Vapor-phase infrared laser spectroscopy: a complementary method for detecting doping agents in urine

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“A picture may be worth a thousand words, but a spectrum is worth a thousand pictures.”

— J. S. Miller
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Summary

Doping controls are conducted in accredited laboratories worldwide. They present considerable analytical challenges, not only because sensitive and selective instrumentation is required, but also because results must be available quickly. Prohibited substances are typically detected by a mass spectrometer after painstakingly long sample preparations and chromatographic separation. Complementary methods would be welcome, e.g., to deliver results more quickly and to differentiate isomers or isotopes more accurately. To illustrate, ephedrine is prohibited for its stimulatory effects when its concentration in urine is greater than 10 µg/mL, whereas pseudoephedrine, its rather inactive diastereoisomer, is legal.

This thesis focuses on the development of novel infrared laser-based instruments designed to detect organic molecules, such as doping agents, in the vapor phase. The in-house-built instruments consist of an OPG-based photoacoustic spectrometer and a DFG-based multipass transmission spectrometer. The completion of these spectrometers relies on two critical developments: the construction of a widely and continuously tunable laser source in the 3 to 4 µm fingerprint region and the adaptation of sensitive trace-gas sensing devices for measurements in vapors.

The optical parametric generator (OPG) produces several mW of mid-IR radiation. Over 300 cm^{-1} can be scanned continuously by tuning the temperature of a periodically poled lithium niobate crystal (PPLN). Vapor-phase infrared spectra of pure low-melting point (< 160 °C) solid organic compounds are recorded in a heated photoacoustic cell, stabilized at 60 °C. Represen-
tative samples of various doping substance classes are examined, including metoprolol tartrate (beta-blocker), methandienone (anabolic), etacrynic acid (diuretic), nikethamide and mephentermine sulfate (stimulants). They show specific absorption bands between 2800 and 3100 cm$^{-1}$, where molecular C-H stretching vibrations are excited. Further measurements are taken on Ephedra alkaloids. On the one hand, the optical resolution of 8 cm$^{-1}$ is good enough to discriminate ephedrine from methylephedrine. On the other hand, it is inadequate at distinguishing ephedrine from pseudoephedrine.

The OPG-based photoacoustic spectrometer is a simple and cost-effective instrument to record vapor-phase spectra. However, the risk of contamination due to condensation inside the photoacoustic cell is high, because the cell cannot be heated above 60 °C, the maximum permissible temperature of high-responsivity miniature microphones.

The PPLN-based difference frequency generator (DFG) has an improved linewidth of only 150 MHz ($5 \cdot 10^{-3}$ cm$^{-1}$). In gas sensing applications, such devices are usually tuned over 1-2 cm$^{-1}$ as a result of a simple piezo scan. To meet our requirements, the continuous tuning range of the DFG was extended to 329 cm$^{-1}$ by simultaneously tuning the PPLN temperature and the step motor of an external cavity. The tuning mechanism is fully automated. Vapor-phase infrared spectra are recorded in a in-house-built high-temperature multipass cell (HTMC).

The HTMC is a novel type of compact long-path absorption cell that can withstand temperatures up to 723 K. The mirrors are heated separately and may be kept at a higher temperature than the rest of the cell in order to prevent condensation. Therefore, measurements on condensable vapors are feasible, in addition to measurements on heated gases. The HTMC has a compensation mechanism for thermal expansion that prevents fatal optical misalignments. The HTMC is successfully tested by acquiring methane and water vapor absorption lines at different temperatures. The wide tuning capabilities of the DFG are demonstrated by recording the whole C-H stretching absorption band of acetone vapor at 296, 400 and 500 K.
The HTMC combined with the widely and continuously tunable DFG offers a unique analytical tool for probing liquid and solid organic samples in the vapor phase. In order to display the large selective potential of the spectrometer, quantitative measurements, reported for the first time, are taken on ephedrine and pseudoeophedrine. Despite featuring similarities, the vapor-phase infrared spectra of these diastereoisomers are clearly distinguishable with respect to a vibrational band centered at 2970.5 and 2980.1 cm\(^{-1}\), respectively. The detection limits (SNR=3) of pure ephedrine and pure pseudoeophedrine are 12 and 6 µg, respectively. Their effective absorption cross sections are as strong as the main C-H stretching vibrational peak of acetone (\(\tilde{\sigma} = 1.5 \cdot 10^{-19} \text{ cm}^2\)).

Direct examination of urine with the DFG-based multipass transmission spectrometer is hindered by the strong water content of urine and the thermal degradation of urea. For this reason, urine samples are first prepared by means of liquid-liquid extraction, then evaporated in the HTMC. Ephedrine-positive and pseudoeophedrine-positive human urine samples have distinguishable vapor-phase infrared spectra. The detection limit of ephedrine in human urine is 50 and 25 µg/mL, when 10 mL and 20 mL urine are prepared, respectively. In principle, it can be improved to approximately 1 µg/mL with an appropriate sample preparation procedure.

The analytical method developed in this thesis could readily serve as a complementary tool to confirm certain positive findings, using the “B” probe of an athlete. The majority of doping agents, however, must be traceable in urine at ng/mL concentrations, requiring 3 orders of magnitude lower detection limits. Temperature deviations of the PPLN currently limit the instrumental noise to 0.3–0.5 % and the minimum detectable absorption coefficient to \(2.6 \times 10^{-6} \text{ cm}^{-1}\) (SNR=3). The laser spectrometer has room for much improvement. Its potential is discussed with respect to doping agents detection and further applications.
Résumé

Les contrôles anti-dopage sont effectués dans des laboratoires accrédités à travers le monde. Ils posent un défi considérable de par la sensibilité et la sélectivité requises des instruments de mesure, mais aussi du fait que les résultats doivent être prêts rapidement. Les substances interdites sont en général détectées par un spectromètre de masse après de longues et pénibles préparations et une séparation chromatographique. Des méthodes complémentaires seraient la bienvenue, par exemple, afin de différencier des isomères ou des isotopes plus précisément, ou bien encore pour fournir des résultats plus rapidement. Pour citer un exemple, l’éphédrine est interdite pour ses effets stimulants lorsque sa concentration dans l’urine est supérieure à 10 µg/mL, tandis que la pseudoéphédrine, sa diastéréoisomère plutôt inactive, est légale.

Le sujet de cette thèse est le développement de nouveaux spectromètres à laser infrarouge qui permettent de détecter des molécules organiques, telles que les produits dopants, en phase vapeur. Ces spectromètres consistent en un générateur optique paramétrique (OPG) couplé à un système de détection photoacoustique, ainsi qu’un générateur de différence de fréquence (DFG) couplé à une cellule de détection à passages multiples. La réalisation de ces instruments repose sur deux développements critiques : la construction d’un laser continûment accordable dans l’infrarouge moyen entre 3 et 4 µm et l’adaptation pour des mesures en phase vapeur d’appareils utilisés en principe pour la détection de traces de gaz.

L’OPG délivre plusieurs mW de radiation dans l’infrarouge moyen. Une
plage de plus de 300 cm\(^{-1}\) est continûment accordable par le biais du réglage de la température d’un cristal de niobate de lithium à polarisation périodiquement inversée (PPLN). Les spectres infrarouges en phase vapeur de substances organiques à point de fusion relativement bas (< 160 °C) sont enregistrés dans une cellule photoacoustique dont la température est stabilisée à 60 °C. Des échantillons représentatifs de différentes classes de substances dopantes sont examinés. Ils révèlent des bandes d’absorption spécifiques entre 2800 et 3100 cm\(^{-1}\). Des mesures supplémentaires sont effectuées sur des alcaloïdes de plantes du genre *Ephedra*. La résolution optique de 8 cm\(^{-1}\) est suffisante pour distinguer l’éphédrine de la méthylephédrine, mais elle demeure insuffisante pour distinguer l’éphédrine de la pseudoéphédrine. L’OPG couplé au système photoacoustique est un outil analytique simple et peu coûteux qui permet d’enregistrer des spectres infrarouges de vapeurs. Toutefois, le risque de condensation à l’intérieur de la cellule photoacoustique est élevé, parce que la cellule ne peut pas être chauffée au-delà de la température de bon fonctionnement des microphones miniatures ultrasensibles.

Le DFG, également basé sur un PPLN, a une résolution de 150 MHz seulement (5 \cdot 10^{-3} \text{ cm}^{-1}). Dans l’optique de détecter des traces de gaz, de tels lasers sont généralement accordables sur une plage de 1 à 2 cm\(^{-1}\) à l’aide d’un transducteur piézoélectrique. Pour le besoin de notre application, cette plage d’accord est étendue à 329 cm\(^{-1}\) grâce au réglage continu et simultané de la température du PPLN et du moteur pas à pas d’une cavité externe. Ce réglage est complètement automatisé. Les spectres infrarouges en milieu vapeur sont enregistrés dans une cellule à haute température et à passages multiples (HTMC).

La HTMC est un nouveau type de cellule compacte à long chemin d’absorption optique qui peut supporter des températures allant jusqu’à 723 K. Les miroirs sont chauffés séparément et peuvent être réglés à une température plus élevée que le reste de la cellule afin d’empêcher la condensation de vapeurs. Ainsi, en sus des traditionnelles mesures sur des gaz, des mesures sur des vapeurs sont possibles. La HTMC incorpore un système mécanique
de compensation thermique permettant d’éviter des dérèglements optiques. La HTMC a été testée avec succès en enregistrant des lignes d’absorption de méthane et de vapeur d’eau à différentes températures. La large plage d’accord du laser à été mise à l’épreuve en enregistrant toute une bande d’absorption de vapeur d’acétone à 296, 400 et 500 K.

La HTMC combinée au DFG offre un outil unique d’analyse d’échantillons liquides ou solides en milieu vapeur. Afin de démontrer le caractère très sélectif de la méthode, des mesures infrarouges quantitatives sont entreprises pour la première fois sur des échantillons d’éphédrine et de pseudoéphédrine. Malgré des similitudes, les spectres infrarouges de ces diastéréoisomères sont clairement distinguables, particulièrement par rapport à une bande de vibration centrée respectivement à 2970.5 et 2980.1 cm$^{-1}$. La limite de détection (SNR=3) de l’éphédrine pure et de la pseudoéphédrine pure sont respectivement 12 et 6 µg. Leur section efficace d’absorption effective est aussi élevée que la bande de vibration C-H de l’acétone ($\tilde{\sigma} = 1.5 \cdot 10^{-19}$ cm$^2$).

La forte teneur en eau de l’urine et la dégradation de l’urée ne permettent pas l’examen direct d’un échantillon d’urine avec le DFG couplé à la HTMC. Les échantillons sont de ce fait préparés au préalable à l’aide d’une extraction liquide-liquide avant d’être évaporés dans la HTMC. Les échantillons d’urine humaine positive à l’éphédrine et à la pseudoéphédrine ont des spectres bien distincts. La limite de détection de l’éphédrine dans l’urine humaine est de 50 et 25 µg/mL, lorsque respectivement 10 mL et 20 mL d’urine sont préparés. En principe, la limite de détection peut être améliorée à environ 1 µg/mL à l’aide d’une méthode de préparation appropriée.

La méthode analytique développée dans le cadre de cette thèse pourrait déjà servir de méthode complémentaire pour confirmer certains tests positifs. Néanmoins, la majorité des produits dopants doivent être décelables dans l’urine à des concentrations de l’ordre du ng/mL nécessitant une limite de détection 3 ordres de grandeur inférieure. Le spectromètre est perfectible par bien des aspects. Son potentiel pour la lutte anti-dopage est discuté. D’autres applications sont également suggérées.
Chapter 1

Introduction

Illegal doping substances taken by athletes in sports are currently detected in urine using primarily gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to mass spectrometry (LC-MS) [1–6]. The research presented in this thesis is devoted to preliminary studies towards the development of a new complementary detection method based on infrared laser spectroscopy. This analytical method exploits the fact that an infrared spectrum, which probes vibrational energy levels of a molecule, can be used as a structural fingerprint.

Infrared spectra can be recorded in the vapor, liquid or solid phase. Quantitative measurements are difficult in the solid phase due to scattering effects, whereas liquid phase spectra tend to be broad and unselective. In this thesis, vapor-phase spectra are recorded with laser-based trace-gas sensing devices. Traditionally designed for room temperature operation, they have been adapted to withstand organic vapors at elevated temperatures.

Other sensitive optical-based methods that have gained increasing attention lately are fluorescence spectroscopy and surface-enhanced Raman spectroscopy (SERS). They both have achieved the ultimate sensitivity, namely single molecule detection. Fluorescence probes singlet-singlet electronic transitions of a molecule. The effect is strong, but fluorescence spectra are relatively broad band. They do not provide much structural information. Fur-
thermore, fluorescence is often limited by the need to attach a fluorescent label to the molecule under investigation. In contrast, Raman scattering—like infrared absorption—directly probes vibrational energy levels of a molecule. SERS is an effect resulting in strongly increased Raman signals from molecules attached to metal surfaces or to metallic nanoparticles [7–9]. Due to SERS enhancement factors of about fourteen orders of magnitude, effective Raman cross sections reach the level of fluorescence cross sections \((10^{-16} \text{ cm}^2)\).

1.1 Analytical requirements for doping controls

Forensic and clinical analysis involves generally collection of a sample, sample preparation, separation, detection and data analysis. The collection of a urine sample is rather straightforward, unlike the collection of other body fluids such as blood or exhaled breath. The high water content and complexity of most biological fluids, such as urine, do not permit direct examination of a sample. Before introducing a sample in an analytical instrument, it has to be prepared. This step can be interpreted as a pre-concentration technique or a coarse separation method, in which a group of analytes featuring similar chemical or physical properties are isolated from the matrix (water). The analytes undergo further separation, usually in a chromatographic column. A detection unit, e.g. a mass spectrometer, identifies the analytes one by one. The result is given after data analysis and comparison with a database.

The purpose of this thesis is to investigate the potential of a laser-based detection method for doping agents in urine that does not require chromatographic separation. This method, referred to as vapor-phase infrared laser spectroscopy, shall be evaluated according to the following widespread comparative criteria:

- **Sensitivity**—It defines the lowest amount of a given doping substance
that can be identified in a sample. In GC-MS, routinely achieved detection limits of doping agents in urine are in the ng/mL range. Better sensitivities would enable to perform tests on less invasive human media such as saliva, sweat or breath. Of particular interest is hair samples, where doping agents can be detected even several months after intake [10,11].

- **Selectivity**—It defines the discrimination power of a method, which is of prime importance to distinguish isotopes or closely related chemical structures. Selectivity depends on the resolution of an analytical instrument, but also on the fingerprinting ability of the physical, chemical or biological process upon which the instrument is inherently based. Infrared spectroscopy has long been reported as the analytical technique with the highest selectivity. The infrared spectrum is a unique identification characteristic of molecules that does not only depend on the particular functional groups, but that also reflects the arrangement of these functional groups within the molecule. In contrast to the spectra obtained by mass spectrometry, the IR spectrum is predominantly a property of the whole molecule rather than the sum of the properties of its constituents.

- **Speed**—Taking into account the elaborate sample preparation, the retention time in the chromatographic column and a possible laboratory backlog, current doping tests take several weeks before final results are known. Faster screening techniques would be welcome to deliver results before sporting event ceremonies.

- **Portability and cost**—Doping tests are performed worldwide in one of 34 accredited laboratories. Portable instrumentation would permit rapid, on-site monitoring of suspect samples. Equipment and operating expenses are rather secondary issues for the time being.
1.2 Prohibited classes of substances

Doping controls are carried out in accordance with the World Anti-Doping Code [12], a document harmonizing anti-doping policies. The main prohibited classes of substances are:

- **Anabolic androgenic steroids (AAS)**—AAS are similar to testosterone both in terms of their effects on the human body and their chemical structure. The anabolic effects promote the development of muscle and tissue. AAS are thus abused in order to increase strength and power or to decrease recovery time following injury. Typical side effects are masculinization of female bodies and feminization of male bodies.

- **Peptide hormones**—Prominent banned peptide hormones are erythropoietin (EPO) and growth hormone, which are produced naturally by the human body. EPO stimulates the production of red blood cells and may also increase the body’s capacity to buffer lactic acid. By raising the oxygen-carrying capacity of blood, it improves endurance. Misused to gain competitive edge, EPO leads, by thickening the blood, to an increased risk of heart disease, stroke, cerebral and pulmonary embolism. Human growth hormone (hGH) is known to act on many aspects of cellular metabolism and is also necessary for skeletal growth. In body growth, the major role of hGH is to stimulate the liver and other tissues to secrete insulin-like growth factor (IGF-1). IGF-1 stimulates production of cartilage cells, resulting in bone growth and also plays a key role in muscle and organ growth. Commonly reported side effects for hGH abuse are: diabetes in prone individuals; worsening of cardiovascular diseases; muscle, joint and bone pain; hypertension and cardiac deficiency; abnormal growth of organs; accelerated osteoarthritis. In untreated individuals known for pathological over-production of hGH, life expectancy is significantly reduced.

- **Beta-blockers**—Beta-blockers are a large group of drugs known as
well as beta-adrenergic blocking agents. They act to block beta-1 or beta-2 adrenergic receptors in the nervous system, thus suppressing the action of beta-adrenergic substances such as adrenaline. The effect of beta-blockade results in slowing of the heart rate, reduction in blood pressure and reduced anxiety. Abused in sports for their ability to steady nervous twitches and to improve concentration, beta-blockers are prohibited in precision sports such as, but not limited to, gymnastics, automobile, motorcycling, archery and shooting.

- **Stimulants**— Stimulants act on the nervous system. They are abused to vitalize the body both mentally and physically, thus inhibiting the feeling of pain and fatigue, but also the sense of personal capacity. They have led to occasional death of athletes.

- **Masking agents**—Prominent representatives of this class are plasma expanders, diuretics and epitestosterone. Plasma expanders are abused to increase the water content of blood, e.g., in an attempt to mask the detection of EPO. Diuretics increase the amount of water excreted by the kidneys, resulting in water loss and weight reduction. They are misused in order to reach a lower-weight class, e.g., in rowing or boxing, or to dilute urine in an attempt to avoid detection of anabolic agents. Epitestosterone, an inactive epimer of testosterone, occurs naturally in urine in concentrations similar to testosterone. The usual technique for the detection of testosterone abuse is to compare its concentration to that of epitestosterone (T/E ratio) [13]. Administration of exogenous testosterone does not affect levels of epitestosterone in the body, because epitestosterone is produced through a different bio-synthetic pathway. Therefore, testosterone administration increases the T/E ratio. An increased T/E ratio is also an indirect marker of other anabolic steroid administration (androstenedione and dehydroepiandrosterone). To circumvent the T/E test, athletes have been reported to self-administer epitestosterone.
The intake of synthetic oxygen carriers or methods such as blood transfusions and gene doping are also prohibited.

### 1.3 Challenges in anti-doping practices

Doping controls are conducted worldwide in laboratories accredited by the World Anti-Doping Agency (WADA). During a visit to the WADA-accredited doping laboratory in Lausanne (Switzerland) and a personal meeting with its director M. Saugy in 2005, three general types of problems have been identified in anti-doping practices:

- **Origin differentiation**—Prohibited substances such as EPO, hGH or certain AAS are naturally synthesized in the body. It is not practical to set levels that would be considered unnaturally high and indicative of doping, because natural levels vary in response to factors such as nutritional state or exercise. Special analytical methods are required to distinguish endogenous production from exogenous origin. The direct marker approach focuses on the differences between the natural and synthetic forms, which occur as a result of the different pathways followed during production. For example, the natural and synthetic forms of certain AAS, such as testosterone, present small but measurable differences in the $^{13}\text{C}/^{12}\text{C}$ isotope ratio. The method for determining the isotopic composition of these steroids includes gas chromatography, a subsequent combustion to CO$_2$ and, finally, isotope ratio mass spectrometry (GC-IRMS) [13, 14]. The application of this technique is not simple and the instrumentation is expensive. The T/E ratio continues to be tested in routine screening of urine samples, with GC-IRMS used as a complementary method, particularly to confirm suspicious results.

- **Structural differentiation**—GC-MS relies on the different retention times in chromatographic columns to differentiate isomers. Complementary methods are required to confirm positive findings with better
accuracy and to provide courts with more reliable scientific evidence. Of particular interest is the case of ephedrine, a prohibited stimulant when its concentration in urine is $> 10 \mu g/mL$. In contrast, its inactive isomer pseudoephedrine is considered as legal.

• **Sample preparation times**—Urine samples are separated in several batches. Each batch is screened for substances belonging to a specific doping agent class. The batches undergo different sample preparations that can last 1/2 day. Shorter preparation times are required to deliver quicker results.

After more than 40 years of development, IRMS instrumentation is commercially available. In the last decade, isotope ratio infrared spectrometry has become a serious competitor to IRMS. For doping tests, the $\delta^{13}C$ value of trace-CO$_2$ concentrations must be measured with an accuracy of 1‰ in order to differentiate between AAS of endogenous and exogenous origin [14]. Since 1994, such levels of accuracy are achievable in atmospheric CH$_4$ by means of infrared laser spectroscopy [15]. To determine the isotope ratio in CO$_2$, relatively high CO$_2$ concentrations are required [16–18]. CO$_2$ isotope ratio infrared spectrometry thus represents a powerful tool in fields such as breath analysis or volcanology, where CO$_2$ concentrations typically range between 1 and 20 % [19–21]. Doping analysis, however, remains out of reach due to the high level of sensitivity required, testosterone and epitestosterone concentrations in urine being in the ng/mL range.

Another benefit of laser infrared spectroscopy is structural differentiation. The detection of certain stimulants, such as Ephedra alkaloids, is a more accessible analytical challenge, as legal concentration limits in urine are in the $\mu g/mL$ range.

### 1.4 Ephedra alkaloids

Ephedra is a plant that has been known in China for more than 5,000 years and utilized in traditional Chinese medicine and its Japanese counterpart,
Kampo medicine. Not all species produce alkaloids, i.e. active constituents. There are six known alkaloids including methylephedrine, ephedrine and pseudoephedrine, which are produced in certain Ephedra species. The effects of Ephedra can be explained by its constituent alkaloids, specifically ephedrine, an alpha- and beta-adrenergic agonist. In western medicine, it has been used in the treatment of several disorders including asthma, heart failure, rhinitis, and urinary incontinence, and for its central nervous system stimulatory effects in the treatment of narcolepsy and depression.

Ephedrine and pseudoephedrine are stereoisomers (spatial isomers) of 2-methylamino-1-phenylpropanol (C$_{10}$H$_{15}$NO), i.e. they share the same bonds and have identical atoms sharing the same neighbors. The structural formula of 2-methylamino-1-phenylpropanol has two carbon chiral centers. Each may assume an R and S configuration, so there are four stereoisomeric combinations possible. These are shown in Fig. 1.1, together with the assignments that have been made on the basis of chemical interconversions. Ephedrine and pseudoephedrine each have an enantiomer, i.e. a non-superimposable mirror image of themselves. Although these missing stereoisomers are not present in the natural source, they may be synthetically prepared and exhibit, as expected, opposite-sign specific optical rotations. Either of the ephedrine enantiomers has a diastereoisomeric (non enantiomeric stereoisomeric) relationship with either of the pseudoephedrine enantiomers.

Ephedrine and pseudoephedrine reveal significantly different physical properties: ephedrine has a melting point of 36 °C, is moderately soluble in water and has a specific optical rotation [α]$_D^{22}$ of ±3 ° in EtOH, whereas pseudoephedrine has a melting point of 116 °C, is sparingly soluble in water and has a specific optical rotation [α]$_D^{22}$ of ±53 ° in EtOH [22]. While ephedrine has been banned from sports for its stimulatory effects, pseudoephedrine has different pharmacological properties and is considered as legal. The World Anti-Doping Code prohibits ephedrine when its concentration in urine is > 10 µg/mL [12]. A sensitive and selective analytical method is therefore required to differentiate these two isomers in urine.
Figure 1.1: Stereoisomers of 2-methylamino-1-phenylpropanol. (-)-ephedrine and (+)-pseudoephedrine are found in the Chinese shrub Ma Huang (Ephedra vulgaris), while (+)-ephedrine and (-)-pseudoephedrine can be synthesized.

Famous athletes tested positive to ephedrine include soccer legend Diego A. Maradona and olympic medallist Carl Lewis. Like many other athletes, the latter failed a doping test, but was never prevented to compete, in spite of international rules. Baring in mind that doping fighting is a political issue as well, we shall, however, restrict ourselves in the course of this thesis to its scientific aspect.


Chapter 2

Mid-infrared laser source

Infrared laser spectroscopy—aimed at fingerprinting rather large organic molecules such as doping agents—requires wide continuous tuning of the laser wavelength. Promising broadly tunable mid-IR laser sources—that do not require cryogenic cooling—include optical parametric oscillators [23,24], Cr$^{2+}$-doped solid-state lasers in the mid-IR below 3 $\mu$m [25], and external-cavity quantum-cascade lasers in the mid-IR range above 4 $\mu$m [26,27]. An optical parametric generation-based (OPG) laser and a difference frequency generation-based (DFG) laser are further developed in this thesis to reach the 3–4 $\mu$m fingerprint region, where fundamental C-H stretching vibrations can be excited. The OPG laser delivers higher output powers at the cost of a broader linewidth.

In a previous work [28], a mid-IR DFG source, widely and rapidly tunable over 230 cm$^{-1}$, was set up using birefringent phase-matched LiNbO$_3$. The bulk crystal was kept at a fixed angle and at a fixed temperature. The Poynting vector walk-off limited the interaction length between mixed beams inside the crystal. Consequently, the resulting DFG idler output power was too low for sensitive laser spectroscopic applications. In recent years, such limitations have been overcome by quasi phase-matching (QPM), where a grating structure periodically resets the accumulated phase error between propagating and locally generated waves [29]. Such gratings are commercially available...
for certain ferroelectric crystals including LiNbO$_3$. A periodic structure with layers of oppositely oriented spontaneous polarization is formed by applying strong electric fields. QPM in periodically poled materials offers numerous advantages, such as walk-off free non critical phasematching, or access to the large non-phasematchable nonlinear coefficient $d_{33}$, which is associated with light polarized along the crystal polar direction. QPM in periodically poled lithium niobate with a pulsed neodymium-doped yttrium aluminium garnet laser (Nd:YAG)—as pump laser—and a continuous wave (cw) external-cavity diode laser (ECDL)—as signal laser—generates sufficiently high power to operate trace-gas detection schemes such as photoacoustic, multipass transmission, or cavity ring-down [30]. Like other gas sensing devices of the art, the DFG radiation is tuned over 1–2 cm$^{-1}$ as a result of a simple piezo scan. To meet our requirements, the continuous tuning range is improved in this thesis to over 300 cm$^{-1}$ by simultaneously tuning the crystal temperature and the step motor of the external cavity.

2.1 Optical parametric generation (OPG)

The mid-infrared fingerprint region between 3 and 4 $\mu$m is accessed by means of an optical parametric generation (OPG) laser source, pictured in Fig. 2.1. The pump source is provided by a $Q$-switched diode-pumped Nd:YAG laser (InnoLight GmbH, Hannover, Germany). The Nd:YAG laser is built as a non-planar ring oscillator with a Cr$^{4+}$:YAG saturable absorber [31]. The Nd:YAG laser pulses are about 6 ns long and have a peak-power of about 5 kW. The pulse repetition rate can be altered by changing the current applied to the two pump laser diodes of the Nd:YAG laser. The wavelength temperature dependence of the Nd:YAG laser has been measured previously [32]. For frequency conversion, the Nd:YAG pump is focussed into one of the eight grating periods of a 50 mm long periodically poled lithium niobate crystal (PPLN). The polarization of the Nd:YAG pump is adapted by a quarter- and a half-wave plate so that the beam enters the PPLN extraordinarily polarized.
A trigger signal is generated by a small portion of the pump beam, which is collected by a Si photodiode placed before the PPLN. The PPLN crystal is fixed inside a temperature-controlled oven. Quasi phase-matching in the crystal is achieved by temperature tuning and delivers several mW of OPG average power. Unwanted radiation after the PPLN, such as the pump or its second harmonic, is removed by a germanium filter. The linewidth of the OPG idler is $8 \text{ cm}^{-1}$. It has been determined by analyzing absorption peaks of 100 ppmV methane diluted in synthetic air at 1 bar total pressure.

![Experimental setup of the tunable OPG laser.](image)

**Figure 2.1**: Experimental setup of the tunable OPG laser.

### 2.2 Difference frequency generation (DFG)

The rather large temperature acceptance bandwidth of the PPLN at our wavelength range is responsible for the broad OPG radiation. By introducing a signal laser to trigger a DFG optical process, a nearly Fourier-limited idler radiation as narrow as 150 MHz can be generated [33]. The idler wavelength must satisfy the energy conservation law

$$\frac{1}{\lambda_i} = \frac{1}{\lambda_p} - \frac{1}{\lambda_s},$$

where $\lambda_i$, $\lambda_p$ and $\lambda_s$ are the idler, pump and signal wavelengths, respectively. The idler linewidth is given by the convolution of the signal and pump laser linewidths. Linewidth broadening due to competing OPG processes can be hindered by operating the DFG at reduced average powers below 1 or 2 mW [34].
Figure 2.2: Experimental setup of the tunable DFG laser. A pulsed Nd:YAG laser and a continuous wave external-cavity diode laser (cw ECDL) are mixed in a periodically poled lithium niobate crystal (PPLN).

The DFG system pictured in Fig. 2.2 was built. In this optical arrangement, the Q-switched Nd:YAG described in Sect. 2.1 serves as pump, while the signal beam is provided by a continuous-wave (cw) external-cavity diode laser (ECDL) mounted in a Littman/Metcalf configuration (Santec, Komaki, Aichi, Japan). Tuning of the ECDL step motor enables to scan a wide idler range of 329 cm$^{-1}$. Another ECDL (EOSI, Boulder, CO, USA) is employed for narrow piezo scans over 1–2 cm$^{-1}$. The fiber-coupled Santec laser is equipped with an optical isolator to prevent disturbances from possible backreflections. Fiber-coupling and optical isolation are mounted externally when the EOSI laser is in use. By realigning the fiber-coupling unit, an output power, three times higher than reported in [35], has been retrieved. A wider tuning range of the EOSI ECDL is also observed. About 1% of the ECDL signal beam is coupled into a wavemeter (Burleigh Instruments, Fishers, NY, USA) for constant wavelength control. The optical fibers and the coupler are all polarization maintaining. Difference frequency conversion—from the pump and signal beam into the mid-infrared idler beam—takes place in the 50 mm long PPLN crystal. Like in the OPG system, a small portion of the pump beam is collected for the purpose of a trigger signal. After the PPLN, a germanium filter lets only the mid-infrared idler beam
pass through.

In a separate experiment, the ECDL was tuned to 1560.271 nm and mixed with the Nd:YAG laser emitting at 1064.555 nm. The beam profile of the sum frequency generation (SFG), situated at 632.8 nm, was analyzed on a CCD camera. An interference filter for a He-Ne laser line cut unwanted radiation after the PPLN. The beam had several peaks that could interfere with each other. The SFG beam profile and pointing varied as the temperature of the PPLN was tuned. A similar behavior is expected for the DFG beam. With the help of a Hg-Cd-Zn-Te detector mounted on a x- and y-direction translation stage, the pointing of the DFG was also seen to vary as the PPLN temperature was tuned. This is why, in certain experiments, a pinhole is placed after the PPLN to spatially clean the DFG beam.

2.3 Laser features and tuning mechanism

All major laser features are reported in Table 2.1. The mid-IR range accessible by OPG is only limited by the available set of grating periods. In this thesis, we restrict ourselves to the C-H stretching region between 2800 and 3100 cm\(^{-1}\), which requires grating periods of e.g. 29.5, 29.7 and 29.9 µm. Provided the PPLN temperature is tuned between 42 and 173 °C, a single grating period change is actually sufficient, namely from 29.7 to 29.9 µm. The temperature step (typically 0.1 °C) defines the idler tuning step.

In the case of DFG, the accessible idler range is limited by the tuning range of the ECDL. The tuning mechanism for wide DFG scans requires simultaneous tuning of the PPLN temperature and the ECDL wavelength. A Labview driven tuning program is implemented to automate the whole procedure. At first, the ECDL is adjusted to a given wavelength. The wavemeter reads the value of the ECDL wavelength with an accuracy of 1.5 pm. The optimal temperature of the PPLN is determined according to the relation derived in Fig. 2.3. The crystal oven is set to the optimal temperature. Before data acquisition, a control loop ensures that the difference between the set
Table 2.1: OPG laser and DFG laser features.

<table>
<thead>
<tr>
<th></th>
<th>OPG</th>
<th>DFG narrow scan</th>
<th>DFG wide scan</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pump:</strong></td>
<td>Nd:YAG</td>
<td>Nd:YAG</td>
<td>Nd:YAG</td>
</tr>
<tr>
<td>- manufacturer</td>
<td>InnoLight</td>
<td>InnoLight</td>
<td>InnoLight</td>
</tr>
<tr>
<td>- pulse duration</td>
<td>6 ns</td>
<td>6 ns</td>
<td>6 ns</td>
</tr>
<tr>
<td>- repetition rate</td>
<td>4–8 kHz</td>
<td>4–8 kHz</td>
<td>4–8 kHz</td>
</tr>
<tr>
<td>- peak power</td>
<td>5 kW</td>
<td>5 kW</td>
<td>5 kW</td>
</tr>
<tr>
<td><strong>Signal:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- manufacturer</td>
<td>EOSI</td>
<td>Santec</td>
<td></td>
</tr>
<tr>
<td>- range</td>
<td>1510–1580 nm</td>
<td>1520–1600 nm</td>
<td></td>
</tr>
<tr>
<td>- linewidth</td>
<td>&lt; 4 MHz</td>
<td>&lt; 1 MHz</td>
<td></td>
</tr>
<tr>
<td>- power</td>
<td>5 mW</td>
<td>5–9 mW</td>
<td></td>
</tr>
<tr>
<td><strong>Idler:</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- range</td>
<td>&gt; 2800–3100 cm$^{-1}$</td>
<td>2771–3064 cm$^{-1}$</td>
<td>2815–3144 cm$^{-1}$</td>
</tr>
<tr>
<td>- linewidth</td>
<td>240 GHz</td>
<td>150 MHz</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(8 cm$^{-1}$)</td>
<td></td>
<td>(5 · 10$^{-3}$ cm$^{-1}$)</td>
</tr>
<tr>
<td>- power</td>
<td>&gt; 1 mW</td>
<td>&lt; 1 mW</td>
<td></td>
</tr>
<tr>
<td>- continuous</td>
<td>&gt; 300 cm$^{-1}$</td>
<td>1–2 cm$^{-1}$</td>
<td>329 cm$^{-1}$</td>
</tr>
<tr>
<td>tuning range</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- stepwidth</td>
<td>0.15 cm$^{-1}$</td>
<td>≦ 0.04 cm$^{-1}$</td>
<td>0.04–0.4 cm$^{-1}$</td>
</tr>
<tr>
<td><strong>Tuning elements</strong></td>
<td>PPLN temp.</td>
<td>piezo</td>
<td>step motor and PPLN temp.</td>
</tr>
</tbody>
</table>
Figure 2.3: At fixed ECDL wavelengths, the DFG power was maximized by adjusting the PPLN temperature. An optimal polynomial temperature curve was thus determined for the 29.5 and 29.9 µm grating periods. A reciprocal relation holds for the optimal signal wavelength at given PPLN temperatures. Together with Eq. (2.1), it can be used to determine the OPG idler wavelength.

temperature and the actual temperature is within a user-defined tolerance. After data acquisition, the wavelength of the ECDL is increased by a user-defined step and the procedure described above is repeated. As the DFG efficiency may vary across the wide tuning range, an automated scale change is implemented for optimal data acquisition with an oscilloscope (TDS 644A, Tektronix, Beaverton, OR, USA). The whole procedure takes about 5 s per step. Data acquisition is the most time-consuming module of the program, because 100 to 150 pulses per step are averaged on the oscilloscope. The
oscilloscope has a waveform acquisition rate\textsuperscript{i} of about 30 acquisitions per second depending on the chosen time scale. Therefore, if the pulse repetition rate of the laser is 6 kHz and if, let's say, a single averaged laser pulse is displayed on the oscilloscope, only one out of 200 pulses is acquired.

In narrow scans, the voltage accuracy applied to the piezo element defines the smallest possible stepwidth. In wide scans, the ECDL step motor cannot perform steps that are smaller than 0.01 nm. Furthermore, in transmission spectroscopy, the maximum tuning step is limited by the presence of fringes, as explained in Sect. 4.4.

\textsuperscript{i}A waveform is the digital curve displayed on the screen of a digital oscilloscope. The waveform acquisition rate must not be confused with the sample rate, which defines the time resolution of an acquired waveform.
Chapter 3

OPG-based photoacoustic spectrometer

3.1 Introduction

In forensic drug testing, the standard method of choice for recording IR spectra is gas chromatography coupled to Fourier transform infrared spectroscopy (GC/FTIR) [36,37]. The basic design of a GC/FTIR spectrometer consists of a heated fused silica transfer line that directs the GC effluent to flow through a narrow 10–20 cm long tube, the inner walls of which are gold coated. This interface, known as a lightpipe, allows to record vapor-phase IR spectra, but suffers of a lack of sensitivity in comparison with recent developments in FTIR interfaces such as matrix isolation and direct deposition. The latter deliver cryogenic infrared spectra, which are very similar to the widespread condensed-phase infrared spectra from KBr disks. The largest currently available IR vapor-phase library, marketed by Sadtler Research Laboratories [38], covers only 9000 compounds, which is a small number compared to other IR, UV or MS libraries. There is therefore a great need for a low-cost and sensitive method to measure IR spectra of solid organic compounds in the vapor phase. For this purpose, an OPG-based photoacoustic spectrometer is presented in this chapter. Initially designed for trace gas
analysis in the gas phase \([34, 39]\), the photoacoustic cell has been adapted to measure vapors of organic compounds.

### 3.2 Photoacoustic cell

#### 3.2.1 Microphone signal generation

A photoacoustic (PA) cell is an acoustic unit consisting of a resonator, microphones, windows, gas inlets and gas outlets. It may also include buffer volumes and an electronic board. In PA trace gas analysis, an infrared laser beam is usually guided through a PA cell filled with a buffer gas, in which an absorbing species has been diluted. Absorbed infrared laser radiation results in the excitation of upper vibrational energy levels. Collisional deactivation processes lead to localized transient heating, i.e. to localized kinetic energy of the buffer gas molecules. The resulting expansion launches acoustic waves. If the pulse repetition rate of the laser is adjusted to one of the eigenresonances of the PA cell, standing acoustic waves build up. Such acoustic waves may be detected by placing one or several microphones at the waves antinodes. This resonant mode of operation is usually achieved by modulating a cw laser at one of the eigenfrequencies of the PA cell.

In literature \([40]\), pulsed photoacoustics refers to acoustic generation with a single laser pulse. The single short pulse acts as a broadband source that excites all eigenmodes of the PA cell simultaneously. In the present work, standing acoustic waves are generated by adjusting the repetition rate of short laser pulses to an acoustic resonance frequency of the PA cell. A theoretical model of this particular case was not found in literature. From the theory of modulated resonant photoacoustics and pulsed photoacoustics with single pulses \([41, 42]\), one would expect a PA signal \(S\) (microphone signal) proportional to the laser pulse energy \(E_L\) and to the absorption coefficient \(\alpha\) of the absorbing species. Furthermore, \(S\) should be proportional to the quality factor \(Q\) as the PA cell is operated at one of its eigenresonances.
Hence, we may write
\[ S = C \cdot E_L \cdot \alpha \cdot Q, \]  
(3.1)

where \( C \) is the PA setup constant. In general, \( C \) depends on the adiabatic coefficient of the buffer gas, the PA cell geometry, the number of microphones \( N_{\text{mic}} \), the responsivity of the microphones, and the spatial overlap between the laser beam and the standing acoustic wave. The absorption coefficient \( \alpha \) is given by \( \alpha = n \tilde{\sigma} \), where \( n \) is the number density of absorbing species in the cell and \( \tilde{\sigma} \) an effective cross section per molecule. The physical significance of the latter quantity is discussed in Sect. 4.2.

A selected acoustic resonance profile centered at \( \nu \) with a full width at half maximum (FWHM) \( \Delta \nu \) is described by its quality factor \( Q \) given by \( Q = \nu / \Delta \nu \). If the selected resonance is not well enough separated from other resonances or if its Q factor is too low, the contributions of other resonances must be taken into account in the expression of the PA signal. As long as the microphones are small compared to the acoustic resonator, the PA signal scales with \( N_{\text{mic}} \), whereas the signal-to-noise ratio increases by a factor of \( \sqrt{N_{\text{mic}}} \).

3.2.2 Experimental setup

As pictured in Fig. 3.1, the doping substances are heated in a specially designed glass sample holder in order to increase their vapor pressure. The sample holder is coupled to a in-house-built open-ended PA cell. Inside the cell, vapors of a compound under investigation are excited in the mid-infrared fingerprint region between 3 and 4 \( \mu \)m by means of the Nd:YAG laser-pumped OPG source described in Sect. 2.1. The pulse repetition rate of the Nd:YAG laser is matched to the first longitudinal resonance frequency of the PA cell, through which the OPG beam is directed.

The sample holder consists of two glass vessels inside each other. The sample is placed in the inner vessel, while the outer vessel is connected to a hot silica oil bath. The temperature of the bath can be adjusted up to 150 °C. For each measurement, the glass holder containing several mg of the
sample and the PA cell are evacuated and filled with synthetic air. Then, the valve between the sample holder and the PA cell is closed. The PA cell is evacuated, while the sample holder is heated. The valve between the PA cell and the sample holder is reopened again in order to allow the hot sample vapor to flow into the evacuated cell. Finally, the PA cell is filled with synthetic air up to a total pressure of 500 mbar. At lower pressures, the responsivity of the microphones decreases [43].

In order to hinder condensation as much as possible, the PA cell and the transfer tubes are heated with a band heater up to 60 °C. The microphone signal is read by a lock-in amplifier, while the average OPG power is controlled by a Hg-Cd-Zn-Te reference detector, linked to an oscilloscope. A small portion of the Nd:YAG pulses, which is directed towards a Si photodiode by means of a beam splitter inserted before the PPLN, serves as a reference signal for the lock-in amplifier and as a trigger signal for the oscilloscope.

The maximum permissible temperature of 60 °C (333 K) is limited by the high-responsivity miniature microphones (EK-3024, Knowles Acoustics, West Sussex, UK). Microphones that can withstand high temperatures up to 573 K are commercially available: the microphone type 1223 from Norsonic
(Tranby, Lier, Norway) or the type 4938 from Brüel & Kjær (Nærum, Denmark). Such devices, however, reveal reduced responsivity or have a large nominal diameter, making them less suitable for implementation within a PA cell. There are also so-called high-temperature-resistant probe microphones, such as the type 4182 from Brüel & Kjær, consisting of a pipe connected to the microphone diaphragm. While the tip of the probe pipe, which acts as an acoustic channel, withstands temperatures up to 973 K, the microphone has to be placed outside the measurement area, at around 323 K maximum. Despite the lower responsivity of this device, it can provide an interesting alternative for measurements in very hot gases. For vapors, however, it is not suitable, as condensation on the microphone diaphragm would occur. The same limitation applies to a PA cell using a Helmholtz resonator [44], where a pipe separates the sample and the detection chambers.

### 3.2.3 Resonance frequency

Two in-house-built open-ended photoacoustic cells were used alternatively for our measurements. The first one, previously described, has four microphones and reveals a resonance frequency of 5.7 kHz [39]. The resonance profile has a FWHM of 1.37 kHz, resulting in a $Q$-factor of 4.2. It was measured with a chopper-modulated high power CO$_2$ laser at ambient temperature. At the operating temperature of 52 °C, a resonance frequency of $6.15 \pm 0.01$ kHz, pictured in Fig. 3.2, was measured with the pulsed OPG laser after filling the PA cell with 1000 ppm methane buffered in synthetic air. (The total pressure was 500 mbar.) Surprisingly, a $Q$-factor of 8.7 is obtained (FWHM = 0.71 kHz). It remains unclear if the origin of the higher $Q$-factor lies in the fact that the resonator responds differently to pulsed and modulated excitations. The second cell is a modification of the first one with eight microphones instead of four and an NTC temperature sensor built inside. It has a resonance frequency of $5.95 \pm 0.01$ kHz and $6.41 \pm 0.02$ kHz at 21 °C and 53 °C, respectively (Fig. 3.2). The properties of both cells are summarized in Table 3.1.
Figure 3.2: Resonance profiles of the PA cells recorded at different temperatures. The strongest resonance can be assigned to the first longitudinal mode. The second resonance of the PA cell with eight microphones could not be identified. Given the geometry of the open-ended cylindrical resonator, any combination of longitudinal, azimuthal or radial modes would give much higher resonance frequencies [45]. A theoretical approach including the buffer volumes would be required for a proper assignment.

At a temperature $T$ given in Kelvin, the resonance frequency shifts to $\nu_T$ as a result of the temperature dependence of sound velocity. It can easily be derived and yields

$$\nu_T = \sqrt{\frac{T}{T_0}} \nu_0,$$  \hspace{1cm} (3.2)

where $\nu_0$ is the resonance frequency at a reference temperature $T_0$. Given the coefficient of dilatation of stainless steel, the shift due to the cell dilatation is negligible at our temperatures. The measured resonance frequency shift
Table 3.1: Open-ended photoacoustic (PA) cell specifications.

<table>
<thead>
<tr>
<th></th>
<th>PA cell with 4 microphones</th>
<th>PA cell with 8 microphones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resonator length</td>
<td>29 mm</td>
<td>28 mm</td>
</tr>
<tr>
<td>Resonator diameter</td>
<td>6 mm</td>
<td>3 mm</td>
</tr>
<tr>
<td>Max. operating temp.</td>
<td></td>
<td>60 °C</td>
</tr>
<tr>
<td>Resonance frequency</td>
<td>5.7 kHz</td>
<td>5.95 kHz at 21 °C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.15 kHz at 52 °C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.41 kHz at 53 °C</td>
</tr>
</tbody>
</table>

is slightly higher than expected from Eq. (3.2). It yields 450 Hz instead of 300 Hz for the PA cell with four microphones, and 460 Hz instead of 315 Hz for the PA cell with eight microphones.

### 3.3 Infrared spectroscopy of selected doping agents

To demonstrate the feasibility of vapor-phase PA measurements on large organic molecules, pure doping agents from different classes were analyzed. The substances were purchased from Sigma-Aldrich at the highest level of purity available. As far as a research on all major databases including Sadtler [46], NIST [47], Beilstein [48], and FDM [49], has given, the vapor-phase IR spectra of these compounds are unreleased up to date. Solid-phase spectra can be found in the FDM database. Unfortunately, nujol was employed as a mulling agent. Therefore, spectral information within the C-H stretching region is lost because of the absorption of nujol, a mineral oil with a long hydrocarbon chain.
As a representative of the beta-blocker, anabolic and diuretic class, metoprolol tartrate \((\text{C}_{15}\text{H}_{25}\text{NO}_3 \cdot 1/2 \text{C}_4\text{H}_6\text{O}_6; \text{CAS No. 56392-17-7})\), metandienone \((\text{C}_{20}\text{H}_{28}\text{O}_2; \text{CAS No. 72-63-9})\), and etacrynic acid \((\text{C}_{13}\text{H}_{12}\text{Cl}_2\text{O}_4; \text{CAS No. 58-54-8})\) were analyzed, respectively. These compounds exist in the solid phase at room temperature and have been chosen for their relatively low melting point \((\leq 165 \, ^\circ\text{C})\). When possible, the substances were melted, but never heated above \(150 \, ^\circ\text{C}\). Metoprolol tartrate and etacrynic acid have a melting point of about \(122 \, ^\circ\text{C}\). In the glass sample holder, they could be melted. Metandienone has a melting point of \(165 \, ^\circ\text{C}\) and remained solid. Therefore, a reasonably low vapor pressure (concentration) of metandienone is expected in the PA cell. It is not excluded that the doping substances recondensed partly before reaching the PA cell. Furthermore, nothing is known about their adsorption behavior. The determination of a concentration in the PA cell based upon the mass loss in the glass sample holder would be, therefore, purely speculative.

Vapor-phase PA spectra, pictured in Fig. 3.3, were recorded with a resolution of \(8 \, \text{cm}^{-1}\) in a spectral region between \(2800\) and \(3100 \, \text{cm}^{-1}\). They are composed of several measurement series taken on different days with different grating periods of the PPLN. The PA signal is normalized to the laser power and expressed as an absorption coefficient in arbitrary units. Each plotted data point represents an average of 24 adjacent measured data points (12 towards higher wavelengths and 12 towards lower wavelengths). This smoothing procedure is justified by the fact that the stepwidth \((0.15 \, \text{cm}^{-1})\) is much smaller than the linewidth of the OPG source \((8 \, \text{cm}^{-1})\). An averaging time of 3 seconds for each measured data point was selected on the lock-in amplifier.

The measured spectra significantly differ from each other, which shows that they originate from the species under investigation rather than impurities such as water vapor. Another source of error investigated was the PA cell outgassing. This effect is amplified when the cell is operated at elevated temperatures and when the wrong type of epoxy is used for sealing [50]. In our
Figure 3.3: C-H stretching absorption band of selected doping agent vapors. The PA cell was filled with N₂ up to a total pressure of 500 mbar at 60 °C.
case, preliminary measurements have indicated that outgassing is not negligible anymore after several hours of continuous operation without evacuating the PA cell.

3.4 Infrared spectroscopy of selected stimulants

In this section, measurements were taken on prohibited stimulants, such as nikethamide, mephentermine sulfate and Ephedra alkaloids. These drugs all act on the central nervous system. Nikethamide ($C_{10}H_{14}N_{2}O$; CAS No. 59-26-7) is a synthetic nicotine derivative that was formerly used in the treatment of barbiturate overdose, while mephentermine sulfate ($C_{11}H_{17}N \cdot 1/2 H_{2}SO_{4}$; CAS No. 1212-72-2) is used to maintain blood pressure in hypotensive states, for example, following anesthesia. The latter acts mainly indirectly on adrenergic receptors by releasing norepinephrine.

PA measurements of nikethamide and mephentermine sulfate are pictured in Figs. 3.4 and 3.5, respectively. For comparison purposes, Fig. 3.4 depicts also the FTIR condensed-phase spectrum of nikethamide, which was recorded by the Canadian Forensic Laboratory at a resolution of 2 cm$^{-1}$ [51].

The melting point of nikethamide is 23 °C; it is unclear whether the thin nikethamide film, deposited to record the FTIR spectrum, was solid or liquid. The FTIR spectrum exhibits notable differences with respect to the vapor-phase PA measurements. For molecules without intermolecular H-bonding, one reason is the lack of reaction field in the gas phase [52], resulting in a shift of several bands, as clearly seen in Fig. 3.4. Furthermore, internal rotations, which are hindered in the solid phase at ambient temperature, are activated in the vapor phase. The absorption features of nikethamide are also narrower in the vapor phase. This effect cannot be imputed to the spectral resolution, for the condensed-phase data were actually recorded with a higher resolution, i.e. 2 cm$^{-1}$ instead of 8 cm$^{-1}$. In addition, Fig. 3.4 also depicts a spectrum of water vapor simulated with Hitran [53]. It is the most
Figure 3.4: C-H stretching absorption band of nikethamide. Vapor-phase PA measurements are compared to a condensed-phase FTIR spectrum of nikethamide. Water vapor absorption lines, simulated with the Hitran database, are also depicted. The PA cell was filled with N$_2$ up to a total pressure of 500 mbar at 60 °C.

Figure 3.5: C-H stretching absorption band of mephentermine sulfate vapor. The PA cell was filled with N$_2$ up to a total pressure of 500 mbar at 60 °C.
likely impurity that can occur in the PA cell, along with end products of oxidation or thermal decomposition. Whereas some of the peaks measured between 3010 and 3080 cm\(^{-1}\) might be explained by the presence of water vapor, the major peaks measured at 2937 cm\(^{-1}\), 2955 cm\(^{-1}\) and 3003 cm\(^{-1}\) can definitely be assigned to nikethamide.

Fig. 3.6 compares the IR spectra of some Ephedra alkaloids: methyle-
phedrine ($\text{C}_{11}\text{H}_{17}\text{NO}$; CAS No. 552-79-4), (-)-ephedrine (CAS No. 299-42-3), (+)-pseudoephedrine (CAS No. 90-82-4) and (-)-pseudoephedrine (CAS No. 321-97-1). Methylephedrine and ephedrine are closely related structures but are not linked by any kind of isomerism; methylephedrine has an additional CH$_3$ group. As explained in Sect. 1.4, ephedrine and pseudoephedrine are stereoisomers of 2-methylamino-1-phenylpropanol ($\text{C}_{10}\text{H}_{15}\text{NO}$), i.e. they share the same bonds and have identical atoms sharing the same neighbors.

In Fig. 3.6, ephedrine and methylephedrine may clearly be distinguished, but the diastereoisomeric pair ephedrine and pseudoephedrine fail to differentiate, the small shifts observed being within the resolution errors. The IR spectra of the pseudoephedrine enantiomers are indistinguishable, as expected for mirror images. Due to its low melting point, ephedrine is one of the few stimulants that have been measured in the vapor phase before. In Fig. 3.6, PA measurements are thus compared to a commercially available vapor-phase spectrum provided by the NIST database at the same resolution as the PA data (8 cm$^{-1}$). The PA and FTIR spectra only agree well with respect to the main absorption peak. They are discussed in more detail along with measurements presented in the following chapter (Sect. 4.6).
Chapter 4

DFG-based multipass transmission spectrometer

4.1 Introduction

Sensitive laser-based detection schemes for infrared spectroscopy such as photoacoustic (PA), cavity ring-down (CRD) or multipass transmission (MT) have been implemented in a countless number of gas sensing devices. CRD is a relatively new technique. Originally designed to measure precisely the high optical reflectivity of laser mirrors [54, 55], it was applied in 1988 as a spectroscopic tool using a pulsed laser [56]. Continuous-wave (cw) cavity ring-down was demonstrated in 1992 [57]. In order to match the frequency of a cw laser with a longitudinal cavity mode, the cavity length was scanned with a piezoelectric transducer, mounted behind one cavity ring-down mirror. PA, CRD and MT are reviewed in several publications [58, 59]. In a recent dissertation [30], they are compared to each other using the same pulsed mid-infrared laser source. They offer different advantages such as excellent detection limits with high pump powers in the watt range (PA), extremely long effective optical path lengths (CRD), and the need of only a few $\mu$W IR radiation (MT). To record vapor-phase spectra of doping agents, the suitability of the instrumentation to elevated operating temperatures must be
addressed first.

In Chapt. 3, we saw that the miniature microphones prevented operation of the photoacoustic cell above temperatures of 333 K. Vapor-phase infrared spectroscopy was thus limited to measurements on low-melting-point doping agents, which were presented in Sect. 3.3 and 3.4.

In the case of CRD, condensation on the high reflectivity mirrors must be avoided. The dielectric mirror layers are typically deposited at a substrate temperature between 500 and 700 K. Then, they are often annealed for several hours between 700 and 800 K, in order to reduce tensile stress and prevent delamination at room temperature. Hence, operation of the mirrors up to 800 K is, in principle, possible. Thermal expansion of the layers will however result in a shift of the center wavelength and a change of reflectivity. The mirror reflectivity and separation determine in turn the ring-down time, thus making a CRD setup very sensitive to temperature change. Furthermore, in continuous-wave CRD spectroscopy, a piezoelectric transducer is usually required to match the cavity modes, which complicates the design of a high-temperature resistant experimental scheme. Instead of modulating the cavity length, one can modulate the frequency of the laser. However, applications using such an approach are limited [60]. Operation of CRD at elevated temperatures is, therefore, not straightforward.

The metallic mirrors are critical in the case of MT as well. Condensation on the optical surface must be absolutely avoided. Furthermore, thermal expansion may lead to fatal misalignments. In the Herriott configuration, for example [61], the re-entrant condition depends on the distance between the mirrors. These technical difficulties have restricted measurements in the vapor phase to using heatable single-pass gas cells. The purpose of the present chapter is to present the design and the implementation of a novel type of long-path cell, which can—among other advantages—withstanding harsh environments in addition to temperatures up to 723 K.
4.2 Multipass transmission theory

4.2.1 A new insight into Beer-Lambert’s law

A laser absorption spectrometer for ratiometric detection of a gaseous species is schematically shown in Fig. 4.1. In this simple scheme, a laser beam is passed through a cell and the transmitted intensity through the cell is recorded as a function of the frequency of the incident light $\nu$. One detector controls the intensity of the incoming light $I_{in}$, while the other controls the intensity of the outgoing light $I_{out}$. Atomic or molecular species in the cell, characterized by lower and upper energy levels $E_i$ and $E_f$, absorb a fraction of the light according to Beer-Lambert’s law [62]:

$$I_{out}(\nu) = I_{in}(\nu) \cdot B(\nu) \cdot e^{-A}, \quad (4.1)$$

where $A$ is the absorbance and $B(\nu)$ is the background signal due to the several optical elements placed between the two detectors. The absorbance $A$ is determined by the absorption path length $L$, the cross section $\sigma$ for the transition $(E_i \rightarrow E_f)$ and by the population densities $n_i$, $n_f$ in the energy levels $E_i$, $E_f$ with statistical weights $g_i$, $g_f$:

$$A = n_i \cdot \left(1 - \frac{g_in_f}{g_f n_i}\right) \cdot \sigma \cdot L. \quad (4.2)$$

The factor $\left(1 - \frac{g_in_f}{g_f n_i}\right)$ accounts for the effect of stimulated emission, which causes an effective decrease of absorption. From (4.2), the cross section $\sigma$
must have the unit of m$^2$ or, as often found in literature, cm$^2$. It can be interpreted as the surface of a completely absorbing disc that has the same stimulated absorption effect as a species in the lower level $E_i$. Furthermore, it can be described in terms of a line strength $S_{if}$ and a normalized profile $g(ν, p, T)$, which generally depends on the temperature $T$ and the pressure $p$:

$$σ = S_{if} g(ν, p, T).$$

The line strength and the normalized line profile can thus be defined by

$$S_{if} = \int σ(ν) dν \text{ and } \int g(ν, p, T) dν = 1.$$

It is important to understand that the line strength $S_{if}$ is temperature independent, irrespective of the broadening mechanism responsible for the line profile $g(ν, p, T)$. In literature, other definitions of the line strength may occur. In all cases, it is related to the probability that a species in the lower level experiences a transition, i.e. it is related to the Einstein coefficients or the transition moment [62]. For example, if $B''_{if}$ is the Einstein coefficient of stimulated absorption, we may write

$$S_{if} = \frac{hν_{if}}{c} B''_{if},$$

where $ν_{if} = (E_f - E_i)/h$. The superscript in $B''_{if}$ refers to the fact that, in the definition of the Einstein coefficients, the energy density is defined per unit frequency interval. Omitting the superscript leads to a great deal of confusion in literature [63, 64]. Experimentally, it is often more practical to express the absorbance $A$ as a function of the total density of species $n$ present in the cell:

$$A = n \cdot \tilde{σ} \cdot L,$$

especially for vapors of large molecules when the energy levels and their population densities are unknown. By comparing (4.2) and (4.6), the effective cross section $\tilde{σ}$ can be expressed as

$$\tilde{σ} = \frac{n_i}{n} \left(1 - \frac{g_i n_f}{g_f n_i}\right)σ.$$
It may be interpreted as the surface of a completely absorbing disc that has the same effective absorption effect as a species in the cell. Similarly to the cross section $\sigma$, the effective cross section $\tilde{\sigma}$ can also be written as

$$\tilde{\sigma} = I_{if}(T) g(\nu, p, T),$$

(4.8)

where the line intensity $I_{if}(T)$ is given by

$$I_{if}(T) = \int \tilde{\sigma}(\nu) \, d\nu.$$  

(4.9)

Experimentally, the line intensity can easily be determined:

$$I_{if}(T) = \frac{\int A(\nu) \, d\nu}{nL} = \int \tilde{\sigma}(\nu) \, d\nu.$$  

(4.10)

By inserting first (4.7), then (4.4) and (4.5) in (4.9), the line intensity becomes

$$I_{if}(T) = \frac{h\nu_{if} n_i}{c} \frac{g_i n_f}{g_f n_i} \left(1 - \frac{g_i n_f}{g_f n_i}\right) B_{if}^\nu.$$  

(4.11)

Assuming local thermodynamic equilibrium (LTE), the population partition between states is governed by Boltzmann statistics:

$$\frac{g_i n_f}{g_f n_i} = e^{-h\nu_{if}/k_B T} \quad \text{and} \quad \frac{n_i}{n} = \frac{g_i e^{-E_i/k_B T}}{Z(T)},$$  

(4.12)

where the partition function $Z(T)$ is given by $Z(T) = \sum_i g_i e^{-E_i/k_B T}$. Hence, the line intensity $I_{if}$ is a temperature-dependent quantity, unlike the line strength $S_{fi}$. This is sometimes a source of confusion in literature. In the Hitran database, the line intensity of various transitions, defined like in (4.11) [65], can be found in units of $\text{cm}^{-1} \text{molecule}^{-1} \text{cm}^{-2}$. This is the physical quantity that we refer to as line intensity as well throughout this work.

In summary, $\sigma$ usually denotes the stimulated absorption cross section per molecule in the lower energy level. Frequency integration yields a temperature-independent quantity related to the Einstein coefficient. From an experimental point of view, it is more practical to define an effective absorption cross section per molecule in the cell $\tilde{\sigma}$, the frequency integration of which yields the temperature-dependent line intensity.
4.2.2 Detection limit

To determine experimentally the transmission \( T = e^{-A} \) through the cell pictured in Fig. 4.1, two measurements are usually carried out: a measurement with a sample in the cell and a background measurement without any absorbing species, which provides the factor \( B(\nu) \) in (4.1). The relative error \( \frac{\Delta T}{T} \) is given by the relative standard deviation \( \sigma_{\text{stat}} \), which can be determined by repeating a measurement at a constant frequency many times. To ensure that the measured signal \( T \) does not originate from noise, it must differ from 1 (i.e. no absorbing species in the cell) by a value equal to at least three times the error of \( T \) (SNR=3). In other terms, we must have

\[
1 - T \geq 3\Delta T. \tag{4.13}
\]

Therefore, the detection limit—i.e. the minimal detectable number density \( n_{\text{min}} \) of a species in the cell—is

\[
n_{\text{min}} = \ln \left(1 + 3\sigma_{\text{stat}}\right) \simeq \frac{3\sigma_{\text{stat}}}{\bar{\sigma}(\nu)L}, \quad \text{when} \quad 1 \gg \sigma_{\text{stat}}. \tag{4.14}
\]

From (4.14), we can conclude that the detection limit may be improved for a given absorbing species by reducing the noise \( \sigma_{\text{stat}} \) or by increasing the absorption path length \( L \). If the absolute number of species is limited, the volume \( V \) of the cell will have to be taken into account. By detection limit one should then understand the minimal absolute detectable number \( N_{\text{min}} \) of an absorbing species, which may be improved by reducing the noise or by optimizing the \( L/V \) ratio.

4.2.3 Two-mirror multipass cell

To increase the absorption path length of the system described in Fig. 4.1 despite limited laboratory space, folding of the light in a multipass absorption cell is necessary. For this purpose, a White [66] or Herriott [61] optical arrangement may be chosen. In the Herriott configuration—which yields a better \( L/V \) ratio—two identical concave spherical mirrors are placed facing
each other at a distance $d$ nearly equal to their radius of curvature $R$. The laser beam is injected with a slope $(x'_0, y'_0)$ into the cavity through an entry hole drilled through one mirror at coordinates $(x_0, y_0)$. The beam travels back and forth between the mirrors before exiting the cavity through the entry hole. The beam spots on the two mirrors $(x_n, y_n)$ after $n$ passes lie in general on ellipses. In terms of ray optics, the multipass cell is equivalent to a series of equally spaced thin lenses. The lenses have a focal length $f$ and are spaced at a distance $d$. We postulate that the lens system is stable, i.e. that $d < 4f$. Using matrix formalism [67], one can show that the coordinates of the beam spots after $n$ passes are

$$x_n = x_0 \cos n\theta + \sqrt{\frac{d}{4f-d}}(x_0 + 2fx'_0) \sin n\theta,$$

with \( \cos \theta = 1 - \frac{d}{2f} \) and \( R = 2f \). \hspace{1cm} (4.15)

This relation can be rewritten in the form

$$x_n = A \sin n\theta + \alpha,$$

with \( A^2 = \frac{4f}{4f-d} (x^2_0 + dx_0x'_0 + dfx'^2_0) \) \hspace{1cm} (4.16)

and

$$\begin{cases} 
\tan \alpha = \sqrt{\frac{4f}{d} - 1} / \left(1 + 2f \frac{x'_0}{x_0}\right) \\
\sgn(\sin \alpha) = \sgn(x_0). 
\end{cases} \hspace{1cm} (4.17)$$

The problem is fully separable and a corresponding relation holds for the $y$ coordinate:

$$y_n = B \sin n\theta + \beta,$$

with \( B^2 = \frac{4f}{4f-d} (y^2_0 + dy_0y'_0 + dfy'^2_0) \) \hspace{1cm} (4.18)

and

$$\begin{cases} 
\tan \beta = \sqrt{\frac{4f}{d} - 1} / \left(1 + 2f \frac{y'_0}{y_0}\right) \\
\sgn(\sin \beta) = \sgn(y_0). 
\end{cases} \hspace{1cm} (4.19)$$
When projected onto an x-y plane, $\theta$ is the angle between two successive points $(x_n, y_n)$ and $(x_{n+1}, y_{n+1})$, whereas $2\theta$ is the angle between two successive points on one mirror. $A$ and $B$ are the maximum possible excursions of the ray in the x and y direction, respectively. As previously described, the projections of the intersection points $(x_n, y_n)$ into a x-y plane lie, in general, on an ellipse. In special cases, they are on a circle of radius $r$, namely when

$$A = B = r,$$

and

$$\tan \alpha \cdot \tan \beta = -1.$$ (4.24)

The distance between the beam spots are optimized by choosing $r$ slightly smaller than the actual radius of the mirrors. This is to avoid fringes due to overlapping of the beams. Furthermore, for practical reasons, we impose the input beam on a horizontal plane (parallel to the optical table on which the cell is mounted). With (4.23) and (4.24), we can then derive simple relations for the input beam conditions:

$$x_0 = \pm \frac{r}{2} \sqrt{\frac{d}{f}},$$ (4.25)

$$y_0 = \pm |x_0| \sqrt{\frac{4f}{d} - 1},$$ (4.26)

$$x'_0 = -\frac{2x_0}{d},$$ (4.27)

$$y'_0 = 0 \text{ (imposed)}.$$ (4.28)

From (4.27), (4.28) and the lens matrix formalism, one can also derive the slopes of a ray that exits the cell after an even number of passes $N$ [67]:

$$x'_{N-1} = \frac{x_0}{f} + x'_0 = \frac{x_0}{f} - \frac{2x_0}{d},$$ (4.29)

$$y'_{N-1} = \frac{y_0}{f} + y'_0 = \frac{y_0}{f}.$$ (4.30)

We notice from (4.29) and (4.30) that the slopes of the exiting beam do not depend on the number of passes or the path length, but that changes of the
entrance slopes are directly transferred to the exit slopes. A beam injected in the cell returns exactly to its entrance point after \( N \) passes and \( M \) complete orbits in the x-y plane, provided the following reentrant condition is satisfied:

\[ N\theta = 2M\pi. \] (4.31)

The angle \( \alpha \) between two neighboring spots on one mirror is \( \frac{2\pi}{N/2} \). As seen on Fig. 4.2, it must not be confused with \( 2\theta \), which is the angle between two consecutive spots on one mirror.

\[ (x_0, y_0) \quad \bullet \quad (x_4, y_4) \quad \alpha \quad \frac{2\theta}{r} \quad (x_2, y_2) \]

Figure 4.2: Beam spots on the entry mirror lying on a circle of radius \( r \). A laser beam enters the cavity through a hole drilled at coordinates \((x_0, y_0) = (x_N, y_N)\) and exits the cavity through the same hole after \( N = 18 \) passes and \( M = 4 \) complete orbits in the x-y plane. The beam spot diameter decreases with the number of passes if the beam is continuously refocussed \((d < 2f)\). The angle \( \alpha \) between two neighboring beam spots on one mirror should not be confused with the angle \( 2\theta \) between two consecutive spots on one mirror.

According to (4.16), the reentrant condition is satisfied for discrete values of the mirror separation \( d_N \) given by

\[ d_N = 2f \left( 1 - \cos \frac{2M\pi}{N} \right). \] (4.32)

The path length \( P_N \) is approximatively \( Nd_N \), but a more accurate value must
be computed from
\[ P_N = \sum_{n=0}^{N} \sqrt{d^2 + (x_n - x_{n-1})^2 + (y_n - y_{n-1})^2}. \] (4.33)

To maximize the path length and to ensure that the beam is continuously refocussed in the cell, we look for solutions close to the confocal position \( d_N \lesssim 2f \) i.e. \( \frac{2M\pi}{N} \lesssim \pi/2 \). The combinations of \( M \) and \( N \) giving such reentrant paths can conveniently be expressed in terms of families \((N, M)\) with
\[ N = 4M + K, \quad K = 2, 4, 6, \cdots . \] (4.34)

Eq. (4.32) can now be rewritten as
\[ d_N = 2f \left[ 1 - \cos \left( \frac{\pi}{2} \left( 1 - \frac{K}{N} \right) \right) \right]. \] (4.35)

It is important to understand that the total number of passes \( N \) is determined by the mirror separation \( d_N \). Changes in the input beam will only reflect on the shape of the ellipse. In the following, we will restrict ourselves to the \( K = 2 \) family, for which \( d_N \) and \( P_N \) are maximum given \( N \). For a complete discussion of all families \( K \), one can refer to [68]. For a large number of passes \( N \gg K \), the mirror separation, the input beam conditions and the output beam slopes become \( d_N \simeq 2f \), \( x_0 \simeq \pm \frac{\sqrt{2}}{2} r \), \( y_0 \simeq \pm \frac{\sqrt{2}}{2} r \), \( x'_0 \simeq -\frac{x_0}{f} \), \( y'_0 = 0 \), \( x'_{N-1} \simeq 0 \) and \( y'_{N-1} \simeq \pm \frac{y_0}{f} \). Therefore, if the input beam enters the cell in a horizontal plane under a certain angle, it exits the cell in a vertical plane under the same angle.

### 4.3 High-temperature multipass cell (HTMC)

This section presents the design of the high-temperature multipass cell, a novel type of heatable long-path cell. A general description of the cell is covered in Sect. 4.3.1 and the main properties are highlighted in Sect. 4.3.2. The experimental arrangement employed in the present and subsequent chapter

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**CHAPTER 4. DFG-BASED MT SPECTROMETER**

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is described in Sect. 4.3.3. It features a tunable DFG laser with an in-house-built HTMC. In Sect. 4.3.4, initial measurements illustrate the performance of the cell.

4.3.1 Description

The main components of the in-house-built high-temperature multipass cell (HTMC), depicted in Fig. 4.3, consist of two spherical or astigmatic mirrors facing each other (M1 and M2), a bellows (Be), a viewport (Vi), an entry window (W), a tube (T) with fittings and electric feedthroughs, and a small vessel (Ve). One mirror (M1) is fixed, while the other (M2) can be tilted or translated in the x, y or z direction without disassembling the HTMC. Each mirror is separately heated by a cable heater (H1 and H2) and can easily be maintained at a higher temperature than the rest of the cell, in order to prevent condensation on the optical surface. As mirror substrate material, synthetic fused silica was chosen for its high softening point, good chemical resistance and relatively low thermal expansion. The latter is critical, because alignments depend on the radius of curvature of the mirror as seen in Sect. 4.2.3. The choice of a glass substrate rather than a metallic one was also driven by the fact that the coating can easily be dissolved and redeposited in-house, in the event of an accidental contamination. If, however, the mechanical robustness of a metallic substrate is desired, the mirrors may be replaced, for the cell can fully be dismantled, i.e. no mechanical components are glued to each other. Each mirror is mechanically held on an ultra high vacuum Conflat flange (CF1 and CF2) by means of a spring washer, allowing thermal dilatation to be compensated. The cable heater for the mirror is directly incorporated into the flange and exits the latter through a Swagelok tube fitting, to which it is sealed. (At the exit, a tube was initially brazed around the cable heater in order to match a standard Swagelok diameter.) In this manner, the heater, which usually has a limited lifetime, can easily be replaced, independent of the flange.

On the side of the tilt mirror (M2), a ceramic disk (C3) thermally isolates
Figure 4.3: Exploded view of the high-temperature multipass cell consisting of mirrors (M1 and M2), cable heaters (H1 and H2), Conflat flanges (CF1 and CF2), ceramic spacers (C1, C2, and C3), a tilt plate (TP), a translation stage (TS), a bellows (Be), a viewport (Vi), an entry window (W), a tube (T) with fittings (F1 and F2) and electric feedthroughs (EF), supports (S1 and S2), a base plate (BP), two linear bushings (Bu), and a vessel (Ve).
the flange from a tilt plate (TP), allowing the user to perform adjustments under any thermal condition. The tilt plate is mounted on an x-, y- or z-direction translation stage (TS). A bellows (Be), sealed to the flange, compensates for thermal expansion, once the distance between the two mirrors is fixed. Furthermore, it enables one mirror to be tilted and translated under any vacuum condition.

On the side of the fixed mirror (M1), a viewport (Vi) offers a line of sight on the whole optical surface. This permits an easy alignment, e.g. by observing the beam spots pattern of a He-Ne laser beam. The optical beam is coupled in and out of the cell through an entry window (W) and a hole drilled through the fixed mirror.

A flexible wire heater (not shown in Fig. 4.3) heats the bellows and the main body of the cell. The latter is composed of a tube (T) with fittings (F1 and F2) and electric feedthroughs (EF). Two supports (S1 and S2) hold the tube in place on each side. On one side, the support (S2) is mounted on two linear bushings (Bu) in order to allow thermal expansion of the tube. The supports are thermally isolated from a base plate (BP) by thick ceramic layers (C1 and C2). The base plate is a piece of aluminum with three slots for mounting on optical tables without distorting the mirror alignment. One ceramic layer (C2) is placed in such a manner that the bushings, the lubricant of which should be kept below 393 K, are thermally isolated from the heated body. The fittings may be used to connect a vacuum system or may serve as an inlet port. The electric feedthroughs can be used, for example, to connect resistance temperature detectors placed inside the cell. If wiring inside the cell is required, ceramic beads provide a flexible solution for electric insulation at high temperatures.

Finally, a small vessel (Ve) in which a solid or liquid probe can be placed is mounted on a small Conflat flange. The flange, around which a ring heater can be tightened, is sealed to the cell body. Two electric feedthroughs are also welded to the flange, thus allowing the temperature of the vessel to be controlled separately.
Unless otherwise specified, all metallic vacuum components are of 316L stainless steel for maximum corrosion resistance, while silver-coated copper gaskets are used for sealing. Heating is separately controlled for the two windows, the vessel and the rest of the cell.

4.3.2 Properties

The HTMC is a heatable long-path absorption cell, where the mirrors are separately heated in order to avoid condensation on their optical surface. Furthermore, the cell is designed to compensate thermal expansion by the simultaneous action of a bellows and linear bushings. No cold spots appear inside the vacuum components. Therefore, measurements on condensable vapors are possible, in addition to traditional measurements on gases. All materials employed have good resistance to aggressive chemicals. The maximum achievable operating temperature (723 K) is limited by the glass-to-metal hermetic seals. Whereas sapphire can be readily metalized and sealed to ultra high vacuum (UHV) flanges by brazing, most optical materials are not suitable for high temperature joining processes. In those cases, Kalretz O-rings may be employed. In spite of performing up to 600 K only, Kalretz offers more flexibility in the choice of the window material, antireflection coating and wedge angle. In the future, with the advent of new sealing techniques, the maximum operating temperature of the HTMC could be improved, as the design of the cell and all material used both allow much higher temperatures.

The HTMC is designed for optical configurations using spherical mirrors or astigmatic mirrors, as described, for the first time, by Herriott et al. [61] and McManus et al. [69], respectively. With spherical mirrors, several stable configurations satisfy the reentrant condition given by (4.31). The path length is thus variable by an amount that depends on the travel range of the translation stage that governs the mirror spacing. For a given cell volume, the maximum achievable path length is, however, shorter than obtained with astigmatic mirrors. In both configurations, alignments may be performed un-
der any thermal or vacuum condition without dismantling the cell. Detailed cell specifications are summarized in Table 4.1.

Table 4.1: High-temperature multipass cell (HTMC) specifications.

<table>
<thead>
<tr>
<th></th>
<th>HTMC with sapphire window</th>
<th>HTMC with other window materials</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum path length</td>
<td>~10–60 m for 1–4 liters</td>
<td>~40–100 m for 1–4 liters</td>
</tr>
<tr>
<td>and cell volume using:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- spherical mirrors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- astigmatic mirrors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maximum operating</td>
<td>723 K</td>
<td>600 K</td>
</tr>
<tr>
<td>temperature</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transmission range</td>
<td>0.25–4 µm</td>
<td>window material dependent</td>
</tr>
<tr>
<td>Window sealing type</td>
<td>hermetic</td>
<td>Kalrez O-ring</td>
</tr>
<tr>
<td></td>
<td>glass-to-metal</td>
<td>Kalrez O-ring</td>
</tr>
<tr>
<td></td>
<td>for UHV</td>
<td></td>
</tr>
</tbody>
</table>

4.3.3 Experimental setup

In Chapt. 3, infrared spectra of doping agents were recorded with an OPG-based photoacoustic spectrometer. The resolution of the spectra was limited by the linewidth of the OPG laser source. It was sufficient to distinguish drugs belonging to different doping classes or even closely related structures such as methylephedrine and ephedrine. However, it was too poor to differentiate ephedrine from pseudoephedrine. For this reason, the OPG-based laser was replaced by the DFG laser source presented in Sect. 2.2. In this manner,
Figure 4.4: Experimental setup of the DFG-based multipass transmission spectrometer.

A DFG-based multipass transmission spectrometer, pictured in Fig. 4.4, was built in-house.

A HTMC in the Herriott configuration with spherical gold-coated mirrors is implemented in the spectrometer. The spherical mirrors have a nominal diameter of 70 mm and a radius of curvature of 400 mm. An entry hole is drilled through the fixed mirror 30 mm from the center. Two entry windows are available: a hermetically sealed 1.6 mm thick sapphire window for applications up to 723 K and an elastomer-sealed CaF$_2$ window for applications up to about 600 K. The CaF$_2$ window is 2 mm thick and weaged by an angle of 2° to hinder interference fringes due to overlapping multiple reflections. The cell has a total inner volume of 2.0 liters, including all fittings and a high-temperature-resistant pressure transducer. The total optical path length can be varied from about 9 to 35 m. According to (4.33), the path lengths for $N = 74, 82$ and $90$ are $28.5, 31.7$ and $34.9$ m, including twice the distance between the entry hole and the entry window (approx. $2 \times 50$ mm).

An in-house-built rack consisting of four PID temperature controllers monitors the mirror heaters, the wire heater of the cell body and the ring
heater of the vessel separately. In this way, the temperature of the cell, the mirrors and the vessel can be adjusted between room temperature and 573 K with an accuracy of 1 K. The maximum achievable temperature of 573 K is limited here by the total heating power available and not by the materials used as described in Table 4.1.

The laser beam is split before entering the HTMC by a 6.35 mm thick CaF$_2$ window, whose faces are wedged by an angle of 30’. The mid-infrared power entering and exiting the cell is recorded by two room temperature Hg-Cd-Zn-Te detectors (Vigo System S.A., Warsaw, Poland). When spatial cleaning of the idler through a pinhole is required, two thermoelectrically cooled Hg-Cd-Zn-Te detectors (Vigo System S.A., Warsaw, Poland) are used instead. The effect of the pinhole and the issue of interference fringes will be detailed in Sect. 4.4.

The transmitted and reflected portion of the beam after the splitter travel approximately the same distance in open space before reaching the detectors. This arrangement prevents unwanted absorption lines in the background due to the presence of water vapor in ambient air.

After each measurement cycle, condensates are captured in a cooling trap prior to evacuating the cell through a high-vacuum system. Background measurements with pure nitrogen (99.999 %) are performed before or after each measurement series. To prevent oxidation of the mirrors at elevated temperatures, nitrogen rather than synthetic air is always employed as non-absorbing buffer gas.

4.3.4 Test measurements

The HTMC described in Sect. 4.3.3 is assembled in a way that the spherical mirrors are separated by approximately their radius of curvature $R$. With the help of the translation stage, the mirror spacing $d$ is then adjusted to discrete values that fulfill (4.35) ($K = 2$). A comparison between the measured and calculated mirror separation is shown in Fig. 4.5. The experimental error bars, of the order of 100 $\mu$m, illustrate the amount required to completely
misalign the cell.

The thermal expansion of the cell from room temperature to 548 K can be easily verified by measuring the displacement of the bushings, which yields 850 ± 50 µm. This confirms our initial assumption: without a compensation mechanism, fatal misalignments would occur when exposing the cell to appreciable temperature changes.

In Fig. 4.6, the detector signal ratio is plotted as a function of the number of passes $N$. The mirror reflectivity $R_e$ can be deduced from a power fit of the form $f(N) = a \cdot R_e^N$, where $a$ and $R_e$ are the coefficients of the fit. The measurement shown in Fig. 4.6 was taken on 8 June 2006 at a fixed idler wavelength $\lambda_i = 3.50$ µm after filling the HTMC at room temperature with 100 mbar N$_2$. A mirror reflectivity $R_e = 94.5 \pm 0.1$ % is obtained, in agree-
Figure 4.6: Mirror reflectivity of $94.5 \pm 0.1\%$ at $\lambda_i = 3.50 \, \mu m$ deduced by fitting the detector signal ratio as a function of the number of passes in the cell.

This measurement is in good agreement with an angle of $6.2^\circ$, calculated on the basis of equations derived in Sect. 4.2.3.
4.4 Fringe artifacts and instrumental noise

When the temperature of the PPLN crystal is tuned, the efficiency of the DFG conversion changes, but the generated idler wavelength stays constant. Therefore, the detector signal ratio must not vary as a function of the crystal temperature. The contrary is, however, observed in Fig. 4.7, which features a 10 °C temperature scan at $\lambda_i = 3.34 \ \mu m$. As discussed in Sect. 2.2, the beam profile of the sum frequency generation varies strongly with temperature changes. A similar behavior can be expected for the DFG beam, making the detector signal ratio temperature-dependent as well. This feature is troublesome when performing wide scans requiring simultaneous tuning of the signal wavelength and the crystal temperature, because the noise is then

![Figure 4.7: Temperature scan at a fixed idler wavelength $\lambda_i = 3.34 \ \mu m$ with and without a pinhole in the idler beam path. The two y-axes are scaled in a way that allows direct comparison of the two scans.](image)
determined by the beam profile fluctuations. This drawback is overcome by spatially cleaning the idler with a pinhole placed in the beam path. Fig. 4.7 illustrates how such a pinhole makes the setup considerably less sensitive to crystal temperature fluctuations.

Typical wide scan measurements with a pinhole placed in the beam path are shown in Figs. 4.8 and 4.9. The idler was scanned from 2942 to 3024 cm\(^{-1}\) with 0.08 cm\(^{-1}\) steps by simultaneously tuning the ECDL wavelength (1550–1570 nm) and the crystal temperature (70.7–120.1 °C). The HTMC was mounted with the wedged CaF\(_2\) entry window and filled with 100 mbar N\(_2\) at room temperature (Fig. 4.8), and with 130 mbar N\(_2\) at 400 K (Fig. 4.9). The optical path length of the cell was 31.7 m (N = 82). Two scans were performed for each of the measurements (room temperature and 400 K). In such successive scans, the wavelength of the ECDL is not reproducible.
Therefore, the detector signal ratios cannot be directly divided from one another. Instead the data from the first scan have to be interpolated, e.g. with a Smoothing Spline algorithm [70], to compute data at the same $\lambda$-values as the second scan. The division of the 2nd scan (raw data) by the 1st scan (interpolated values) is—as expected—scattered around 100 % transmission, with a routinely achieved standard deviation $\sigma_{\text{stat}}$ of 0.3–0.5 %, limited by temperature deviations, as already explained. Given an instrumental noise $\sigma_{\text{stat}}$ of 0.3 % and a maximum absorption pathlength $L$ of 34.9 m, the minimum detectable absorption coefficient (SNR=3) $\alpha_{\text{min}} = \frac{\ln(1+3\sigma_{\text{stat}})}{L}$ is $2.6 \times 10^{-6}$ cm$^{-1}$. It can be improved up to 2 orders of magnitude at the expense of long averaging times (20 s per step) and a limited continuous tuning range [71] (the temperature of the PPLN is fixed).

The measurement performed at 400 K is not more noisy than the room
temperature measurement, despite a measurable constant blackbody radiation. The latter was subtracted from the detectors signal by operating the oscilloscope in AC mode. Isolated data points in the transmission spectra indicate the presence of moisture. In routine measurements, the cell is shortly evacuated before introducing pure N\textsubscript{2} and up to 1000 ppm water vapor may still subsist.

A distinctive fringe pattern with a period $\Delta \tilde{\nu} = 3.8 \text{ cm}^{-1}$ can be seen in each scan. It is due to a 800 $\mu$m thick BaF\textsubscript{2} protective window mounted in front of each detector. The fringes can be reduced by placing the detectors in the focal spot of the idler and by orienting them diagonally with respect to the beam propagation direction. In this manner, multiple reflections within the BaF\textsubscript{2} window no longer overlap and cannot interfere with each other. When the transmission is calculated in routine measurements, fringes can be canceled out in most cases. An additional fringe structure with a period $\Delta \tilde{\nu} = 1.8 \text{ cm}^{-1}$ is superposed to the regular one when the 1.6 mm thick sapphire entry window is mounted on the HTMC, in agreement with the relation $d = \frac{1}{2n\Delta \tilde{\nu} \cos \theta}$ [72]. Here $d$ is the thickness of the window, $n$ is the index of refraction and $\theta$ the incident angle given by Eq. 4.27. These fringes are not problematic for narrow scans up to 3 cm\textsuperscript{-1}, but limit the maximum tuning step in wide scans, as at least 10 data points within one fringe are required.

4.5 Infrared spectroscopy of heated gases and vapors

To demonstrate the performance of the DFG-based multipass transmission spectrometer, infrared spectra of methane (CH\textsubscript{4}), water vapor (H\textsubscript{2}O) and acetone vapor (CH\textsubscript{3}COCH\textsubscript{3}) were taken at different temperatures in the fingerprint region between 3 and 4 $\mu$m. In Sect. 4.5.1, single absorption lines of methane and water vapor are presented. In Sect. 4.5.2, a whole absorption band of acetone vapor was measured.
4.5.1 Narrow scan over methane and water vapor absorption lines

Before each measurement series, the background was measured with pure nitrogen (99.999%). Methane samples were taken from a certified mixture of 1000 ppm methane diluted in nitrogen, while water was directly injected into the HTMC through a septum adapter with the help of a microliter syringe. Only the piezo of the ECDL (EOSI) was scanned to cover a continuous tuning range of up to 1 cm$^{-1}$, as described in Sect. 2.2.

In Fig. 4.10, a measurement on methane clearly evidences different temperature effects on line intensity in a spectral range free from interferences with strong water absorption lines. The cell was filled at room temperature

![Absorbance vs Wavenumber](image)

Figure 4.10: Measurements of 1000 ppm methane buffered in 100 mbar N$_2$ at 296 K and in 185 mbar N$_2$ at 548 K, respectively. The path length of the HTMC was maintained at 24 m.
with 1000 ppm methane up to a total pressure of 100 mbar N$_2$. A measurement was then performed at room temperature and at 548 K, using the same gas sample, thus keeping the methane concentration constant. In agreement with the latest Hitran predictions [53], the $P(16)$ lines of the $\nu_3$ band at 2853.70 cm$^{-1}$ and 2853.90 cm$^{-1}$ increase in intensity by a factor of 8–10. The $\nu_2 + \nu_4$ sum band $Q$ branch transition at 2853.94 cm$^{-1}$ originates from a lower lying rotational level, i.e. $J = 3$ instead of $J = 16$. The intensity of the line at 2853.94 cm$^{-1}$ is therefore expected to decrease as a result of lower state depopulation. In fact, it appears to remain constant in Fig. 4.10 due to an overlap with the weak nearby $P(16)$ line of the $\nu_3$ band at 2853.93 cm$^{-1}$ whose intensity increases with temperature. The multiple absorption lines were best fitted with a sum of Voigt convolutions, because they are pressure-broadened and Doppler-broadened under our reduced pressure conditions. Line intensities were derived from the parameters of the fit. The effect of the mid-infrared laser line width is negligible [32]. The room temperature and high-temperature spectrum were slightly shifted in the wavenumber-axis to match the expected peak at 2853.70 cm$^{-1}$, because we did not have any wavelength control over the Nd:YAG pulses. A good estimation based upon previous measurements was, however, available [32]: shifts were therefore less than 0.04 cm$^{-1}$.

Another measurement, depicted in Fig. 4.11, was performed on weak 2$\nu_2$ overtone transitions of water vapor. Here, 9.6 $\mu$L water were evaporated in nitrogen at room temperature, thereby raising the total pressure to 205 mbar and the water vapor concentration in the HTMC to 3.2 %. A measurement was first taken at room temperature, then at 545 K using the same sample. The $P(5)$ line at 3022.37 cm$^{-1}$ hardly changes in intensity, in good agreement with Hitran calculations. In contrast, the $P(9)$ line at 3022.66 cm$^{-1}$ increases by a factor of 3.8, which is less than the factor 5.7 predicted by semi-empirical Hitran calculations. Again, the spectra were slightly shifted in the wavenumber-axis, as a control over the pump wavelength was not available.
Figure 4.11: Measurements of 3.2 % water vapor buffered in N\textsubscript{2} at 296 K and 545 K. The total pressure was 205 mbar and 377 mbar, respectively. The path length of the HTMC was maintained at 28.5 m.

The presented measurements demonstrate the feasibility of high-temperature experimental investigations of gases and vapors with long-path length systems. An equivalent measurement to the one performed on 1000 ppm methane could be undertaken using a commercially available single-pass heated cell, provided the methane concentration is increased by two orders of magnitude in order to compensate for the reduced absorption path length. Such an attempt would present a safety issue though, for the lower and upper inflammability limit of methane in air is 5 % and 15 %, respectively. An equivalent measurement to the one performed with 3.2 % water vapor can however not be realized using a single-pass heated cell. Hence, a whole new range of low-intensity transitions becomes accessible using a HTMC at elevated temperatures.
4.5.2 Wide scan over an acetone vapor absorption band

Before performing measurements on vapors of doping agents, we wish to test the reliability of the spectrometer and to demonstrate its wide tunability. For this purpose, infrared spectra of acetone were recorded over the whole available tuning range.

Acetone \((\text{CH}_3)_2\text{CO}\) is a molecule with \(C_2v\) symmetry comprised of one carbonyl group a two methyl groups. It has 24 vibrational modes, which are reduced by group theory into 8 of symmetry species \(A_1\), 4 of \(A_2\), 7 of \(B_1\) and 5 of \(B_2\) [73]. Acetone is of spectroscopic interest for several reasons. It is an atmospheric trace gas that plays an important role in tropospheric chemistry [74, 75]. It is also found in interstellar space [76–78]. Last but not least, patients with diabetes or on ketogenic diets have higher acetone concentrations in their breath. It is well known that human breath contains several hundred volatile organic compounds (VOCs) [79]. Some of them have been identified as particular diagnostic agents of specific illnesses such as acetone, which is referred to as a “biomarker” for diabetes. In a study performed by GC-MS with a solid-phase microextraction technique, the acetone concentration in diabetic breath was found to be higher than 1.71 ppmv, while its concentration in normal breath was lower than 0.76 ppmv [80]. This is in agreement with an other diagnostic method based on a quartz crystal microbalance, which yielded an effective acetone concentration in diabetic breath of 0.26–4.9 ppm (compared to non-diabetic persons) [81]. Furthermore, a laser-based acetone breath analyzer is reported in the ultraviolet and near-infrared spectral regions; the limit of detection of acetone was 1.5 ppmv in pure nitrogen [82]. In real breath samples, laser-based detection of acetone has not yet been reported.

The measurements presented in this section are the first of its kind, i.e. using a widely continuously tunable laser rather than an FTIR to record the whole C-H stretching absorption band of acetone. As described in Sect. 2.3, the whole available wavenumber range of 329 cm\(^{-1}\) was covered with a step width of 0.08 cm\(^{-1}\) by simultaneously tuning the step motor of the ECDL and
the temperature of the crystal; furthermore, the grating period was changed once from 29.5 to 29.9 µm. One microliter pure acetone (Merck, 99.8 %) was directly injected into the HTMC (2.0 liters volume) through a septum adapter with the help of a microliter syringe. Acetone spectra were then recorded at 296, 400 and 500 K at 100, 130 and 170 mbar total pressure, respectively. Each time, the acetone concentration was 1665 ppm and the total optical path length was 31.7 m.

In Fig. 4.12, the measurements taken at room temperature and 400 K are compared to a high-resolution FTIR spectrum (resolution = 0.125 cm\(^{-1}\)). The latter was recorded at 296 K under a total pressure of 1 atm [83]. No

![Figure 4.12: C-H stretching absorption band of acetone at 296 and 400 K. A room temperature FTIR recording of acetone is compared to single scans taken on 1665 ppm acetone buffered in 100 mbar N\(_2\) at 296 K and in 130 mbar N\(_2\) at 400 K, respectively. The path length of the HTMC was 31.7 m. An inset of the main peak is displayed.](image)
deviations due to the different pressure conditions are expected, as the rotational lines are not resolved. The rotational constants of acetone are small enough for its rotational lines to overlap, even under our reduced pressure conditions. In this manner, the contour of the absorption spectrum is continuous over the whole wavelength range, despite the narrow linewidth of the laser. The FTIR spectrum was only scaled in the absorbance axis to fit our experimental conditions. It agrees very well with the room temperature measurement. Three distinctive absorption features in the measured spectra were assigned to C-H stretching vibrational modes taken from theoretical work [84]. The possible assignments and measured effective cross sections are summarized in Table 4.2. The weak mode at 2929.4 cm$^{-1}$ hardly appears in Fig. 4.12, but can be clearly distinguished in the data. Another weak peak, which appears at 2942.0 ± 0.1 cm$^{-1}$, and the broad band, centered around 2947 cm$^{-1}$, could not be assigned. The error of the observed vibrational wavenumbers is given by the chosen step width (0.08 cm$^{-1}$), whereas the injected volume of acetone is the principal source of error in the determination of the effective cross sections. Given the effective cross section at 2970.0 cm$^{-1}$ derived in Table 4.2 and a noise level of 0.3 % (Sect. 4.4), a detection limit of 760 ppbv acetone in 1 atm N$_2$ can be projected (SNR=3).

Based on our previous discussion on diabetes diagnostics, this represents a promising result towards the development of a laser-based acetone detector. The strongest absorption features, however, lie in the carbonyl stretching

### Table 4.2: C-H stretching vibrational modes, assignments and measured effective cross sections of acetone.

<table>
<thead>
<tr>
<th>assignments</th>
<th>$\tilde{\nu}$ [cm$^{-1}$]</th>
<th>$\tilde{\sigma}(\tilde{\nu}, T)$ [10$^{-21}$ cm$^2$]</th>
</tr>
</thead>
<tbody>
<tr>
<td>calculated</td>
<td>measured</td>
<td>$T = 296$ K</td>
</tr>
<tr>
<td>$\nu_2(A_1), \nu_{14}(B_1)$</td>
<td>2926</td>
<td>2929.4 ± 0.1</td>
</tr>
<tr>
<td>$\nu_9(A_2), \nu_{20}(B_2)$</td>
<td>2973</td>
<td>2970.0 ± 0.1</td>
</tr>
<tr>
<td>$\nu_1(A_1), \nu_{13}(B_1)$</td>
<td>3020</td>
<td>3017.2 ± 0.1</td>
</tr>
</tbody>
</table>
region around 1740 cm\(^{-1}\), where detection limits of 4.2 ppbv acetone in N\(_2\) have been reported using a CO laser photoacoustic detection scheme [85].

At 400 K, the main peak at 2970.0 cm\(^{-1}\) decreases as a result of lower state depopulation. A separate measurement taken with an identical acetone sample in the cell has shown that \(\frac{\sigma(2970.0\text{ cm}^{-1}, 296\text{ K})}{\sigma(2970.0\text{ cm}^{-1}, 400\text{ K})} = 1.40 \pm 0.1\).

![Figure 4.13: Signs of degradation in the C-H stretching absorption band of acetone. The measurement was taken on 1665 ppm acetone buffered in 170 mbar N\(_2\) at 500 K. The path length of the HTMC was 31.7 m.](image)

Fig. 4.13 features first signs of acetone degradation at 500 K, which cannot be investigated with a common FTIR. Thanks to the narrow linewidth of the laser, unexpected narrow absorption lines appear in addition to the absorption due to acetone. The measured lines above 3000 cm\(^{-1}\), e.g. at 3010.231 or 3056.357 cm\(^{-1}\), could be assigned to water vapor (H\(_2\)O), whereas the numerous lines below 3000 cm\(^{-1}\), e.g. at 2824.658 or 2830.652 cm\(^{-1}\), are predominantly due to formaldehyde (CH\(_2\)O). Water vapor and formaldehyde
seem to come from acetone oxidation as a result of an increased amount of \( \text{O}_2 \) in the cell [86]. The reproducibility of this measurement was not investigated. It merely shows how severe the consequences of a leak may be at elevated temperatures. In the subsequent experiments, special attention was paid to avoid such occurrences.

### 4.6 Infrared spectroscopy of ephedrine and pseudoephedrine

In Chapt. 3, closely related doping agents could be distinguished. The OPG-based photoacoustic spectra, however, failed to discriminate ephedrine from pseudoephedrine. The infrared spectra that are presented in the current section were recorded with a DFG spectrometer at 100-times higher resolution, i.e. \( 0.08 \text{ cm}^{-1} \) instead of \( 8 \text{ cm}^{-1} \). Furthermore, quantitative measurements were taken, allowing effective cross sections and absolute detection limits to be derived.

A few mg of pure ephedrine (Sigma-Aldrich, Inc.) were placed in the vessel and evaporated in the HTMC at 333 K. This is the same temperature at which the photoacoustic measurements were recorded. A vapor-phase infrared spectrum was recorded over the whole available wavenumber range of 329 cm\(^{-1}\) and is compared in Fig. 4.14 to an FTIR spectrum. The latter was measured in the gas phase at an unspecified temperature and at a resolution of \( 8 \text{ cm}^{-1} \) by a GC-FTIR instrument [47]. The FTIR data provide only relative absorption coefficients as a function of wavelength that cannot be used for quantification, unlike the DFG-based multipass transmission measurements.

In Fig. 4.15, vapor-phase infrared measurements of \((-\text{-})\)ephedrine, \((+\text{-})\)pseudo-ephedrine and \((-\text{-})\)pseudoephedrine are compared to each other. They were recorded at 423 K, i.e. above the melting point of pseudoephedrine. Low

\(^{1}\)The linewidth of the DFG laser \((5 \cdot 10^{-3}\text{cm}^{-1})\) is actually much lower than the chosen stepwidth \((0.08 \text{ cm}^{-1})\).
Figure 4.14: C-H stretching absorption band of ephedrine vapor. DFG measurements were taken in 100 mbar N\textsubscript{2} at 333 K and are compared to a lower-resolution FTIR database.

amounts of substances were introduced, so that the partial pressure of our compounds at 423 K stayed below their saturation vapor pressure. In this manner, the measured absorbance at a given wavelength increased linearly as a function of the probe mass introduced in the vessel, as seen on the right hand side of Fig. 4.15. The horizontal error bars depict the reproducibility of the weighing procedure, which was of the order of 70 µg. From the linear fit, effective cross sections of ephedrine and pseudoephedrine were calculated, as displayed on the y-axis of the spectra (left hand side of Fig. 4.15). Given an optical path length of 31.7 m and an average noise level of 0.3 %, the detection limits of ephedrine and pseudoephedrine are 12 and 6 µg, respectively.

The measured data points were interpolated with a Smoothing Spline algorithm in both figures (Figs. 4.14 and 4.15) [70]. All observed vibrational
Figure 4.15: C-H stretching absorption band of ephedrine and pseudoephedrine vapor. Quantitative DFG measurements were taken in 100 mbar N₂ at 423 K. The path length of the HTMC was 31.7 m. Ephedrine and pseudoephedrine detection limits are 12 and 6 µg, respectively.
bands $\nu_i$ at 333 K and 423 K are summarized in Table 4.3, along with the OPG-based photoacoustic measurements depicted in Fig. 3.6. The interpolation has a profile that allows most of the bands $\nu_i$ to be determined with an accuracy of 0.1 cm$^{-1}$. Exceptions are indicated separately at the bottom of Table 4.3.

Table 4.3: C-H stretching vibrational bands $\nu_i$ of ephedrine and pseudoephedrine. The FTIR data, the OPG-based photoacoustic measurements (Fig. 3.6) and the DFG-based multipass transmission measurements (Figs. 4.14 and 4.15) are summarized.

<table>
<thead>
<tr>
<th></th>
<th>ephedrine</th>
<th>pseudoephedrine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FTIR</td>
<td>OPG</td>
</tr>
<tr>
<td></td>
<td>333 K</td>
<td>333 K</td>
</tr>
<tr>
<td>$\nu_1$</td>
<td>2855.8</td>
<td>2856.0</td>
</tr>
<tr>
<td>$\nu_2$</td>
<td>2866.6</td>
<td>2885.6</td>
</tr>
<tr>
<td>$\nu_3$</td>
<td>2886</td>
<td>2915.4$^a$</td>
</tr>
<tr>
<td>$\nu_4$</td>
<td>2950</td>
<td>2941.7</td>
</tr>
<tr>
<td>$\nu_5$</td>
<td>2974</td>
<td>2981</td>
</tr>
<tr>
<td>$\nu_6$</td>
<td>3034</td>
<td>3037.2</td>
</tr>
<tr>
<td>$\nu_7$</td>
<td>3070</td>
<td>3073.7</td>
</tr>
<tr>
<td>$\nu_8$</td>
<td>3093.1</td>
<td></td>
</tr>
</tbody>
</table>

$^a\pm 0.5$ cm$^{-1}$; $^b\pm 0.9$ cm$^{-1}$; $^c\pm 0.7$ cm$^{-1}$; $^d\pm 0.2$ cm$^{-1}$

Since ephedrine counts 13 C-H bonds without any particular degree of symmetry, we expect—from a classical point of view—13 modes of vibration of the molecule in the C-H stretching region. From Figs. 4.14 and 4.15, up to 9 bands can be identified between 2815 and 3144 cm$^{-1}$. At least one additional band lies below the range accessible by the ECDL. Weak bands could also be
hidden in the measured infrared spectra. It is not clear whether the observed band $\nu_2$ is actually a wide band superposition of other bands, because it is only present in the DFG spectrum of ephedrine at 333 K. All other bands appear in each of the high-resolution DFG spectra. Sometimes the bands are not strong enough to determine a center frequency with sufficient accuracy, such as $\nu_9$ in Fig 4.15.

Except for $\nu_3$, the bands observed by FTIR match well with those observed by DFG. The bands $\nu_5$ and $\nu_9$ are unresolved as a result of the low FTIR resolution. Furthermore, other bands appear flattened compared to the DFG spectrum taken at 333 K. In fact, the FTIR spectrum should rather be thought of as a convolution between the instrumental resolution and the intrinsic absorption features of ephedrine. (Strictly speaking, the FTIR spectrum is not suitable for comparison as the temperature at which it was recorded is unknown.)

The OPG measurements seem to be shifted by 8–10 cm$^{-1}$ towards higher wavenumbers. The $\nu_5$ and $\nu_6$ bands in the OPG measurements suggest that ephedrine was effectively detected in the photoacoustic cell. It remains, however, unclear why $\nu_7$ and $\nu_8$ were not observed. (Other bands were not within the spectral range of the OPG scan.)

The observed bands in the DFG spectrum of ephedrine taken at 333 K all lie within less than 1 cm$^{-1}$ from the corresponding bands in the spectrum taken at 423 K. The population densities of levels vary with temperature. The intensities of the observed bands vary in turn from 333 K to 423 K. As expected, the infrared spectra of the pseudoephedrine enantiomers overlap. This also gives an idea of the reproducibility of measurements. The infrared spectrum of ephedrine clearly differs from the spectrum of its stereoisomer pseudoephedrine, especially with respect to the band $\nu_6$. The latter is shifted by 9.6 cm$^{-1}$ and is considerably weaker in ephedrine. Together with the derived detection limits in the low-$\mu$g range, the strong discriminating power of the DFG spectrometer has motivated further studies with urine samples. The results of this research is presented in the following chapter.
In the OPG-based measurements, water lines altered the profile of the spectra. It was not certain if some absorption features were due to water or to the doping agent under investigation. Such impurities or solvent effects are also problematic in FTIR recordings. In the DFG recordings presented here, water absorption lines appeared in the form of isolated data points that significantly deviated from the continuous absorption profile. Around 30 of such data points (out of 4000 recorded) could be univocally assigned to water vapor and were deleted from Figs. 4.14 and 4.15. Furthermore, around 2942 cm$^{-1}$, narrow absorption features appeared when pseudoephedrine was kept for several hours in the heated HTMC. This effect is examined more carefully in Fig. 4.16, where a narrow piezo scan was conducted around 2942 cm$^{-1}$ as a function of time. Whereas the background absorption level diminishes with time, narrow unassigned absorption features appear. This may be a re-
sult of pseudoephedrine degradation into smaller molecules at an estimated rate of 1% per hour at 423 K. For this reason, measurements were started shortly after the HTMC reached the desired temperature.
Chapter 5

Urinalysis

5.1 Introduction

The majority of vibrational analyses has targeted the quantitative determination of main urine constituents. Urea, creatinine, uric acid, sulfate, phosphate, glucose, and protein content have been quantified by Raman and mid-IR spectroscopy [87]. The presence of doping agents among all these components usually requires sample preparation and chromatographic separation. In this thesis, prepared ephedrine and pseudoephedrine-positive urine samples are directly investigated with the DFG-based multipass transmission spectrometer described in the previous chapter. The detection of such spatial isomers in urine at relevant concentrations for anti-doping agencies is challenging with respect to the instrumental sensitivity and selectivity that are required.

First, a brief review of urine composition and medical urine tests is given. Based on the vapor-phase spectra of main urine constituents taken in the C-H stretching region, an appropriate preparation method is selected. The vapor-phase spectra of prepared positive and negative (blank) human urine probes are compared to each other.
5.1.1 Urine composition

Urine is a complex aqueous solution whose pH normally varies from 4.5 to 8. Solutes account for about 4% of the total mass. The volume and composition of urine can vary greatly depending on an individual’s diet, physical activity or health, to name a few. Because of these variables, a normal value in urine for each constituent is difficult to establish. In Fig. 5.1, normal ranges were defined by comparing different literature sources [88–92]. Correlations between the individual constituents are not mentioned.

Figure 5.1: Urine composition of healthy subjects. Normal ranges are explicitly given, while average values of mass are represented on the graph.

Although the average daily volume of urine for a healthy individual is 1.2 to 1.5 L, the normal range is about 0.6 to 2 L. The amount of water excreted is usually determined by the body’s state of hydration. The majority of solutes are either waste products of cellular metabolism or products derived directly from certain foods that are eaten. These solutes may be divided into the following categories:

- **Nitrogenous waste products**—Cellular metabolism results in the buildup of nitrogen compounds, which include:
  
  - *Urea* \( (\text{NH}_2\text{CO}) \)—Toxic ammonia produced during the deamination of amino acids is converted to urea. The amount of urea
in urine is related to the quantity of dietary protein. Urea accounts for about 50% of the total mass of solutes. Aged urine is particularly odorous because of the conversion of urea back to ammonia by bacterial action. Normal bacterial flora from the skin may already contaminate urine as it passes from the urethra out of the body.

- **Creatinine** \((\text{C}_4\text{H}_7\text{N}_3\text{O})\)—Creatinine is mainly a breakdown product of creatine phosphate in muscle tissue. It is usually produced by the body at a fairly constant rate, which depends on the muscle mass.

- **Uric acid** \((\text{C}_5\text{H}_4\text{N}_4\text{O}_3)\)—Uric acid is an end-product of the oxidation of purines in the body. Purine-rich food sources include liver, beef kidney or canned fish [93].

- **Dissolved inorganic salts**—The principle inorganic ions in urine are chloride \((\text{Cl}^-)\), sodium \((\text{Na}^+)\), potassium \((\text{K}^+)\), phosphate \((\text{PO}_4^{3-})\), sulphate \((\text{SO}_4^{2-})\) and ammonium \((\text{NH}_4^+)\). They make up for more than one third of the total mass of solutes.

- **Glucose**—Glucose \((\text{C}_6\text{H}_{12}\text{O}_6)\) in the blood is freely filtered by the glomeruli, the basic filtration units of the kidney. It is then reabsorbed by the renal tubules, so that urine normally contains only small amounts of glucose. The tubular reabsorption of glucose is a threshold-limited process. When the level of glucose in the blood exceeds its renal threshold level, the glucose spills over into the urine. High glucose levels in urine (glucosuria) may be caused by a renal condition, or by a prerenal condition, i.e. high blood glucose levels (hyperglycemia). Diabetes is the most common disease that results in hyperglycemia and glucosuria.

- **Proteins**—High-molecular-weight proteins (> 90000) are unable to penetrate the glomerular filtration barrier. Proteins of low molecular
weight (< 40000) pass through the glomeruli, but are normally reab-sorbed by the renal tubules. The tubular reabsorption of proteins is a non-selective, threshold-limited process so that, similarly to glucose, only small amounts of proteins are found in urine. In normal urine, they comprise about one third albumin and two thirds small globulins. Human albumin has a moderate molecular weight and, therefore, a limited ability to pass the filtration barrier. Its high plasma concentration accounts for its presence in urine. Increased amounts of protein in urine (proteinuria) can be caused by a renal condition, a prerenal condition or even a postrenal condition, where the urinary tract itself produces proteins. Depending on the condition, different proteins are found in urine.

- **Ketone bodies**—Normally, the end products of fatty acid metabolism are adenosine triphosphate, carbon dioxide and water (Krebs cycle). When carbohydrate availability is limited, fat metabolism increases. The Krebs cycle becomes overwhelmed and large amounts of ketone bodies are released in the blood (ketonemia). When blood ketone concentrations exceed the renal threshold level, ketones are excreted in the urine (ketonuria). The average distribution of ketone bodies in serum and urine is 78 % β-hydroxybutyrate, 20 % acetoacetate and 2 % acetone. In addition to ketonuria, trace acetone concentrations are found in human breath. (This issue is addressed in Sect. 4.5.2.) Causes of ketonuria can be the inability to use carbohydrates, an inadequate carbohydrate intake or the loss of carbohydrates. Diabetes is by far the most common clinical condition responsible for ketonemia and ketonuria. In this case, the body cannot use available carbohydrates.

The characteristic yellow color of normal urine is principally due to the presence of urochrome. This pigment is just one of many other substances present in too small quantities to be listed in Fig. 5.1. Finally, other solutes may be due to chemicals that originate from drug intake. Detecting such substances among all the constituents of normal urine is the complex task
addressed by forensic urinalysis.

5.1.2 Medical and forensic urinalysis

Examination of urine specimens (urinalysis) may be conducted to screen for pregnancy, infections, diseases or drugs of abuse. Depending on the application, various types of tests are performed. They may be divided into the following categories:

- **Medical urinalysis**—Urinalysis is one of the most common methods of medical diagnosis, which typically includes:

  - *Physical examination of urine*—This study is the oldest clinical test still being performed. Historically, urine was qualitatively evaluated by its color, clarity, odor, and taste. Urine tasting has not been performed for several centuries, but the evaluation of the other physical properties continues to play an important role in routine urinalysis. Furthermore, if urine is agitated sufficiently, foam develops at its surface due to the presence of protein. This characteristic of urine is also investigated today along with the concentration of solutes, which is often expressed as specific gravity.

  - *Urine dipstick chemical analysis*—A urine dipstick is a paper or plastic strip on which reagent-impregnated test pads are fixed. Once the strip has been dipped in urine, a chemical reaction causes the pad to change color. This micro-chemical system allows a semi-quantitative analysis by simple observation of color change. Commercial reagents strips screen urine for protein, glucose, ketones, blood, bilirubin, urobilinogen, nitrite, and leukocyte esterase [89]. In addition, the pH and the specific gravity may be determined. A reagent strip is typically less sensitive than a forensic test. It is also less selective and, therefore, more prone to false
positive or false negative results. This is, however, the most cost-effective and rapid way to screen urine for its basic constituents. Some examples of urine dipsticks are given in Table 5.1.

- **Microscopic urinalysis**—A 10–15 mL sample of a well-mixed urine specimen is centrifuged in a test tube. The supernate is decanted until a volume of 0.2–0.8 mL is left. The sediment is resuspended in the remaining supernate. With a calibrated pipette, a 5–20 µL volume is withdrawn for examination under a conventional light microscope. The whole procedure is standardized in commercial urinalysis systems to ensure reproducibility. A wide range of elements may be encountered in the microscopic examination of urine such as red blood cells, white blood cells, epithelial cells, casts, bacteria, or yeast. The various types of elements in the field of vision are counted and values for the whole sample are extrapolated (many fields may be viewed and averaged for better accuracy). These elements can originate from throughout the urinary tract or result from contamination. Not all elements indicate a pathologic process, but the presence of certain large amounts is diagnostically significant. Microscopic urinalysis is a semi-quantitative method that continues to play an important role in the initial diagnosis of a renal disease or in monitoring its progression.

- **Quantitative forensic urinalysis**—Drug screening in urine requires quantitative forensic urinalysis. Such tests are not part of a routine medical urinalysis. They are conducted, e.g., to test for the presence of recreational drugs or, in sports, to detect doping agents. Depending on the substance, typical detection periods are 1–4 days in urine. In blood, most of the abused drugs, including their metabolites, decay to low levels a few hours after intake. Long detection periods of up to several months may be found in hair, but concentrations are also lower. Standard methods of detection are gas chromatography coupled to mass spectrometry (GC/MS) and high-performance liquid chromatography.
(HPLC). In the following sections, the feasibility of a novel laser-based method for forensic drug testing is investigated.

Table 5.1: Sensitivity and specificity of selected Chemstrip urine test strips (Roche Diagnostics).

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>400 mg/L</td>
<td>affected by high specific gravity</td>
</tr>
<tr>
<td>Protein</td>
<td>60 mg/L</td>
<td>more sensitive to albumin than other proteins;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>false results if pH $\geq 9$ or</td>
</tr>
<tr>
<td></td>
<td></td>
<td>in the presence of certain compounds</td>
</tr>
<tr>
<td>Ketone</td>
<td>acetoacetate:</td>
<td>does not detect $\beta$-hydroxybutyrate;</td>
</tr>
<tr>
<td></td>
<td>90 mg/L</td>
<td>false results in highly pigmented urines or</td>
</tr>
<tr>
<td></td>
<td>acetone:</td>
<td>in the presence of certain compounds.</td>
</tr>
<tr>
<td></td>
<td>700 mg/L</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ in 90 % of urine samples tested

5.2 Infrared spectroscopy of urine constituents

The high water content of biological fluids strongly limits direct mid-infrared spectroscopic measurements. For example, in the spectral region accessible by the DFG laser, measurements would be completely hindered if only 10 $\mu$L water were evaporated in the HTMC. Assuming a cell volume of 2.0 liters, this would correspond to 10 % water vapor buffered in an ideal gas at 423 K and 100 mbar total pressure. Such conditions are simulated with Hitran in Fig. 5.2. As clearly seen, there is hardly any spectral window free from strong
Figure 5.2: Hitran simulation of 10% water vapor buffered in nitrogen at 423 K and 100 mbar total pressure. The optical path length is 31.7 m.

water vapor absorption lines. Consequently, initial urine measurements were recorded on dried samples.

Dried human urine was obtained by means of lyophilization (Medichem, Steinenbronn, Germany). In a first series of measurements (Fig. 5.3), 3 mg of lyophilized urine were placed in the HTMC vessel. The vessel was sealed to the cell and the latter was evacuated and filled again with 70 mbar nitrogen at room temperature. Measurements were recorded after heating the vessel and the cell to 333, 398 and 423 K, respectively. Another measurement was taken at 423 K after having introduced a 10 times higher amount of lyophilized urine in the vessel. Each time, the mirrors were kept at a slightly higher temperature than the rest of the cell to prevent condensation on the optical surface. The pathlength was 31.7 m. The DFG source was tuned over 244 cm$^{-1}$ with 0.4 cm$^{-1}$ steps using only the 29.9 µm grating period.
Figure 5.3: Vapor-phase infrared spectra of lyophilized human urine samples. Single scans were recorded in 94 mbar N\textsubscript{2} at 398 K and in 100 mbar N\textsubscript{2} at 423 K. The path length of the HTMC was maintained at 31.7 m.
The vapor-phase spectra of 3 mg dried urine taken at 398 and 423 K exhibit narrow absorption lines, especially above 3050 cm$^{-1}$. With the help of Hitran, they could be assigned to more than 2000 ppm water vapor in the cell, and to more than 4000 ppm ammonia. When 31 mg of dried urine were heated to 423 K, more than 7000 ppm H$_2$O and about 3 % NH$_3$ accounted for the observed absorption lines in Fig. 5.3. Despite the tuning step width of 0.4 cm$^{-1}$, these narrow absorption features could be resolved—unlike in a conventional FTIR acquisition—thanks to the narrow line width of the laser. Piezo scans over absorption lines were not conducted. The narrow features observed in Fig 5.3 are merely due to single data points. Such data points may be centered on absorption lines, but may also be situated at the wings of such absorption lines. Therefore, one can only derive a lower limit for the concentration of an absorbing species in the cell. By comparing several data points, one can, however, get a fairly good idea of the concentration of an absorbing species without performing a piezo scan.

Besides narrow lines, a broad absorption feature appears between 2900 and 3010 cm$^{-1}$. This band is not present at 333 K, but appears at 398 and 423 K. It may be due to the absorption of an organic molecule that has a melting point between 333 and 398 K.

The measurement at 333 K (not pictured in Fig. 5.3) does not reveal any particular absorption features, apart from three isolated lines due to less than 1000 ppm water vapor. As mentioned on page 59, the cell is shortly evacuated before introducing pure N$_2$ and up to 1000 ppm water vapor may subsist in routine measurements. Therefore, the water vapor, at least at 333 K, does not necessarily come from the urine sample itself. However, the increased water vapor concentration at 398 K may originate from the evaporation of residual water left after the lyophilization process. Ammonia, a gas at room temperature, is not observed at 333 K. It must be due to the breakdown or thermal degradation of a urine constituent. From these initial measurements, we can conclude that ammonia is the limiting factor when recording vapor-phase mid-infrared measurements on dried urine samples.
To understand the origin of this ammonia, further studies were undertaken on urea, the main solute of urine.

Pure urea (≥ 99.5 %) was purchased from Sigma-Aldrich. It accounts for about 50 % of the total mass of solutes in urine. Hence, 15 mg were introduced in the vessel, in order to reproduce the amount expected in the 31 mg of dried urine (Fig. 5.3). The measurement procedure, the wavelength coverage and the step width were all the same as employed for measurements with dried urine. The urea vapor-phase spectra taken at 398 K and 423 K are pictured in Fig. 5.4.

Urea does not have any C-H bond; its infrared spectrum does not feature any intrinsic absorption band in the spectral region covered by Fig. 5.4 [94]. Besides a fringe artifact between 2960 and 3040 cm$^{-1}$, the measurement at 398 K only exhibits some weak absorption lines. For example, the measured line at 3064.42 was assigned to a water vapor line situated at 3064.404 cm$^{-1}$. It is due to a residual concentration of 400 ppm in the cell. Another line, measured at 3054.34 cm$^{-1}$, could not be assigned to any molecule listed in the Hitran database. In the measurement taken at 423 K, further lines, e.g., measured at 3054.33 or 3080.35 cm$^{-1}$, could not be assigned. Ammonia absorption lines appear as well, in addition to the regular water vapor absorption lines. For example, the measured lines at 2979.97, 2985.31, 3127.13, 3138.88, and 3143.17 cm$^{-1}$ could all be assigned to the ammonia lines centered at 2979.943, 2985.272, 3127.100, 3138.831, and 3143.125 cm$^{-1}$, respectively. The small discrepancies between the wavenumber of measured and assigned lines are within 0.05 cm$^{-1}$. As explained previously, only one data point per line is available in a wide scan. Therefore, the lines pictured in Fig. 5.4 are not necessarily centered on gaseous transitions. Deviations depend on the line profiles, which are not resolved in Fig. 5.4. Furthermore, the wavelength of the YAG is not permanently controlled, unlike the wavelength of the ECDL. The DFG idler wavenumber may in turn be shifted by less than 0.04 cm$^{-1}$ [32].

Urea pyrolysis has been extensively studied in literature [95–98]. No
significant reaction takes place when urea is heated from room temperature to its melting point (mp), situated at 406 K. Around 425 K, it is generally accepted that urea thermally decomposes into ammonia and isocyanic acid as follows:

\[(\text{NH}_2)_2\text{CO} \text{ (l or g)} \rightarrow \text{NH}_3 \text{ (g)} + \text{HNCO} \text{ (g)}\].

(5.1)

This could explain the significant amount of ammonia measured when urea was heated up to 423 K in 100 mbar N\(_2\). By comparing the intensity of
several lines, it was found that the concentration of ammonia in the cell was ≥ 1.6 %, i.e. the total amount in the 2.0 L volume was ≥ 9.1 \cdot 10^{-5} moles. Therefore, of the 15 mg urea (25.6 \cdot 10^{-5} moles), at least 35 % decomposed. The decomposition product isocyanic acid is not listed in the latest Hitran edition, but has been the subject of other rotational-vibrational studies [99, 100]. The latter do not report significant isocyanic acid absorption in the spectral region covered by Fig. 5.4. However, the elevated HNCO concentration in the cell and the long absorption path length could explain the presence of unassigned weak HNCO absorption lines. One should also be careful not to confuse isocyanic acid (HNCO) with its less stable isomer cyanic acid (HOCN). A good insight on stable CHNO species can be found in literature [100].

Other major urine organic constituents—including creatinine, uric acid, glucose and human albumin—were investigated spectroscopically at 423 K. Uric acid does not have any C-H bond and did not reveal any absorption features in the spectral region covered by Fig. 5.4. The texture of creatinine (mp = 528 K) and human albumin after heating up to 423 K did not show any signs of decomposition. Glucose has a melting point around 423 K. Creatinine, human albumin and glucose had too low vapor pressures at 423 K to produce any significant absorption when 1 mg of each constituent was introduced in the HTMC vessel.

In summary, vapor-phase infrared analysis of human urine in the C-H stretching region is primarily limited by the two main urine constituents, i.e. water vapor and urea. Water has strong absorption lines throughout the spectral range accessible by the DFG source, while urea thermally decomposes into ammonia and isocyanic acid. An extraction technique is therefore required for forensic analysis of urine samples.
5.3 Liquid-liquid extraction

Sample preparation is an essential step, because most analytical instruments cannot handle a sample matrix directly. In the previous section, we saw that an extraction method is required to separate water and urea. Current sample preparation procedures rely on standard liquid-liquid extraction (LLE), solid-phase extraction (SPE) [101], solid-phase microextraction (SPME) [102,103], and stir bar sorptive extraction (SBSE) [104]. While LLE, also known as solvent extraction, is a method to separate compounds based on their solution preferences for two immiscible liquids, SPE is based on the preferential affinity of desired or undesired solutes for a solid material. As extraction medium, SPME uses a thin layer of polydimethylsiloxane (PDMS) on the outside of a needle device. After sorption, the compounds are thermally desorbed in the inlet port of an analytical instrument. SBSE is a similar technique based on PDMS-coated stir bars. The amount of PDMS is typically ≤ 0.5 µL and 50–300 µL in SPME and SBSE, respectively. In the following discussion, the potential of liquid-liquid extraction as a sample preparation technique for ephedrine-positive urine is investigated.

Let us assume a volume $V_{aq}$ of water (urine). We propose to extract a solute (ephedrine) with a volume $V_s$ of organic solvent. The partition coefficient $P$ is the ratio of concentrations of un-ionized solute between the two phases. For ionizable systems, such as the majority of drugs found in urine, the correct descriptor is the $pH$-dependent distribution coefficient $D$. If $N_{aq}$ and $N_s$ are the number of all forms of solute (ionized plus un-ionized) in water and in the extraction solvent, $D$ is given by

$$D = \frac{N_s}{N_{aq}/V_{aq}}. \quad (5.2)$$

When the solute is extracted with a single large batch of solvent, we can easily show that the recovery $R$ is related to $D$ according to

$$R = \frac{N_s}{N_s + N_{aq}} = \frac{\alpha D}{1 + \alpha D}, \quad \text{where} \quad \alpha = \frac{V_s}{V_{aq}}. \quad (5.3)$$
Multiple extractions using \( m \) equal volumes of solvent batches yields a better recovery, given by

\[
R = 1 - \left( \frac{m}{m + \alpha D} \right)^m.
\] (5.4)

At a given \( p\text{H} \), \( \log D \) is estimated from \( \log P \) according to

\[
\log D_a = \log P - \log \left(1 + 10^{\pm(p\text{H} - pK)}\right),
\] (5.5)

where \( pK \) is the acid dissociation constant, and where \( D_a \) and \( D_b \) are the distribution coefficients for acids and bases, respectively.

In Fig. 5.5, water-octanol distribution coefficients \( D \) are pictured as a function of the \( p\text{H} \), using \( \log P \) and \( pK \) values compiled in Table 5.2. The situation is somewhat more complex in reality, because some compounds have different ways of dissociation with respective \( pK \) coefficients. Only one \( pK \) per urine constituent is given in Table 5.2, merely to provide recovery estimates at alkaline \( p\text{H} \). Thereby, we can see how solvent extraction at alkaline \( p\text{H} \) is particularly appropriate to recover ephedrine-like drugs without any detectable amount of urea. A certain amount of creatinine may also be

Table 5.2: Reported acid dissociation constants \( pK \) and octanol-water partition coefficients \( P \) of ephedrine and main organic urine constituents. After \( m = 8 \) solvent extractions with a total amount of 20 mL octanol, the relative and absolute recovery \( R \) and \( m_{\text{max}} \) are estimated for a 10 mL alkaline urine sample (\( p\text{H} = 14 \)). We assume a maximum concentration of each constituent in urine, within normal ranges given by Fig. 5.1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>( pK )</th>
<th>ref.</th>
<th>( \log P )</th>
<th>ref.</th>
<th>( R ) [%]</th>
<th>( m_{\text{max}} ) [( \mu \text{g} )]</th>
</tr>
</thead>
<tbody>
<tr>
<td>ephedrine</td>
<td>9.52</td>
<td>[105]</td>
<td>1.13</td>
<td>[106]</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>urea</td>
<td>0.10</td>
<td>[105]</td>
<td>-2.11</td>
<td>[107]</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>creatinine</td>
<td>9.2</td>
<td>[108]</td>
<td>-1.77</td>
<td>[109]</td>
<td>33 ( \cdot ) ( 10^{-3} )</td>
<td>1000</td>
</tr>
<tr>
<td>uric acid</td>
<td>10.60</td>
<td>[105]</td>
<td>-2.17</td>
<td>[110]</td>
<td>13 ( \cdot ) ( 10^{-3} )</td>
<td>134</td>
</tr>
<tr>
<td>glucose</td>
<td>12.34</td>
<td>[105]</td>
<td>-3.02</td>
<td>[111]</td>
<td>2 ( \cdot ) ( 10^{-3} )</td>
<td>6</td>
</tr>
</tbody>
</table>
recovered. However, we do not expect any measurable amount in the vapor phase below a temperature of 528 K, as seen in the previous section.

Preliminary studies served to select the most suitable solvent and the optimal number of solvent batches for extraction. Preparation of urine samples was as follows:

- **Collection of 10 mL of human urine**—Fresh human urine samples came from well-known personnel claiming abstinence from drugs; they were prepared shortly after collection. For comparison purposes, reconstituted lyophilized human urine samples (Medichem, Steinenbronn, Germany) were also employed. The advantage of dried human urine is that it may be refrigerated and reconstituted simply by adding water. Larger urine volumes result in larger extracted amounts. In general, urine is screened for different compounds. When a urine specimen is sent to a forensic laboratory, it is divided into several batches; each batch undergoes a specific preparation. The volume of a single batch must therefore not exceed 10 or 20 mL.
- **Adjustment to alkaline pH**—The pH of urine, normally situated between 4.5 and 8, was increased to more than 12 by adding NaOH 10 M.

- **Multiple extraction with 8 batches of 2.5 mL chloroform (20 mL in total)**—Water-octanol and water-chloroform partition coefficients of ephedrine are similar [106]. Another frequently used extraction solvent is tert.-butyl methyl ether (boiling point = 55 °C) [112, 113]. Chloroform was chosen instead, because it has a lower solubility in water (8 g/L instead of 50 g/L) and is as easy to evaporate (bp = 61 °C). Furthermore, chloroform does not present any significant absorption feature in the C-H stretching region, unlike ethers. This has also been confirmed by a preliminary measurement on pure chloroform.

- **Centrifugation at 2000 g during 2 min**—The water-chloroform interface is not clearly defined. The presence of albumin in urine is responsible for an intermediary layer. The latter was investigated separately and proved to be chloroform dispersed in water (emulsion). This emulsion was broken by centrifugation.

- **Solvent evaporation and transfer to a sample holder**—The 8 batches of extraction solvent were combined with the chloroform recuperated from the emulsion. The chloroform was then slowly evaporated. The last few drops were transferred to a small sample holder designed to fit inside the HTMC vessel. Measurements were undertaken after the chloroform had completely evaporated in ambient air.

## 5.4 Ephedrine and pseudoephedrine screening in human urine

Blank human urine matrices came from reconstituted lyophilized or fresh specimens. Positive samples were obtained by adding the desired amount of analyte in a blank. Negative (blank) and positive urine specimens un-
derwent the same preparation procedure described in the previous section. The vessel containing the prepared probe was sealed to the HTMC. The cell was evacuated at room temperature below $10^{-3}$ mbar for a few minutes, thereby evaporating eventual chloroform or water traces that remained in the prepared probe. The cell was filled again with 70 mbar N$_2$ and heated up to 423 K, always keeping the mirrors at a slightly higher temperature to avoid condensation on their optical surface. Vapor-phase spectra were recorded between 2900 and 3025 cm$^{-1}$ with steps of 0.4 cm$^{-1}$. The total optical path length was 31.7 m. The wide tuning range encompassed the $\nu_6$ vibrational band of ephedrine, which is clearly distinguishable from the $\nu_6$ band of pseudoephedrine. As seen in Fig. 4.15 and listed in Table 4.3, it is centered at 2970.5 and 2980.1 cm$^{-1}$, respectively.

Measurements on prepared negative (blank) and positive urine samples are compared to each other in Fig. 5.6. Urine matrices were collected from well-known personnel briefly before preparation. For further comparison, an ephedrine-positive probe (348 $\mu$g/mL) was prepared in reconstituted lyophilized human urine. The spectra pictured in Fig. 5.6 do not depend on the type of human urine.

According to current WADA regulations [12], ephedrine is illegal in sports when its concentration in urine is $\geq 10$ $\mu$g/mL. Ephedrine-positive probes were prepared at concentrations that were one order of magnitude above the legal limit. Within this range, the spectra of prepared ephedrine-positive urine samples clearly have stronger absorption features than those of a blank. The spectra also exhibit a characteristic peak at 2969.4 $\pm$ 1 cm$^{-1}$, mainly due to the $\nu_6$ band of ephedrine. A pseudoephedrine-positive urine sample was prepared as well. It reveals strong absorption features. The main peak, situated at 2980.0 $\pm$ 0.5 cm$^{-1}$, is in good agreement with measurements taken on pure pseudoephedrine (Fig. 4.15). Therefore, the two diastereoisomers are distinguishable even in urine.

In contrast to the discussion presented in the previous section, a residual absorption is present in the blank. Its intensity varies among the different
Figure 5.6: Vapor-phase spectra of prepared positive and negative (blank) human urine samples. Single scans were taken in 100 mbar N₂ at 423 K. The spectrum of the blank exhibits fringe artifacts. The path length of the HTMC was 31.7 m. The DFG laser was tuned with steps of 0.4 cm⁻¹.
Figure 5.7: The vapor-phase spectra of two negative (blank) human urine samples are compared to the spectrum of an aqueous solution of urea. Before recording the spectra, each probe underwent the same preparation procedure described in Sect. 5.3. The spectra were scaled with respect to the y-axis only. When required by the presence of fringes, the data were interpolated with a Smoothing Spline algorithm [70]. Experimental conditions were the same as in Fig. 5.6.

e urine specimens collected, but its main peak, situated at 2966.3 ± 0.5 cm⁻¹, is reproducible. By means of home-made synthetic urine preparations, the origin of this absorption was investigated. In Fig. 5.7, it appears that an aqueous solution of urea (25 g/L as in normal urine) has the same effect as a blank in terms of absorption, whereas a prepared alkaline water solution does not produce any measurable absorption. Based on the results presented in Fig. 5.4, it is unlikely that the measured spectrum of a blank originates from urea itself. The product of a reaction involving urea and, possibly, NaOH or
Table 5.3: Extracted and evaporated masses $m_{\text{extr.}}$ and $m_{\text{evap.}}$ after preparation of human urine samples. The spectra of ephedrine-positive (EPH) and pseudoephedrine-positive (PEPH) samples were subtracted by the spectrum of the blank, which was taken for each urine specimen prior to adding the doping agent. The recovery was then determined after comparison with quantitative measurements taken in Fig. 4.15.

<table>
<thead>
<tr>
<th>blank</th>
<th>positive human urine</th>
<th>recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m_{\text{extr.}}$</td>
<td>$m_{\text{evap.}}$</td>
<td>solute</td>
</tr>
<tr>
<td>± 0.2 mg</td>
<td>± 0.2 mg</td>
<td></td>
</tr>
<tr>
<td>1.7</td>
<td>0.9</td>
<td>EPH</td>
</tr>
<tr>
<td>1.2</td>
<td>0.7</td>
<td>EPH</td>
</tr>
<tr>
<td>0.7</td>
<td>0.5</td>
<td>EPH</td>
</tr>
<tr>
<td>1.2</td>
<td>0.7</td>
<td>PEPH</td>
</tr>
</tbody>
</table>

chloroform, seems rather to be the cause of the unknown substance extracted from a blank.

For each preparation, the mass extracted from 10 mL urine and the mass evaporated in the HTMC were measured with a balance. The recovery may be calculated by comparing the mass extracted from a blank with the mass extracted from a positive urine sample. In certain cases, it yields more than 100 % recovery, because the adjunction of an additional solute in a solution may affect the recovery of other solutes. For this reason, the recovery was determined with the absorbance values measured in Figs. 5.6 and 4.15 instead. We assume thereby that the additional absorbance in the case of a positive sample is solely due to the doping agent. Results are summarized in Table 5.3.

After a measurement on one ephedrine-positive sample (258 µg/mL), a small ephedrine absorption was left in the subsequent background measurement taken in pure N$_2$, because the HTMC was insufficiently evacu-
ated between both recordings. As a result, the corresponding spectrum in Fig. 5.6 has an absorption level that is 5% lower than what should have been achieved. The recovery in Table 5.3 is corrected accordingly, but the raw data is pictured in Fig. 5.6.

Given an instrumental noise level of 0.3% and an optical path length of 31.7 m, the absolute detection limit of pure ephedrine is 12 µg (Fig. 4.15). With an average ephedrine recovery of 44 ± 3%, the detection limit in urine would be, in principle, 2.7 and 1.4 µg/mL if 10 mL and 20 mL urine were prepared, respectively. In practice, the absorption level of a blank, however, limits the achievable detection limit to 50 and 25 µg/mL, respectively.

After solvent extraction of an ephedrine-positive urine sample, the chloroform and urine phases were allowed to rest for a few minutes until separation.

Figure 5.8: Vapor-phase spectrum of the water-chloroform emulsion, obtained after solvent extraction of an ephedrine-positive human urine sample. Experimental conditions were the same as in Fig. 5.6.
A metastable substance was observed between the two immiscible phases. For the purpose of an independent measurement, this intermediary substance was extracted separately and transferred to the vessel. After evaporation in ambient air, a vapor-phase infrared spectrum, pictured in Fig. 5.8, was recorded at 423 K. It features an ephedrine absorption band that is heavily altered by the presence of more than 1.2 % water in the cell. Given the relatively wide step width of 0.4 cm$^{-1}$, only a few measured data points actually coincide with water absorption lines. Nevertheless, the nature of the intermediary substance becomes apparent: it consists of a water-chloroform emulsion, as already mentioned on page 90. The chloroform evaporates readily at ambient pressure, leaving the ephedrine it contains in the sample holder. The amount of water is too important to evaporate completely, even after evacuating the cell for a few minutes. For better ephedrine recovery, the chloroform dispersed in water was extracted after centrifugation in all measurements presented earlier.
Chapter 6

Conclusions

Novel infrared laser-based instruments were successfully developed to detect doping agents in the vapor phase. They consist in (a) an OPG-based photoacoustic spectrometer and (b) a newly-designed DFG-based transmission spectrometer with a high-temperature multipass cell (HTMC).

The OPG produces several mW of mid-IR radiation in the fingerprint region between 3 and 4 µm, where molecular C-H stretching vibrations take place. A wavenumber range of 300 cm\(^{-1}\) can be continuously scanned by tuning the temperature of a PPLN only. Vapor-phase infrared spectra of pure low-melting point (< 160 °C) solid organic compounds were recorded in a heated photoacoustic cell, stabilized at 60 °C. A representative of each main doping substance class was investigated including metoprolol tartrate (beta-blocker), methandienone (anabolic), etacrylic acid (diuretic), nikethamide and mephentermine sulfate (stimulants). They all show specific absorption bands between 2800 and 3100 cm\(^{-1}\). The sensitivity decreases drastically when trying to measure higher melting point (> 160 °C) substances with even lower vapor pressures. Further measurements were taken on Ephedra alkaloids. The optical resolution of 8 cm\(^{-1}\) is good enough to distinguish closely related chemical structures, such as ephedrine and methylephedrine. Nevertheless, it is too poor to differentiate between ephedrine and pseudoephedrine, which differ only by their geometrical configuration around one
chiral carbon center. Furthermore, the presence of water complicates the data evaluation in spectral regions of strong water absorption, because the linewidth of the laser is much broader than single doppler-resolved roto-vibrational transitions. The OPG-based photoacoustic spectrometer is a simple and cost-effective way to record vapor-phase spectra of solid-organic compounds. However, the risk of contamination due to condensation inside the photoacoustic cell is important, because the cell cannot be heated above 60 °C, the maximum permissible temperature of high-responsivity miniature microphones.

The DFG employs a unique combination of a Q-switched Nd:YAG laser as pump, and a cw external-cavity diode laser as signal, both operating at room temperature. The pump and signal laser beams are mixed in a quasiphased matched PPLN. The DFG has an improved linewidth of only 150 MHz ($5 \cdot 10^{-3}$ cm$^{-1}$). In gas sensing applications, such devices are usually tuned over 1-2 cm$^{-1}$ as a result of a simple piezo scan. To meet our requirements, the continuous tuning range of the DFG laser is extended to 329 cm$^{-1}$ by simultaneously tuning the PPLN temperature and the step motor of the external cavity. The tuning mechanism is fully automated. Vapor-phase infrared spectra are recorded in a home-built high-temperature multipass cell (HTMC). The HTMC is a novel type of compact long-path absorption cell that enables sensitive spectral measurements at adjustable temperatures in a wide temperature range from room temperature up to 723 K. The mirrors are heated separately and may be kept at a higher temperature than the rest of the cell, in order to prevent condensation on their optical surface. The HTMC parts are thermally interconnected such that no cold spots inside vacuum components appear. Therefore, measurements on condensable vapors are feasible, in addition to measurements on heated gases. Furthermore, optical adjustments, e.g. in order to vary the optical pathlength, may be performed under any thermal or vacuum condition without dismantling the cell. Once adjustments are made, thermal expansion is mechanically compensated. This mechanism has proven to be essential for preventing fatal
optical misalignments when exposing the HTMC to significant temperature changes. The HTMC was thoroughly tested under various conditions for several months and its feasibility was demonstrated by obtaining methane and water vapor absorption spectra at room temperature and high temperature in the mid-infrared spectral range between 3 and 4 µm.

The high-temperature multipass cell combined with the widely continuously tunable DFG source offers a unique analytical tool for probing liquid and solid organic samples in the vapor phase. The wide tuning capabilities of the DFG laser were demonstrated by recording the C-H stretching absorption band of acetone vapor at 296, 400 and 500 K. In order to unveil the large selective potential of the spectrometer, quantitative measurements, reported for the first time, were taken on the diastereoisomeric pair ephedrine and pseudoephedrine. Despite featuring similarities, the vapor-phase IR spectra of ephedrine and pseudoephedrine can clearly be distinguished with respect to a vibrational band centered at 2970.5 and 2980.1 cm\(^{-1}\), respectively. The detection limits (SNR = 3) of pure ephedrine and pure pseudoephedrine are 12 and 6 µg, respectively. Surprisingly, the absorption features of these two stimulants are as strong as the main C-H stretching vibrational peak of acetone (\(\tilde{\sigma} = 1.5 \cdot 10^{-19} \text{ cm}^2\)). The cross section of the main pseudoephedrine peak, centered at 2980.1, is even \(2.5 \cdot 10^{-19} \text{ cm}^2\) at 423 K. While Ephedrine has been banned from sports for its stimulatory effects, pseudoephedrine, which is rather inactive, is considered as legal. The World Anti-Doping Code prohibits ephedrine when its concentration in urine is greater than 10 µg/mL. A sensitive and selective analytical method is therefore required to differentiate these two isomers in urine.

Preliminary studies have shown that direct examination of urine in the DFG-based multipass transmission spectrometer is hindered by the strong water content of urine and the thermal degradation of urea into ammonia and isocynanic acid. For this reason, urine samples were first prepared by means of liquid-liquid extraction, than evaporated in the HTMC. Ephedrine and pseudoephedrine-positive human urine samples were prepared at concen-
trations that were one order of magnitude above the legal limit of ephedrine. With an average ephedrine recovery of 44% after solvent extraction, the detection limit of ephedrine in human urine is 50 and 25 µg/mL, when 10 mL and 20 mL urine are prepared, respectively. The detection limit is limited by the absorption level of a negative (blank) sample. In principle, the detection limit can be improved to approximately 1 µg/mL with an appropriate sample preparation procedure. The vapor-phase IR spectra of prepared pseudoephedrine and ephedrine-positive urine samples are consistent with the previous results obtained on pure samples. A laser-based analytical method has thus been achieved to differentiate between these two isomers in urine at relevant concentrations.

Many publications emphasize the merits of state-of-the-art instrumentation, while neglecting to mention some significant limitations. The skilled scientist is left to his own to unearth the critical issue that would render the allegedly promising instrumentation inapplicable. For the avoidance of figuring among the growing list of such works, the laser-based spectrometer developed in this thesis is fairly evaluated on the basis of comparative criteria introduced on page 8:

- **Sensitivity**—The method developed in this thesis could already serve—in its present state—as a complementary tool to confirm certain positive findings, using the “B” probe of an athlete. The majority of doping agents, however, must be detectable in urine at ng/mL concentrations, requiring 3 orders of magnitude lower detection limits. The laser spectrometer has still room for much improvement: other sample preparation methods can be investigated; the length/volume ratio of the cell can be optimized; the noise level, of the order of 0.3 %, is not inherently limited by the method, but by temperature deviations of the PPLN only. Given the current noise level and a maximum path length of 34.9 m inside the cell, the minimum detectable absorption coefficient (SNR=3) is \(2.6 \times 10^{-6} \text{ cm}^{-1}\), as derived on page 58.

- **Selectivity**—The main advantage of infrared spectroscopy resides in
its fingerprinting ability. Isomer discrimination is more accurate than in mass spectrometry. In the presence of drug mixtures, chromatographic separation could, however, turn out to be necessary.

- **Speed**—With a mid-IR source that does not rely on temperature tuning of an optical element, the speed of a wide scan could be considerably reduced. The total time to analyze a urine probe would ultimately be limited by the sample preparation procedures which, for the time being, seem inevitable.

- **Portability**—The reputation of laser systems as portable devices is justified. The whole system presented in Sects. 2.2 and 4.3.3 could indeed fit in a suitcase. Further miniaturization would require a direct mid-IR laser source such as an external cavity QCL.
Chapter 7

Further applications

In high-resolution spectroscopy, high-temperature measurements give the opportunity to study hot band transitions and the temperature dependence of pressure-induced line broadening and line shifts. Measurements at different temperatures can also facilitate line assignments. Such measurements are welcome to improve current databases, which often rely on semi-empirical data for hot band predictions. This would in turn provide valuable information to the astronomical community, particularly in connection with atmospheric studies of Venus (surface temperature 733 K) or of extrasolar planets [114].

An HTMC could present a competitive advantage wherever trace gases must be analyzed in the presence of condensable vapors such as in volcano emissions, exhaust fumes, human breath or smoke produced during electro surgery, to name a few. Waste incinerators, for example, emit trace gases that must be monitored in an environment consisting typically of 15% water vapor at 600 K and 1 atm [115]. Other harsh environments requiring a cell that can withstand elevated temperatures are found in aircraft turbine gas exhausts [116]. The standard procedure for certification purposes is described in regulations of the International Civil Aviation Organization [117]: to avoid condensation of water and volatile hydrocarbons between the sampled exhaust probe and the analytical instrument, transfer lines must be
maintained at 423 K. Industrial applications implementing an HTMC are also conceivable. Whether in a petrochemical plant, a gas turbine, a combustion engine or a fuel cell [118], there are many processes that require robust analytical instruments. In the semiconductor manufacture industry, the moisture background level is one of the main limitations of current analytical systems employing multipass cells [119]. Because water quite strongly adsorbs on metallic surfaces due to its polarity, this contaminant cannot be removed by simply purging the cell with a dry inert gas. Desorption at elevated temperatures could, however, provide an effective alternative.

In the food industry, infrared spectral analysis of liquid fats, oils or beverages is a widespread technique [120]. Despite its success, the full discriminatory potential of infrared spectroscopy is often hindered by the fact that liquids present broad spectral features. Perhaps more selective measurements could be achieved on evaporated prepared samples, which are expected to deliver narrower absorption features [121].

This thesis demonstrates the feasibility of laser-based forensic urinalysis without preliminary chromatographic separation. Urine metabolites excreted at µg/mL concentrations may be identified. Besides the detection of doping substances investigated in the coarse of this thesis, clinical applications are conceivable as well. Ketone bodies and, in certain medical conditions, amines may be found in urine at detectable concentrations.
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Publications

Listed below are all publications that resulted from the present thesis. The work presented in Chapt. 3 was published in [v], [vi] and [vii]. The work presented in Sect. 4.3 and in Sect. 4.5.1 was published in [iii]. The work presented in Sect. 4.5.2 was published in [ii]. The work presented in Sect. 4.6 and in Chapt. 5 was published in [i].

Refereed journals and proceedings

Vapor-phase infrared laser spectroscopy: from gas sensing to forensic urinalysis.

Trace gas monitoring with infrared laser-based detection schemes.

High-temperature multipass cell for infrared spectroscopy of heated gases and vapors.
Spectroscopy: photoacoustic laser-absorption technique identifies sports doping substances.
(Article featuring the following publication.)

The potential of mid-infrared photoacoustic spectroscopy for the detection of various doping agents used by athletes.

Vapor-phase infrared spectroscopy on solid organic compounds with a pulsed resonant photoacoustic detection scheme.

Optoacoustic detection of different doping substances commonly used by athletes with an optical parametric generation laser source.

Oral presentations at conferences

• R. Bartlome and M. W. Sigrist.
Laser-based isomer identification in the vapor phase.
CLEO/Europe-IQEC, Munich, Germany.

• R. Bartlome and M. W. Sigrist.
A home-built infrared laser spectrometer for analytical chemistry.
Annual Meeting, Swiss Physical Society, Zurich, Switzerland.
• R. Bartlome and M. W. Sigrist.

*High-temperature multipass cell for infrared spectroscopy of heated gases and vapors.*
CLEO/QELS, Long Beach, CA (USA).

• R. Bartlome, C. Fischer and M. W. Sigrist.

*Vapor-phase infrared spectroscopy on solid organic compounds with a pulsed resonant photoacoustic detection scheme.*
European Conf. on Biomedical Optics (ECBO), Munich, Germany.
June 12–17, 2005.

**Patent**


**Supervised Master’s thesis and semester works**

• M. Reinhard.

*IR-Spektroskopie: Datennahme und Messung eines Acetonpeaks.*

• R. Joss.

*IR-Spektroskopie von Aceton, Ethanol und Methanol.*
Semester work, 2006.

• Z. Schlegel.

*Eine Hochtemperatur beständige Multipasszelle für Infrarotspektroskopie in der Dampfphase.*
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