[130] N. Fucci, N. De Giovanni, F. De Giorgio, R. Liddi, and M. Chiarotti. An evaluation of the cozart rapiscan system as an on-site screening tool for drugs of abuse in a non-conventional biological matrix: virteous humor. *Forensic Science International*, 156:102–105, 2006.

[131] Urs Beerli. personal communication with urs beerli produktmanager gasmesstechnik/verkauf dräger, July 2009.

[132] Cozart. Cozart dds system. *http://www.cozartgroup.com/dds-info.php*, 2009.

[133] Robert A. Ogert, Anne W. Kusterbeck, Gregory A. Wemhoff, Rachel Burke, and Frances S. Ligler. Detection of cocaine using the flow immunosensor. *Analytical letters*, 25(11):1999–2019, 1992.

[134] Werner A. Baumgartner, Charles T. Black, Peter F. Jones, and William H. Blahd. Radioimmunoassay of cocaine in hair: Concise communication. *Journal of Nuclear Medicine*, 23:790–792, 1982.

[135] John K. Baker and Ronald F. Borne. Carbon-13 magnetic resonance spectra of cocaine metabolites and derivatives. *Journal of Heterocyclic Chemistry*, 15(1):165–166, 1978.

[136] Richard Leute. Experiences with esr as a tool for surveying narcotic addiction. *Annals of the New York Academy of Sciences*, 222(Elerctron Spin resonnce and Nuclear Magnetic Resonance in Biology and Medicine and Magnetic Resonance in Biological Systems):1087–1096, December 2006.

[137] Dennis R. Clark and Tarek M. Hajar. Detection and confirmation of cocaine use by chromatographic analysis for methylecgonine in urine. *Clinical Chemistry*, 33(1):118–119, 1987.

[138] Nidhi Nath, Mohyee Eldefrawi, Jeremy Wright, David Darwin, and Marilyn Huestis. A rapid reusable fiber optic biosensor for detecting cocaine metabolites in urine. *Journal of Analytical Toxicology*, 23(Oct):460–467, 1999.

[139] Milan N. Stojanovic, Paloma de Prada, and Donald W. Landry. Aptamer-based folding fluorescent sensor for cocaine. *Journal of the American Chemical Society*, 123(21):4928–4931, 2001.

[140] Jing-Lin He, Zai-Sheng Wu, Hui Zhou, Hong-Qi Wang, Jian-Hui Jiang, Guo-Li Shen, and Ru-Qin Yu. Fluorescence aptameric sensor for strand displacement amplification detection of cocaine. *Analytical Chemistry*, 82(4):1358–1364, 2010.

[141] Jiwei Chen, Jianhui Jiang, Xing Gao, Guokun Liu, Guoli Shen, and Ruqin Yu. A new aptameric biosensor for cocaine based on surface-enhanced raman scattering spectroscopy. *Chemistry a European Journal*, 14(27):8374, 2008.

[142] Jinwen Zhou, Amanda V. Ellis, Hilton Kobus, and Nicolas H. Voelcker. Aptamer sensor for cocaine using minor groove binder based energy transfer. *Analytica Chimica Acta*, 719: 76–81, 2012.

[143] Chun yang Zhang and Lawrence W. Johnson. Single quantum-dot-based aptameric nanosensor for cocaine. *Analytical Chemistry*, 81:3051–3055, 2009.

[144] Esam M. A. Ali, Howell G. M. Edwards, Michael D. Hargreaves, and Ian J. Scowen. In-situ detection of drugs-of-abuse on clothing using confocal raman microscopy. *Analytica Chimica Acta*, 615(1):63–72, 2008.

[145] Joanna S. Day, Howell G. M. Edwards, Steven A. Dobrowski, and Alison M. Voice. The detection of drugs of abuse in fingerprints using raman spectroscopy ii: cyanoacrylate-fumed fingerprints. *Spectrochimica Acta Part A*, 60:1725–1730, 2004.

[146] Michael D. Hargreaves, Kristian Page, Tasnim Munshi, Richard Tomsett, Gary Lynch, and Howell G. M. Edwards. Analysis of seized drugs using portable raman spectroscopy in an airport environment - a proof of principle study. *Journal of Raman Spectroscopy*, 39:873–880, 2008.

[147] C. Eliasson, N. A. Macleod, and P. Matousek. Non-invasive detection of cocaine dissolved in beverages using displaced raman spectroscopy. *Analytica Chimica Acta*, 607(1):50–53, 2008.

[148] Chetan Shende, Frank Inscore, Paul Maksymiuk, and Stuart Farquharson. Ten-minute analysis of drugs and metabolites in saliva by surface-enhanced raman spectroscopy. *Proceedings of SPIE-The International Society of Optical Engineering*, 6007 (Smart Medical and Biomedical Sensor Technology III):165–171, 2005.

[149] M. Caterina Netti, M. E. Zoorob, Martin D. B. Charlton, Peter Ayliffe, Sven Mahnkopf, Paul Stopford, K. Todd, John R. Lincoln, Nicolas M. B. Perney, and Jeremy J. Baumberg. Probing molecules by surface-enhanced raman spectroscopy. *Proceedings of SPIE-The International Society of Optical Engineering*, 6093 (Biomedical Vibrational Spectroscopy III: Advanced in Research and Industry):60930F/1–60930F/12, 2006.

[150] Rachael E. Littleford, Pavel Matousek, Michael Towrie, Anthony W. Parker, Geoffrey Dent, Richard J. Lacey, and W. Ewen Smith. Raman spectroscopy of street samples of cocaine obtained using kerr gated fluorescence rejection. *The Analyst*, 129:505–506, 2004.

[151] A. Giles Davies, Andrew D. Burnett, Wenhui Fan, Edmund H. Linfield, and John E. Cunningham. Terahertz spectroscopy of explosives and drugs. *Materials Today*, 11(3):18–26, 2008.

[152] Andrew Burnett, Wenhui Fan, Prashath Upadhya, John Cunningham, Howell Edwards, Tasnim Munshi, Michael Hargreaves, Edmund Linfield, and Giles Davies. Complementary spectroscopic studies of materials of security interest. *Proceedings of SPIE-The International Society of Optical Engineering*, 6402 (Optics and Photonics for Counter-Terrorism and Crime Fighting II):64020B/1–64020B/8, 2006.

[153] Andrew Burnett, Wenhui Fan, Prashath Upadhya, John Cunningham, Edmund Linfield, Giles Davies, Howell Edwards, Tasnim Munshi, and Andrew O'Neil. Analysis of drugs of abuse and explosives using terahertz time-domain and raman spectroscopy. *Proceedings of SPIE-The International Society of Optical Engineering*, 6120 (Terahertz and Gigahertz Electronics andPhotonics V):61200M/1–61200M/7, 2005.

[154] William Sweeney, Leo R. Goldbaum, and Nicholas T. Lappas. Detection of benzoylecgonine in urine by means of uv spectrophotometry. *Journal of Analytical Toxicology*, 7(5):235–6, 1983.

[155] Cynthia V. Koulis, John A. Reffner, and Arran M. Bibby. Comparison of transmission and internal reflection infrared spectra of cocaine. *Journal of Forensic Sciences*, 46(4):822–829, 2001.

[156] Mauricio Yonamine, Nadia Tawil, Regina Lucia de Moraes Moreau, and Ovandir Alves Silva. Solid-phase micro-extraction-gas chromatography-mass spectrometry and headspace-gas chromatography of tetrahydrocannabinol, amphetamine, methamphetamine, cocaine and ethanol in saliva samples. *Journal of Chromatography B*, 789:73–78, 2003.

[157] C. Foulon, M. C. Menet, N. Manuel, C. Pham-Huy, H. Galons, J. R. Claude, and F. Guyon. Rapid analysis of benzoylecgonine, cocaine, and cocaethylene in urine, serum, and saliva by isocratic high-performance liquid chromatography with diode-array detection. *Chromatographia*, 50(11-12):721–727, 1999.

[158] Karl B. Scheidweiler and Marilyn A. Huestis. Simultaneous quantification of opiates, cocaine, and metabolites in hair by lc-apci-ms/ms. *Analytical Chemistry*, 76(15):4358–4363, 2004.

[159] P. Fernández, L. Morales, C. Vázquez, M. Lago, and A. M. Bermejo. Comparison of two extraction procedures for determination of drugs in human saliva by high-performance liquid chromatography. *Journal of Applied Toxicology*, 28:998–1003, 2008.

[160] Justin Gombos. Fooling the bladder cops. *http://www.marijuanalibrary.org/bladder.html*, version 3.8, 1995.

[161] Justin Gombos. The drug testing faq - fooling the bladder cops. *http://cocaine.org/drugtestfaq/index.html*, version 4.11, 1996.

[162] Jeff Nightbyrd. Conquering the urine tests. *pamphlet*, 11, 1996.

[163] Ph. Wägli, A. Homsy, and N. F. de Rooij. Norland optical adhesive (noa81) microchannels with adjustable wetting behavior and high chemical resistance against a range of mid-

infrared-transparent organic solvents. *Sensors and Actuators B: Chemical*, 156(2):994–1001, 8 2011.

[164] Marc Ravreby. Quantitative determination of cocaine and heroin by fourier transform infrared spectrophotometry. *Journal of Forensic Sciences*, 32(1):20, 1987.

[165] A. Koçak, J. P. Lucania, and S. L. Berets. Some advances in fourier transform infrared transflection analysis and potential applications in forensic chemistry. *Applied Spectroscopy*, 63(5): 507, 2009.

[166] Cynthia V. Koulis, Kerry J. Hymes, and Jessica L. Rawlins. A new infrared spectral library of controlled and noncontrolled drug standards using internal reflection spectroscopy. *Journal of Forensic Sciences*, 45(4):876–881, 2000.

[167] Roger J. Keller, editor. *The Sigma Library of FT-IR Spectra Edition I.* Sigma Chemical Company, Inc., 1986.

[168] M. G. Pelders and J. J. Ros. Poppy seeds: Differences in morphine and codeine content and variation in inter- and intra-individual excretion. *Journal of Forensic Sciences*, 41(2):209–212, 1996.

[169] Harald Schütz. *Alkohol im Blut, Nachweis und Bestimmung, Umwandlung, Berechnung.* Verlag Chemie, 1983.

[170] Lutz A. Kiesow, Christopher T. Simons, and William B. Long. Quantitative determination and comparison of ethanol in saliva samples of unknown volume with blood ethanol levels in human test subjects following ethanol ingestion. *Annals of the New York Academy of Sciences*, 20(694):293, September 1993.

[171] M. Alvarez, F. J. Bermejo, P. Chieux, E. Enciso, J. Alonso, and N. Garcia. The structure of nonplanar molecular liquids:

tetrachloroethylene. *Journal of Physics: Condensed Matter*, 1 (44):8595–8607, 1989.

[172] Jahanbakhsh Ghasemi and Saadi Saaidpour. Quantitative structure-property relationship study of n-octanol-water partition coefficients of some diverse drugs using multiple linear regression. *Analytica Chimica Acta*, 604:99–106, 2007.

[173] Willfried Schramm, Richard H. Smith, Paul A. Craig, and David A. Kidwell. Drugs of abuse in saliva: A review. *Journal of Analytical Toxicology*, 16:1–9, Jan/Feb 1992.

[174] Darmstadt Reagenzien Merck. *Uvsol- Lösungsmittel und Substanzen für die Spektrosokpie*. Reagenzien Merck, 1960(approx.).

[175] Hubert W. Lilliefors. On the kolmogorov-smirnov test for normality with mean and variance unknown. *Journal of the American Statistical Association*, 62(318):399–402, 1967.

[176] www.drugbank.ca. Open data drug and drug target database. *Drug Bank*, visited in July 2013.

[177] Francisco Torrens. Universal organic solvent-water partition coefficient model. *Journal of Chemical Information and Computer Sciences*, 40:236–240, 2000.

[178] Judith E. Thompson. *A Practical Guide to Contemporary Pharmacy Practice*. Williams and Wilkins, Baltimore, Philadelphia, London, Paris etc., online version visited July 2013.

[179] A. B. A. Boxall, P. Johnson, E. J. Smith, C. J. Sinclair, E. Stutt, and L. S. Levy. Uptake of veterinary medicines from soils into plants. *Journal of Agricultural and Food Chemistry*, 54:2288 – 2297, 2006.

[180] *levamisole*. www.biomedicalpublications.com/levamisole.pdf, last visited July 2013.

[181] L. Rothman, I. Gordon, A. Barbe, D. Benner, P. Bernath, M. Birk, V. Boudon, L. Brown, A. Campargue, J.-P. Champion, K. Chance, L. Coudert, V. Dana, V. Devi, S. Fally, J.-M. Flaud, R. Gamache, A. Goldman, D. Jacquemart, I. Kleiner, N. Lacome, W. Lafferty, J.-Y. Mandin, S. Massie, S. Mikhailenko, C. Miller, N. Moazzen-Ahmadi, O. Naumenko, A. Nikitin, J. Orphal, V. Perevalov, A. Perrin, A. Predoi-Cross, C. Rinsland, M. Rotger, M. Simeckova, M. Smith, K. Sung, S. Tashkun, J. Tennyson, R. Toth, A. Vandaele, and J.V. Auwera. The hitran 2008 molecular spectroscopic database. *J. Quant. Spectosc. Radiat. Transfer*, 110:533–572, 2009.

[182] Philip Wägli, Alexandra Homsy, and Nico F. de Rooij. Norland optical adhesive (noa81) microchannels with adjustable surface properties and high chemical resistance against ir-transparent organic solvents. *Procedia Engineering*, 5:460–463, 2010.

[183] Joaquim A. Ribeiro and Ana M. Sebastião. Caffeine and adenosine. *Journal of Alzheimer's Disease*, 20:S3, 2010.

[184] Diogo R. Lara. Caffeine, mental health, and psychiatric disorders. *Journal of Alzheimer's Disease*, 20:S239– S248, 2010.

[185] M. Casas, J. A. Ramos-Quiroga, G. Prat, and A. Qureshi. Effects of coffee and caffeine on mood and mood disorders. *Coffee, tea, chocolate, and the brain*, pages 73–83, 2004.

[186] RM Gilbert, JA Marshman, M Schwieder, and R Berg. Caffeine content of beverages as consumed. *Canadian Medical Association Journal*, 114(3):205–208, 1976.

[187] G. J. Hulbert, R. N. Biswal, C. B. Mehr, T. H. Walker, and J. L. Collins. Solid/liquid extraction of caffeine from guaraná

with methylene chloride. *Food Science and Technology International*, 4(1):53–58, 1998.

[188] Günter Lehmann, Ingrid Haug, and Regina Schlösser. Über die bestimmung von coffein in kaffee und coffeinhaltigen lebensmitteln mit hilfe der extrelut-fertigsäule. *Zeitschrift für Lebensmittel-Untersuchung und Forschung*, 172(2):87–89, 1981.

[189] Y. Hilal and U. Engelhardt. Characterisation of white tea – comparison to green and black tea. *Journal für Verbraucherschutz und Lebensmittelsicherheit*, 2(4):414–421, 2007.

[190] Atherton Seidell. The solubility of acetanilide, phenacetine, caffeine and salol in several solvents. *Journal of the American Chemical Society*, 29(7):1088–1091, 1907.

[191] OECD-SIDS. *SIDS INITIAL ASSESSMENT PROFILE: Caffeine (CAS 58-08-2)*. http://www.chemicals.moew.government.bg/chemical/site/File/registers/profile/58082p.pdf, August 12th 2013.

[192] Uiram Kopcak and Rahoma Sadeg Mohamed. Caffeine solubility in supercritical carbon dioxide/co-solvent mixtures. *The Journal of Supercritical Fluids*, 34(2):209–214, 2005.

[193] Abebe Belay, Kassahun Ture, Mesfin Redi, and Araya Asfaw. Measurement of caffeine in coffee beans with uv/vis spectrometer. *Food Chemistry*, 108(1):310–315, 2008.

[194] A. N. Paruta and S. A. Irani. Ethanol and methanol. solubility profiles for the xanthines in aqueous alcoholic mixtures. *Journal of Pharmaceutical Sciences*, 55(10):1055–1059, 1966.

[195] Zouhair Bouhsain, Jose M Garrigues, Salvador Garrigues, and Miguel de la Guardia. Flow injection fourier transform infrared determination of caffeine in coffee. *Vibrational Spectroscopy*, 21(1–2):143–150, 1999.

[196] Yuegang Zuo, Hao Chen, and Yiwei Deng. Simultaneous determination of catechins, caffeine and gallic acids in green, oolong, black and pu-erh teas using hplc with a photodiode array detector. *Talanta*, 57(2):307–316, 2002.

[197] H.G.M. Edwards, T. Munshi, and M. Anstis. Raman spectroscopic characterisations and analytical discrimination between caffeine and demethylated analogues of pharmaceutical relevance. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 61(7):1453–1459, 2005.

[198] Nahid Mashkouri Najafi, Ahmadi Seyed Hamid, and Rajabi Khorrami Afshin. Determination of caffeine in black tea leaves by fourier transform infrared spectrometry using multiple linear regression. *Microchemical Journal*, 75(3):151–158, 2003.

[199] Manish M. Paradkar and Joseph Irudayaraj. Rapid determination of caffeine content in soft drinks using ftir–atr spectroscopy. *Food Chemistry*, 78(2):261–266, 2002.

[200] M.M. Paradkar and J. Irudayaraj. A rapid ftir spectroscopic method for estimation of caffeine in soft drinks and total methylxanthines in tea and coffee. *Journal of Food Science*, 67(7):2507–2511, 2002.

[201] Kerstin M.-C. Hans, Markus W. Sigrist, and Nagihan Bostanci. Salivary fingerprinting of periodontitis by ir-atr spectroscopy. *submitted*, 2014.

[202] Amir Savage, Kenneth A. Eaton, David R. Moles, and Ian Needleman. A systematic review of definitions of periodontitis and methods that have been used to identify this disease. *Journal of Clinical Periodontology*, 36(6):458–467, 2009.

[203] Andréa Vieira Colombo, Carina M. Silva, Anne Haffajee, and Ana Paula Vieira Colombo. Identification of oral bacteria as-

sociated with crevicular epithelial cells from chronic periodontitis lesions. *Journal of Medical Microbiology*, 55(5):609–615, 2006.

[204] X. M. Xiang, K.Z. Liu, A. Man, E. Ghiabi, A. Cholakis, and D. A. Scott. Periodontitis-specific molecular signatures in gingival crevicular fluid. *J Periodont Res*, 45:345–352, 2010.

[205] J. A. Loo, W. Yan, P. Ramachandran, and D.T. Wong. Comparative human salivary and plasma proteomes. *Journal of dental research*, 89(10):1016–1023, 2010.

[206] C. A. Ramseier, J. S. Kinnea, A. E. Herr, T. Braun, J.V. Sugai, and C.A. Schelburne. Identification of pathogen and host-response markers correlated with periodontal disease. *Journal of periodontology*, 80(3):436–446, 2009.

[207] M. Hamedi, G.N. Belibasakis, A.T. Cruchley, M. Rangarajan, M.A. Curtis, and N. Bostanci. Porphyromonas gingivalis culture supernatants differentially regulate interleukin-1 and interleukin-18 in human monocytic cells. *Cytokine*, 45(2):99–104, 2009.

[208] Ryan T. Demmer and Panos N. Papapanou. Epidemiologic patterns of chronic and aggressive periodontitis. *Periodontology 2000*, 53(1):28–44, 2010.

Publications

Refereed journals

K.M.-C. Hans, S. Müller and M.W. Sigrist,
Infrared attenuated total reflection (IR-ATR) spectroscopy for detecting drugs in human saliva,
Drug Testing and Analysis, 4:420-429, 2012.

K.M.-C. Hans, M. Gianella and M.W. Sigrist,
Sensing cocaine in saliva with attenuated total reflection infrared (ATR-IR) spectroscopy combined with a one-step extraction method,
Proc. of SPIE, 8229:822919-1/7, 2012.

K.M.-C. Hans, M. Müller, M. Gianella, Ph. Wägli and M.W. Sigrist,
Sensing cocaine in saliva with infrared laser spectroscopy,
Proc. of SPIE, 8591:85910F-1/7, 2013.

K.M.-C. Hans, M. Müller, T. Petrosyan and M.W. Sigrist,
Infrared detection of cocaine and street cocaine in saliva with a one-step extraction,
Analytical Methods, DOI:10.1039/C3AY41285G.

K.M.-C. Hans, M.W. Sigrist and N. Bostanci,
Salivary Fingerprinting of Periodontitis by IR-ATR Spectroscopy,
submitted August 2013

Proceedings

K.M.-C. Hans, S. Müller and M.W. Sigrist,
Infrared spectroscopic determination of drugs in saliva,
Dig. Cleo/Europe 2011, 22-26 May 2011, Munich, Germany

K.M.-C. Hans, M. Gianella and M. W. Sigrist,
Sensing cocaine in saliva with attenuated total reflection infrared (ATR-IR) spectroscopy combined with a one-step extraction method,
Dig. BIOS/SPIE Photonics West, 21-26 January 2012, San Francisco, USA

K.M.-C. Hans, M. Gianella, M. Müller and M.W. Sigrist,
Sensing cocaine in saliva with a Quantum Cascade Laser (QCL),
Book of abstracts: ALT 12 - Advanced Laser Technologies, p.285-286, 2-6 September 2012, Thun, Switzerland

K.M.-C. Hans, M. Müller, M. Gianella, Ph. Wägli and M.W. Sigrist,
Sensing cocaine in saliva with infrared laser spectroscopy,
Dig. BIOS/SPIE Photonics West, 2-7 February 2013, San Francisco, USA

Conference contributions

K.M.-C. Hans and M.W. Sigrist,
IrSens: Detection of cocaine in saliva with MIR spectroscopy (Poster),
Nano-Tera Annual Plenary Meeting, 29 April 2010 Bern, Switzerland

K.M.-C. Hans, S. Müller and M.W. Sigrist,
IRSENS: Sensing cocaine in saliva: pre-studies using mid-infrared spectroscopy (Talk),
Nano-Tera Workshop: "Lab-on-a-chip & remote data analysis" 14-15 June 2010, Rüschlikon (Zurich), Switzerland

K.M.-C. Hans, S. Müller and M.W. Sigrist,

Infrared spectroscopic determination of drugs in saliva (Poster),
Cleo/Europe 2011 22-26 May 2011, Munich, Germany

K.M.-C. Hans, S. Müller and M.W. Sigrist,
IrSens: Sensing cocaine in saliva employing a one-step extraction and MIR spectroscopy (Poster),
Nano-Tera Annual Plenary Meeting, 12-13 May 2011, Bern, Switzerland

K.M.-C. Hans, M. Gianella and M.W. Sigrist,
Sensing cocaine in saliva with attenuated total reflection infrared (ATR-IR) spectroscopy combined with a one-step extraction method (Poster),
Photonics West 2012, 21-26 January 2012, San Francisco, USA

K.M.-C. Hans, M. Gianella, M. Müller and M. W. Sigrist,
Attenuated Total Reflection Spectroscopy on a One-Step-Extract from Saliva utilized for Cocaine Detection (Poster),
Nano-Tera Annual Plenary Meeting, 26-27 April 2012, Zurich, Switzerland

K.M.-C. Hans, M. Müller, M. Gianella and M.W. Sigrist,
Cocaine Detection with Attenuated Total Reflection (ATR) Spectroscopy (Talk),
Swiss Physical Society Annual Meeting, 21-22 June 2012, Zurich, Switzerland

K.M.-C. Hans, M. Gianella, M. Müller and M.W. Sigrist,
Sensing cocaine in saliva with a Quantum Cascade Laser (QCL) (Talk),
ALT 12 Advanced Laser Technologies, 2-6 September 2012, Thun, Switzerland

K.M.-C. Hans, M.Gianella and M.W. Sigrist,
Infrared spectroscopy for detecting cocaine in saliva (Talk),
in the group of Prof. Eustace L. Dereniak, College of Optical Sciences, University of Arizona, Tucson, USA

K.M.-C. Hans, M. Müller and M.W. Sigrist,
Sensing cocaine with infrared spectroscopy (Poster),
International OSA Network of students- IONS 13, 9-12 January 2013, Zurich and Lausanne, Switzerland

K.M.-C. Hans, M. Müller, M. Gianella, P. Wägli and M.W. Sigrist,
Sensing cocaine in saliva with infrared laser spectroscopy (Talk),
Photonics West 2013, 2-7 February 2013, San Francisco, USA

K.M.-C. Hans and M.W. Sigrist,
Sensing cocaine in saliva (Talk),
Optics chapter ETH Zurich: Pizza and Talks, 17 April 2013, Zurich, Switzerland

K.M.-C. Hans, T. Petrosyan and M.W. Sigrist,
Detection of cocaine in saliva with Quantum Cascade Laser Spectrosopy (Poster),
Nano-Tera Annual Plenary Meeting, 30-31 May, 2013, Bern, Switzerland

K.M.-C. Hans, P. Wägli and M.W. Sigrist,
IrSens: Combined Microfluidics with Quantum Cascade Laser Spectroscopy for sensing Cocaine in Saliva (Movie),
Nano-Tera Annual Plenary Meeting, 30-31 May, 2013, Bern, Switzerland

Media presence and exhibitions

Exhibition for the Swiss public: Scientifica 2012,
Alkohol- und Drogentests bald mit dem Laser?,
K.M.-C. Hans, J. Kottmann, J.M. Rey, M. Gianella, D. Marinov, M. Fill, M.W. Sigrist, P. Wägli, J. Faist,
booth with poster, driving simulator, obstacle courses, quiz and short film sequence,
31 August - 2 September, 2012, Zürich, Switzerland

Exhibition for the Swiss public: Scientifica 2013,
Mehr Sicherheit mit Lasern?,
K.M.-C. Hans, Z. Lapin, M. Issler, V. Jain, J. Heinsoo, M. Mangold, T. Thiele, S. Riedi, F. Lindenfelser, A. Hambitzer, R. Landig and U. Grob,
Video presentation of the project IrSens embedded in a booth from the Optics chapter ETH Zurich,
30 August - 1 September 2013, Zurich, Switzerland

Infrarot-Detektor entlarvt Kokser,
ETH life January 06, 2012

Schnelltest der ETH soll Kokser entlarven,
20 minuten January 09, 2012

ETH-Drogen-Schnelltest könnte Polizeiarbeit erleichtern,
http://www.teletop.ch/programm/sendungen/top-news/detail/art/top-news-vom-9-januar-2012-001624459/
(ab 8:27 min) Tele Top News, Jan 09, 2012

ETH entwickelt Koks-Detektor,
20 minuten February 13, 2012

Auf Drogensuche im Speichel,
NZZ (Neue Züricher Zeitung), February 15, 2012 Nr. 38

Acknowledgement

I would like to express my great gratitude towards Prof. Markus Sigrist for giving me the opportunity to write this thesis in his research group. I especially would like to thank him for his always open door, fruitful discussions and the possibility to take part in scientific exchange at conferences.

Furthermore, I would like to gratefully acknowledge the two co-examiners Prof. Jérôme Faist and P.D. Heinz-Detlef Kronfeldt for helpful discussions and their invested time. In addition, I would like to thank Prof. Rainer Wallny for presiding the PhD exam.

I also gratefully acknowledge the whole research group of Prof. Markus Sigrist for their support. Many thanks to Dr. Julien Rey, Dr. Jonas Kottmann, Dr. Vasili Kasiutsich and Dr. Matthias Fill for helpful discussions and a pleasant working atmosphere. Many thanks to Dr. Michele Gianella for his cooperation and time he spent as a post-doc on this project. In addition, I would like to thank the master- and semester students on this project: Susanne Müller, Sebastian Lienert, Matthias Müller and Trigran Petrosyan. A part of the students' measurements are presented in this thesis.

I would like to thank the IrSens cooperation partners. Especially the partners that worked very closely with me. The group of Prof. Jérôme Faist supported this project with a quantum cascade lasers

(designed and manufactured by Dr. Yargo Bonetti). In addition, Dr. Pierre Jouy, Dr. Yargo Bonetti and Prof. Jérôme Faist coordinated the project and supplied many helpful comments as well as measurement equipment. Additionally, I would like to thank Dr. Philip Wägli and Dr. Alexandra Hoemsy from the group of Prof. Nico de Rooij (SAMLAB, EPFL) for the microfluidic education and cooperation. Dr. Lubos Hvozdara, Joab Di Fancesco, Dr. Yu-Chi Chang and Prof. Peter Herzig (Optics and Photonics Technology Laboratory, EPFL) for their stimulating discussions and collaboration. Lukas Emmenegger from the EMPA Dübendorf for measurement equipment. Furthermore, the financial support of nano-tera, the Swiss National Science Foundation and the ETH Zurich are gratefully acknowledged.

I am very greatful for the supply of cocaine street samples by the Forensic Science Institute Zurich. I enjoyed the fruitful cooperation with Dr. Michael Bovens and Esther Schnorr very much.

PD Dr. Nagihan Bostanci (Institute of Oral Biology, University of Zurich) is gratefully acknowledged for the collobaration on the periodontitis project. I want to thank her for all the shared knowledge and the supplied samples.

I would like to thank Christian Berchtold and Prof. Renato Zenobi (Department of chemistry and appied biosciences, Organic Chemistry Laboratory, ETH Zurich) for supporting MS-measurements and access to their scale. In addition, I would like to thank the group of Prof. Atac Imamoglu (Quantum Photonics Group, Institute of Quantum Electronics, ETH Zurich), Dr. Jan Alexander Hiss and Prof. Gisbert Schneider (Department of Chemistry and Applied Biosciences, Institute of Pharmaceutical Sciences, ETH Zurich) for measurement time at their balance.

Prof. Andrew deMello and Dr. Xavier Casadevall i Solvas form the Department of Chemistry and Applied Biosciences at the In-

stiute for Chemical and Bioengineering (ETH Zurich) are gratefully acknowledged for the borrowed syringe pumps and helpful discussions.

Many thanks to all the experts that took time for discussion about drugs and/or saliva. Special thanks go to Dr. Marc Augsburger (Centre Universitaire Romand de Médecine Légale, CHUV and HUG) and Dr. Peter X. Iten (forensic chemist and toxicologist, Institute for Forensic Medicine, University Zurich) for helpful discussions and advice. Many thanks to the enterprise Phocone for the measurement time on their FTIR spectrometer.

In addition, I'd like to thank the ethic committee of the ETH Zurich for their approval and the cantonal drug administration for the authorization to work with drugs. I'd like to thank all test persons for their donated time and saliva. In addition, I would like to thank Christian Beyer, Dr. Julien Rey and Sabine Riedi for proofreading of this thesis.

All my friends and family, who made this time even more enjoyable are gratefully acknowledged. Special thanks go to Dominik, Sabine, Sarah, Pascal, Thomas, Olaf, Esther, Zack and Jan. In addition, I would like to thank my parents and sisters for their continuous support. Last but not least I'd like to express my great gratitude to Christian. Thank you for your support.