

Development of Techniques for Trace Gas Detection in Breath

A thesis submitted for the degree of Doctor of Philosophy

Cathryn E Langley



Jesus College, University of Oxford

Trinity Term 2012

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Abstract

This thesis aims to investigate the possibility of developing spectroscopic techniques for trace gas detection, with particular emphasis on their applicability to breath analysis and medical diagnostics. Whilst key breath molecules such as methane and carbon dioxide will feature throughout this work, the focus of the research is on the detection of breath acetone, a molecule strongly linked with the diabetic condition.

Preliminary studies into the suitability of cavity enhanced absorption spectroscopy (CEAS) for the analysis of breath are carried out on methane, a molecule found in varying quantities in breath depending on whether the subject is a methane-producer or not. A telecommunications near-infrared semiconductor diode laser ($\sim 1.6 \mu\text{m}$) is used with an optical cavity-based detection system to probe transitions within the $2\nu_3$ vibrational overtone of methane. Achieving a minimum detectable sensitivity of 600 ppb, the device is used to analyse the breath of 48 volunteers, identifying approximately one in three as methane producers. Following this, a second type of laser source, the novel and widely tunable Digital Supermode Distributed Bragg Reflector (DS-DBR) laser, is characterised and the first demonstration of its use in spectroscopy documented. Particular emphasis is given to its application to CEAS and to probing the transitions of the two Fermi resonance components of the CO_2 $3\nu_1 + \nu_3$ combination bands found within the spectral range (1.56 - 1.61 μm) of the laser, providing the means to determine accurate $^{13}\text{CO}_2/^{12}\text{CO}_2$ ratios for use in the urea breath test.

Not all molecules exhibit narrow, well-resolved ro-vibrational transitions and the next section of the thesis focuses on the detection of molecules, such as acetone, with broad, congested absorption features which are not readily discernible using narrowband laser sources. To provide the necessary specificity for these molecules, two types of broadband source, a Superluminescent Light Emitting Diode (SLED) and a Supercontinuum source (SC), both emitting over the 1.6 - 1.7 μm region, are used in the development of a series of broadband cavity enhanced absorption (BB-CEAS) spectrometers. The three broadband absorbers investigated here, butadiene, acetone and isoprene, all exhibit overtone and combination bands in this spectral region and direct absorption measurements are taken to determine absorption cross-sections for all three molecules. The first BB-CEAS spectrometer couples the SLED device with a dispersive monochromator, attaining a minimum detectable sensitivity of $6 \times 10^{-8} \text{ cm}^{-1}$, which is further enhanced to $1.5 \times 10^{-8} \text{ cm}^{-1}$ on replacing the monochromator with a Fourier Transform interferometer. The spectral coverage is then extended to 1.5 - 1.7 μm by coupling the first SLED with a second device, providing a demonstration of simultaneous multiple species detection. Finally, a SC source is used to provide greater power and uniform spectral intensity, resulting in an improved minimum detectable sensitivity of $\sim 5 \times 10^{-9} \text{ cm}^{-1}$, or 200 ppb, 400 ppb and 200 ppb for butadiene,

acetone and isoprene respectively. This device is then applied to acetone-enriched breath samples; the resulting spectra are fitted with a simulation to return the acetone levels present in the breath-matrix.

Following this, the development of a prototype breath acetone analyser, carried out at Oxford Medical Diagnostics Ltd. (OMD), is described. To fulfill the requirements of a compact and commercially-viable device, a diode laser-based system is used, which necessitates a thorough investigation into all possible sources of absorption level change. Most notably, this includes a study into the removal and negating of interfering species, such as water vapour, and to a lesser extent, methane. A novel solution is presented, utilising a water-removal device in conjunction with molecular sieve so that each breath sample generates its own background, which has allowed breath acetone levels to be measured within an uncertainty of 200 ppb.

Spectroscopic detection then moves to the mid-infrared with the demonstration of a continuous wave 8 μm quantum cascade laser, which allows the larger absorption cross-sections associated with fundamental vibrational modes to be probed. Following the laser's characterisation using methane, including a wavelength modulation spectroscopy study, the low effective laser linewidth is utilised to resolve rotational structure in low pressure samples of pure acetone. Absorption cross-sections are determined before the sensitivity of the system is enhanced for the detection of dilute concentrations of acetone using two types of multipass cells, firstly a White cell and secondly a home-built Herriott cell. This allows an acetone minimum detectable absorption of 350 ppb and 20 ppb to be attained, respectively. Following this, an optical cavity is constructed and, on treating breath samples in a water-removal device prior to analysis, breath acetone levels determined and corroborated with a mass spectrometer.

Finally, a preliminary study probing acetone in the ultraviolet is presented. Utilising an LED centred at ~ 280 nm with a low finesse optical cavity and an imaging spectrograph, detection of 25 ppm of acetone is demonstrated and possible vibronic structure resolved. Combining large absorption cross-sections with the potential to be compact and commercially-viable, further development of this arrangement could ultimately represent the optimum solution for breath acetone detection.

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I think this is arguably the most important part of my thesis (and not just because it will be the most read bit!) as without the help and support of these people, everything which follows would not have been possible. First and foremost, thank you to Gus for accepting me into his group as a D.Phil student; his experience, insight and great knowledge have been instrumental over the course of my studies. Thank you to Grant, not only for his helpful advice and direction but also for his great enthusiasm - his positivity is infectious! A special thanks has to go to Rob - and not just for helping me fix my bike from time to time! His experimental expertise is second-to-none, and he has been a huge support throughout my D.Phil, from the early days of the SPEX, through to my time on my industrial placement at OMD. Thanks to Wolfgang, for help and guidance as my industrial supervisor - I thoroughly enjoyed my time at OMD (and won't forget his air guitar to Meat Loaf any time soon!). Thanks must also go to Meez, for his support and advice with the broadband work.

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List of Publications and Patents

- **Near-infrared broad-band cavity enhanced absorption spectroscopy using a superluminescent light emitting diode.**

W Denzer, M L Hamilton, G Hancock, M Islam, *C E Langley*, R Peverall, G A D Ritchie

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- **Trace species detection in the near infrared using Fourier transform broad-band cavity enhanced absorption spectroscopy: initial studies on potential breath analytes.**

W Denzer, G Hancock, M Islam, *C E Langley*, R Peverall, G A D Ritchie, D Taylor

The Analyst, **136**, 4, 801-806 (2011)

- **Demonstration of a widely tunable digital supermode distributed Bragg reflector laser as a versatile source for near-infrared spectroscopy.**

L Ciaffoni, G Hancock, P L Hurst, M Kingston, *C E Langley*, R Peverall, G A D Ritchie, K E Whittaker

Applied Physics B, DOI: 10.1007/s00340-011-4869-5 (2012)

- **Demonstration of a Mid-infrared Cavity Enhanced Absorption Spectrometer for Breath Acetone Detection.**

L Ciaffoni, G Hancock, J J Harrison, J H van Helden, *C E Langley*, R Peverall, G A D Ritchie, S Wood

In press

- **WO 2011/117572 A1 (Patent)**

Detection of Acetone in Breath

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Abbreviations

DFB	D istributed F eed b ack
DS-DBR	D igital S upermode- D istributed B ragg R eflector
SLED	S uperluminescent L ight E mitting D iode
SC	S upercontinuum
LED	L ight E mitting D iode
QCL	Q uantum C ascade L aser
NIR	N ear I nfrared
MIR	M id I nfrared
UV	U ltra V iolet
CRDS	C avity R ing D own S pectroscopy
CEAS	C avity E nhanced A bsorption S pectroscopy
BB-CEAS	B road b and C avity E nhanced A bsorption S pectroscopy
FTIR	F ourier T ransform I nfrared interferometer
ppt	p arts p er t rillion (1 in 1×10^{12})
pptv	p arts p er t rillion by v olume (1 in 1×10^{12})
pmol mol⁻¹	p icomol per mol (1 in 1×10^{12})
ppb	p arts p er b illion (1 in 1×10^9)
ppm	p arts p er m illion (1 in 1×10^6)
FWHM	F ull W idth H alf M aximum
HWHM	H alf W idth H alf M aximum
GC-MS	G as C hromatography M ass S pectrometry
PTR-MS	P roton T ransfer R eaction M ass S pectrometry
SIFT-MS	S electd I on F low T ube M ass S pectrometry
IMR-MS	I on-Molecule R eaction M ass S pectrometry
EI-MS	E lectron I onisation M ass S pectrometry

If the preceding subject was fat, our next one, Mrs. B., was a veritable pork barrel...

O. Folin and W. Denis

‘On Starvation and Obesity, with Special Reference to Acidosis’

Journal of Biological Chemistry (1915)

Chapter 1

Breath Analysis for Medical Diagnostics

This chapter begins with a brief overview of breath analysis as a means for the diagnosis of particular metabolic conditions and diseases. A description of the two officially recognised breath tests, namely that for NO and the urea breath test, is then given and a selection of notable other biomarkers are discussed. The second section of the chapter deals with the various methods used to analyse breath samples, including mass spectrometry-based techniques, electrochemical-based sensors and laser spectroscopy-based analyses. The final section of the chapter is devoted to acetone, a molecule which features prominently in this thesis. Its long history of association with diabetes is considered, as are the various means that have been used to detect it, together with a detailed account of its metabolic synthesis from fat deposits. Finally, the chapter concludes with an overview of the content of this thesis.

1.1 Breath Analysis

Ever since the ancient Greeks [1] it has been widely accepted that studies of breath, and breath odour in particular, can provide a means for determining the well-being of a subject [2]. For example, Rollo reported in 1798 on the decaying apple odour on the breath of those suffering from *Diabetes Mellitus* [3], whilst kidney diseases are associated with an ammonia smell and liver complications with a rotten egg odour [4]. However, it was not until 1971 and the ground-breaking discovery by Pauling that breath was composed of hundreds of volatile organic compounds (VOCs) [5], in addition to atmospheric molecules, that breath analysis started to become a viable form of diagnosis. The advent of greater selectivity and sensitivity due to the advancement of gas chromatography (GC) and mass spectrometry

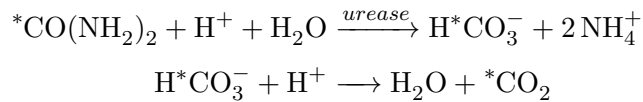
during this period allowed gaseous samples to be separated into their constituent parts and detected. Since then over a thousand compounds have been identified in exhaled human breath [6], with approximately 35 of these believed to be potential biomarkers for certain conditions, some of which are summarised in Table 1.1. These compounds found in breath can be categorised as either endogenously or exogenously synthesised molecules [1]: the latter find their way into breath either through absorption through the skin and then into the bloodstream or through inhalation, whilst the former are a result of metabolism within the body. Once in the bloodstream, these species can diffuse into the lungs and are excreted in exhaled breath; thus the presence of these molecules in a breath sample can provide an insight into body metabolism.

Whilst the presence of certain endogenously synthesised molecules in breath can indicate particular metabolisms taking place in the body, a second type of breath test involves the administration of a known concentration of an isotopically-labelled substance to a subject and the analysis of the subsequently metabolised products [1]. This second type of breath test is perhaps the most widespread (apart from ethanol testing) in use worldwide. The most common compound analysed in this way is carbon dioxide, as it is a major product of metabolism and the initial substrate administered can be readily isotopically labelled. One such example of this is the urea breath test, which is discussed below [7, 8].

The Urea Breath Test

The urea breath test represents a non-invasive tool to diagnose the presence of *Helicobacter pylori* infection [9]. *H. pylori* resides in the stomach and duodenum of over 50% of the world's population and, whilst the vast majority of those people exhibit no symptoms of the infection, it is linked to the development of duodenal ulcers, stomach cancer and gastritis [10, 11]. The most reliable method for detecting the presence of the bacterium is to take a biopsy during an endoscopy, which by definition is a highly invasive technique, whilst the most common techniques for diagnosis involve blood tests for the presence of antibodies to *H. pylori* and stool antigen detection. Therefore, the development of the urea breath test provides a very desirable alternative method for detecting the presence of *H. pylori*, as it is a sensitive, selective and crucially, a non-invasive and rapid technique [12]. The test centres on measuring the isotopic ratio of $^{13}\text{CO}_2/^{12}\text{CO}_2$ in the exhaled breath of the subject, both before and after an isotopically-labelled sample of urea is ingested. The ^{13}C isotope is utilised because it is not radioactive, and so does not pose a health threat to humans. The initial ratio determined acts as a baseline value for comparison with the subsequent ratio obtained following the ingestion of the urea: an increase in the ratio indicates the presence of the bacterium, as only *H. pylori* can metabolise urea. Via the catalytic action of urease, an enzyme produced by *H. pylori*, the isotopically-labelled urea is broken down into H_2O ,

NH_3 and CO_2 :



The CO_2 produced will consequently also contain ^{13}C , and this isotopically-labelled carbon dioxide will then diffuse into the bloodstream, where it will be transported to the lungs and exhaled in breath. Clearly, if *H. pylori* is present in the upper gastrointestinal tract, the isotopic ratio determined from the breath of the subject will be higher than before the isotopically-labelled urea was consumed. The change in the isotopic ratio observed before (R_0) and after (R) the administering of the ^{13}C -urea is commonly reported as follows:

$$\delta^{13}\text{C} = \frac{R - R_0}{R_0} \times 1000 \quad (1.1)$$

This internationally accepted formalism is expressed in ‰, and a 1 ‰ detected change is considered the highest level of accuracy required for diagnosis. The reference ratio, R_0 , can also be provided by the ratio obtained from the marine fossil Pee Dee Belemnite (PDB) [13, 14], which is regarded as the ‘zero-point’ of the $\delta^{13}\text{C}$ ‰ scale. It has an anomalously high ratio so that most natural material will consequently give a negative $\delta^{13}\text{C}$ in comparison to it. Although the presence of *H. Pylori* has been reported with a 2.4 ‰ change in the isotopic ratio, generally, a change of less than 5 ‰ is taken to mean that there is no infection, as much higher changes (often greater than 40 ‰) are observed when *H. pylori* is present [12, 15]. The sensitivities attained by Isotope Ratio Mass Spectrometry (IRMS), the conventional means with which to analyse the breath samples, are comfortably better than the ‘gold standard’ 1 ‰ [16] level required for diagnosis. However, the main problems associated with the technique are that it cannot distinguish between masses of the same molecular weight, such that $^{13}\text{C}^{16}\text{O}_2$ appears the same as $^{12}\text{C}^{16}\text{O}^{17}\text{O}$, a naturally occurring isotopologue, whilst $^{14}\text{N}_2^{16}\text{O}$ interferes with $^{12}\text{CO}_2$; plus there is a need to employ a separation technique on the sample prior to its analysis in the mass spectrometer using gas chromatography, and the equipment required is relatively expensive [17]. In addition, in the UK very few hospitals have access to mass spectrometry facilities so samples have to be sent away to be analysed, meaning patients have to wait a few weeks before getting their test results. These problems can be clearly overcome using a laser spectroscopy-based method, given that it exploits the fact that every molecular species has its own individual spectral fingerprint, providing specificity, and it has the potential to be a relatively quick, cheap and convenient diagnostic tool given the speed at which an absorption spectrum can be obtained and the plethora of lasers available from the telecommunications industry. Many spectroscopic methods have been applied to the determination of $\delta^{13}\text{C}$ ratios, and

some, as will be discussed later in this chapter, approach the levels of precision attained with mass spectrometry [18, 19].

Nitric Oxide

A second clinically recognised [20] breath test involves nitric oxide, with the first breath device for measuring exhaled NO (or fraction of exhaled nitric oxide) approved by the U.S Food and Drug Administration in 2003 [21]. It had been discovered that high levels of NO are found in the breath of asthmatics [22], and furthermore, that the levels detected are reduced following the administration of effective corticosteroids [23]. NO is produced during the conversion of L-arginine to L-citrulline by nitric oxide synthases: inducible (iNOS), endothelial (eNOS), and neuronal (nNOS) [24]. Whilst eNOS and nNOS consistently synthesise NO in their respective tissues (endothelial cell and neurons, respectively) for the many physiological processes in which the molecule is involved [25], the production of NO by iNOS is induced by inflammation [24]. Therefore, iNOS represents the main contributor of the elevated levels of NO found in the breath of asthmatics [26].

Traditionally, breath NO is measured using chemiluminescence [20], whereby the NO in breath reacts with ozone, forming NO₂ in an electronically excited state. On returning to its ground state, radiation is emitted and its intensity is directly proportional to the concentration of the NO. However, alternative methods, including electrochemical sensors and optics-based devices are also being developed [27–33], and this will be discussed in a later section of this thesis.

Selected other biomarkers

Although currently not a clinically approved breath test, ammonia and the structurally-related methylamines represent other nitrogen-based compounds that are found in breath. NH₃ is produced by the catabolism of amino acids, but under normal circumstances most of it is reused in the urea cycle, with what remains excreted in urine. However, in end-stage kidney disease this does not happen and there is a build up of the metabolites from the amino acids in the blood [1]. As well as ammonia, these also include monomethylamine (MMA), dimethylamine (DMA) and trimethylamine (TMA), all of which diffuse into the lungs and are excreted in exhaled breath. Therefore, individuals suffering from kidney disease have elevated levels of these compounds in their breath compared to normal subjects [34, 35]. Similarly, these species can also be found at high levels in the breath of those suffering from liver disease, given that the liver is involved in converting ammonia into urea [36].

Many hydrocarbons are found in breath and one of the most abundant is isoprene. The concentration of isoprene (2-methyl-1,3-butadiene) in human breath (typically ~100-200 ppb [37]) is considered a biomarker of biosynthesised cholesterol and as such, if found at elevated levels, can be used to diagnose those who have an increased risk of heart disease

[1]. Studies [38] have shown that the administering of lovastatin, a pharmacological agent that blocks the enzyme 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), causes a decrease in the levels of isoprene found in exhaled breath [39]. HMG-CoA reductase catalyses the production of mevalonic acid from HMG-CoA during the biosynthesis of cholesterol and it represents the rate-determining intermediate in the pathway, thus indicating that isoprene is produced after this point in the biosynthetic pathway. Deneris *et al.* [40] found that isoprene was produced from the acid-catalysed decomposition of dimethylallyl pyrophosphate (DMAPP) via a carbonium ion mechanism, as illustrated in Figure 1.1. DMAPP is another intermediate in the biosynthesis of sterols and is derived from mevalonic acid.

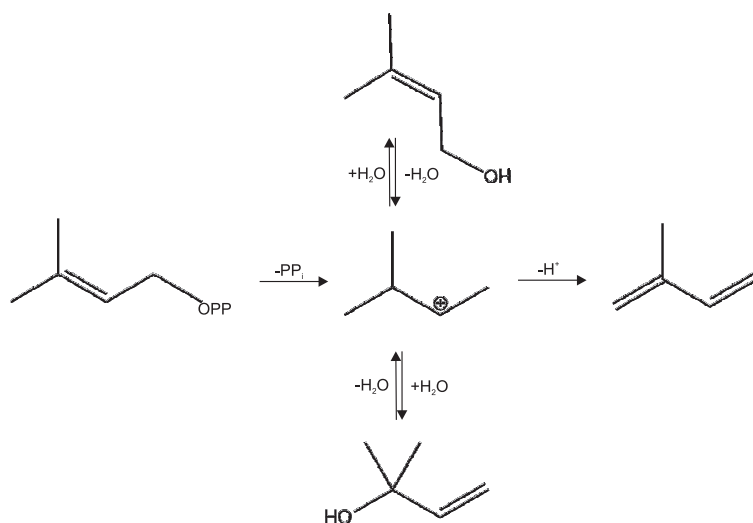


Figure 1.1: A schematic diagram illustrating the synthesis of isoprene from DMAPP (where PP_i is an abbreviation for the anion $P_4O_7^{4-}$); reproduced from [40].

Subjects with hyperlipidemia and hypercholesterolemia, both conditions of which are known to result in an increased risk of coronary artery disease, have been shown to have elevated levels of isoprene in their breath [39], highlighting the potential in utilising the presence of isoprene as a diagnostic tool. However, the levels of isoprene found in breath are known to increase linearly with age, be greater in males than females (prior to the menopause) [39] and, crucially, to vary throughout the day (reaching a maximum at approximately 6 am and a minimum at 6 pm) [41]. Therefore, these factors will need to be considered if the detection of isoprene in breath is to be used as a diagnostic tool.

Other significant hydrocarbons include ethylene, ethane and 1-pentane, which are associated with lipid peroxidation, which in turn signals the presence of reactive oxygen species. These species peroxidise lipids and the resultant radicals formed can undergo a variety of reactions, which can result in a multitude of conditions, ranging from cancers and prematurity through to neurological, cardiovascular and pulmonary diseases [1, 42].

Sulphur-based compounds are also found in breath, with excess levels of these often being an indication of liver-related diseases. These compounds include hydrogen sulphide, methanethiol (methyl mercaptan), ethanethiol (ethyl mercaptan), dimethyl sulphide and dimethyl disulphide, which are produced following the incomplete metabolism of methionine [1, 43] and are generally found at the ~ 10 ppb level (an order of magnitude less than isoprene).

Often a species can be a biomarker for more than one disease, and similarly a particular condition may have more than one biomarker. Acetone (which will be discussed at length later in this chapter), as well as being the signature molecule of diabetes, has also been linked with lung cancer, congestive heart failure and brain seizure [6]. Meanwhile, butane is also a reported marker for lung cancer, as are an array of other hydrocarbons and some sulphur compounds [6, 44]. Schizophrenia is identified by carbon disulphide and pentane [45], whilst the peroxidation of lipids is marked by the presence of pentane and ethane [46]: therefore, a correct diagnosis cannot be given from the elevated presence of pentane alone in a subject's breath, highlighting the need for any breath analysis technique to be specific to the target disease. Of these biomarkers, a handful are found at ppm, or sub-ppm levels (acetone, isoprene, methane, propanol, hydrogen, CO) and a further 400 or so are at the ppb and ppt level.

As the physiological links between certain molecules and conditions are strengthened, so the concept of breath analysis becomes more realistic [2]. In principle it allows for a non-invasive means to diagnose and monitor metabolic statuses by simply collecting a breath sample. In addition to high sensitivity and selectivity, ideally such a device should be low-cost, compact and with a real-time response, thus realising point-of-care disease diagnostics. Currently, there are a number of ways breath samples can be analysed, which can be divided into three main groups: mass spectrometry-based techniques, laser spectroscopy methods and other techniques, including electrochemical-based sensors. These methods are briefly discussed in the following section.

1.2 Techniques for analysing breath

Traditionally, mass spectrometry, coupled with gas-chromatography (GC-MS), has been utilised to detect and analyse the volatile species found in breath samples [5, 43]. The breath sample passes through the gas chromatograph, which separates the constituent molecules based on their chemical properties: different molecules will elute from the column at different times. The separated mix then passes into the mass spectrometer, which ionises the molecules before they are detected and identified using their mass-to-charge ratio. Early mass spectrometers used electron ionisation (EI) to create ions but this frequently leads to

fragmentation of the parent molecule, often making assignments difficult. Softer means of achieving ionisation have been developed, for example, using ion-molecule reaction (IMR) MS. This uses EI to produce primary ions (for example, Hg^+ , Xe^+), which then pass into a sample chamber and, via ion-molecule reactions, cause the formation of ions from the sample under analysis. The mass spectrometer used in this thesis combines both of these techniques and a full description of it will be given in Chapter 5. In addition, often mass spectroscopy is combined with pre-concentration techniques [47, 48] to increase the attainable level of sensitivity.

Further advances have included the development of selected ion flow tube mass spectrometry (SIFT-MS) [49] and proton transfer reaction mass spectrometry (PTR-MS) [50], both of which do not require calibration against standard gas mixtures. These techniques are also based on chemical ionisation, where initial ions are created via a gas discharge source before being used to ionise the sample of interest. However, in contrast to conventional IMR-MS, with SIFT-MS the initial ions are separated out by their mass-to-charge ratio and fed into an inert carrier gas before being transported through the flow tube into which the sample for analysis is injected [49]. The resulting ions formed are then separated by their mass-to-charge ratio and detected via the electrical current that is induced from the ion hitting the detector. In order for the technique to be quantifiable, it is necessary to know the likelihood of an ionisation occurring when an initial ion encounters a sample molecule. Thus, a knowledge of the rate constant of the reaction between the ions and the sample allows the original concentration of the sample to be determined. PTR-MS [50] works along a similar principle, with H_3O^+ representing the primary reactant ion. Proton transfer occurs for every collision as nearly all volatile organic compounds (VOCs) have proton affinities larger than H_2O and the processes tend to be non-dissociative so that cluster ions are less likely to form, resulting in simpler ion chemistry to analyse. In addition, there is no requirement for a mass filter, so a greater flow rate of H_3O^+ ions can enter the flow tube, and the use of an electric field to coax the ions along the length of the tube reduces diffusive losses to the sides of the flow-drift tube. Consequently, higher levels of the H_3O^+ ions are available, resulting in a higher analytical sensitivity than that attainable with SIFT-MS. However, accurate quantification is more difficult to achieve with PTR-MS as, unlike SIFT-MS, the reaction time and the kinetics for the ion-molecule reaction are not well defined [49] and often the sample concentrations are determined using a standardised value for the rate constant. It has been argued that for this reason, PTR-MS has mainly found application in air analysis and environmental studies, rather than in breath analysis where greater accuracy is more vital [49]. Despite this, a number of studies have used PTR-MS for breath profiling [37, 51], though it is SIFT-MS that has really led the way in clinical breath analysis [48, 49, 52].

Despite the advantages associated with mass spectrometry, most notably the very high sensitivities attainable and the fact that multiple species can be monitored, the technique is restricted to use in clinical laboratories, which limits its practicality in routine medical applications to breath. With its high cost and limited portability, it does not currently offer a point-of-care (POC) means for breath diagnostics.

A potential solution includes the use of chemical and electrochemical sensors, which are both compact and relatively inexpensive. One such example is the so-called ‘electronic nose’ which consists of arrays of non-selective chemical sensors, formed from quartz microbalance (QMB) sensors [27, 53]. The application of an alternating current induces oscillations in the quartz crystal; on adsorption to the sensors, the molecules generate a variation in the resonance frequency of the QMB, which is then detected and attributed to the concentration of the sample. However, the very nature of these devices means that they merely detect similarities and differences between samples as they are not sensitive to a particular species. Therefore, they require thorough calibration with other methods and a suitable data analysis to infer the significance of the changes observed [53]. A further disadvantage is that sometimes one does not just want to distinguish between two populations, but actually monitor the absolute levels of a species in a sample, and this technique does not offer this. Other examples of solid state-based sensors utilise metal oxides, such as WO_3 , which in its ϵ -phase demonstrates selective and quantitative detection of acetone [54]. This is a result of the increase in its spontaneous electric dipole moment on interaction with species with high dipole moments, which leads to a measurable decrease in the resistance of the device. Although the sensitivity of the instrument to acetone is much higher than that to other molecules found in breath, it still records signals at 14% and 21% of the signal level due to 600 ppb of acetone for typical water vapour and ethanol levels found in breath. Although the selectivity for acetone is increased under conditions of higher humidity (as would be present in breath), the sensitivity of the device is reduced by 67% [54]. Despite this, against a background of 90% relative humidity, the sensor was able to distinguish 20 ppb levels of acetone (though the question arises how accurate the recorded reading of acetone would be in real breath samples, in which there is not a controlled level of humidity from which to compare). Unfortunately, although chemical-based sensors do offer a compact and relatively cheap method for breath analysis, the lack of absolute selectivity for the target molecule is a common feature of the devices [27].

In contrast, laser spectroscopy offers a very high level of selectivity, whilst still maintaining very good sensitivity. Laser spectroscopy encompasses a wide range of techniques, including laser-induced fluorescence (LIF), where a molecule is excited to a higher electronic energy state by incident radiation before fluorescing; Raman spectroscopy, where following excitation to a virtual energy level, the molecule relaxes to a vibrational or rotational state which

is different from its initial level, emitting radiation at a frequency which is shifted relative to the excitation wavelength by the frequency difference between the initial and final state; and photoacoustic spectroscopy, where the absorption of modulated radiation by the molecular sample is transformed into a sound wave and detected with a microphone. However, absorption spectroscopy, the measurement of the radiation absorbed by a molecule as a function of the wavelength incident on it, forms the focus of this thesis and it will be discussed in detail in the following chapter. Absorption spectroscopy offers a real-time response, is affordable (when based on telecommunications diode laser technology) and has the potential to be compact: as such, its use represents a realistic point-of-care diagnostic device. The technology is not without its drawbacks, most notably that the level of sensitivity attainable depends on the molecule being studied and that generally the number of species that can be monitored simultaneously is restricted. Despite this, there has been a surge of interest in developing breath analyser devices using laser-based techniques in recent years, and a number of excellent reviews have been written on the subject [1, 6, 28, 55]. It is important to note, however, that there are several definitions of sensitivity, so care has to be taken in comparing the results of the different authors in the following discussion.

Nitric oxide has featured heavily in spectroscopic studies of breath as a result of its strong absorption band at 5 μm and due to its role as an officially recognised biomarker for asthma. Techniques have centred on the use of an optical cavity [29, 30, 33, 56, 57] or a multipass Herriott cell [31, 58] to enhance the absorption signal, and modulation spectroscopy to reduce the noise levels in the detected signal [32] (a full description of these techniques will follow in later chapters).

Similarly, measurements of the carbon isotope ratios with CO_2 have also featured prominently, thanks to the wide-spread use of the urea breath test for diagnosing the presence of *H. pylori*. A wide range of techniques have been employed, including Fourier Transform spectroscopy [59], photoacoustic spectroscopy [60] and the use of a multipass Herriott cell with modulation spectroscopy [61]. Several studies with optical cavities have also been reported, such as Kasyutich *et al.* [13], which utilised off-axis Cavity Enhanced Absorption Spectroscopy (CEAS) and Crosson *et al.* [18], who not only reported a precision of 0.22 ‰ with a cavity ring down spectroscopy (CRDS) instrument, but also demonstrated that on real breath samples their sensor matched the levels reported by Isotopic Ratio Mass Spectrometry (IRMS). A number of studies have involved the development of carbon isotope sensors for volcanic gas emissions [62, 63], highlighting a non-breath related use for measuring isotopic ratios. McManus *et al.* [19] designed a neat experiment where a multipass cell was used with a Pb-salt laser to provide two different pathlengths to measure the two isotopic absorptions. This ensured that the absorption depth for the two transitions was approximately the same, and it allowed them to report an impressive 0.2 ‰ precision. A very similar experiment was also undertaken by Uehara *et al.* [64] on isotopic ratios in

methane, and Zare *et al.* [65] demonstrated the use of a continuous flow cavity ring-down spectrometer (CRDS) for carbon isotope measurements on ethane and propane.

The less well-established biomarkers have also been the subject of a number of laser-based investigations. Sigrist *et al.* [66, 67] have conducted studies into detecting the methylamines in breath, utilising a cavity ring down spectrometer in the near infrared (NIR) to probe the first overtone of the N-H stretch vibration at $\sim 1.52 \mu\text{m}$ in monomethylamine (MMA) and dimethylamine (DMA), and a difference frequency generation (DFG) method to probe the 3.5 - 3.9 μm region in the mid-infrared (MIR). The former yielded sensitivities of 350 ppb and 1.6 ppm for MMA and DMA, respectively, in synthetic mixtures of the gases, decreasing to 10 ppm and 60 ppm when interfering species were taken into account. The MIR study allowed trimethylamine (TMA) to also be probed, as the transitions in the 3 μm region correspond to C-N stretches. In addition, the absorption cross-sections are larger than those in the NIR and there is less spectral interference from competing species in breath, which allowed the group to attain sensitivities of 900 ppb, 450 ppb and 120 ppb for MMA, DMA and TMA, respectively. Ammonia itself has been the subject of laser-based spectroscopic studies, with Manne *et al.* [68] demonstrating a device based on cavity ring down spectroscopy (CRDS) with a pulsed quantum cascade laser source at 970 cm^{-1} , achieving a sensitivity of 50 ppb. This was followed with a study based on the use of an astigmatic Herriott cell with the same laser. Using both an interpulse and intrapulse methodology, detection levels for ammonia and ethylene (which was also studied) were found for the interpulse technique to be 4 ppb and 7 ppb, respectively, and 3 ppb and 5 ppb for the intrapulse method. Both of these techniques were then applied to an actual breath sample with promising results.

Using a CO laser, Dahnke *et al.* have developed an instrument capable of real-time monitoring of ethane levels in human breath [69], achieving detection levels of 500 ppt with a cavity leak out spectrometer. This was followed with further studies, which also included the measurement of expirograms [70], and the use of an optical parametric oscillator system at 3 μm to achieve impressive sensitivities of 0.5 ppt [71] for the same molecule.

Carbonyl sulphide, elevated levels of which have been identified in patients who have experienced acute allograft rejection following lung transplantation [72], has been the subject of a number of laser-based studies [73, 74]. For example, Wysocki *et al.* [75] developed a compact Herriott cell in the mid-infrared using a pulsed quantum cascade laser (QCL) at 4.85 - 4.87 μm for the sensing of OCS in breath, achieving a detection limit of 1.2 ppb in a 0.4 s acquisition time.

Although laser-based techniques are limited by the number of species that can be monitored simultaneously, a number of studies have demonstrated that modest multiple species detection can be comfortably achieved. Moskalenko *et al.* [76] monitored ammonia, carbon

monoxide, methane and carbon dioxide in the breath of smokers and non-smokers using tunable diode laser spectroscopy (TDLS) with a multipass cell, whilst Wang *et al.* [14] have presented a continuous wave cavity ring down (cw-CRD)-based sensor for monitoring methane and carbon dioxide isotopes by multiplexing two distributed feedback diode lasers. Utilising optical frequency comb spectroscopy, Thorpe *et al.* [77] were able to cover an impressive 200 nm with 800 MHz resolution. They used the device to record CO₂ isotope measurements, together with CO and NH₃, the latter for which they attained a sensitivity limit of 18 ppb, which although was too high to detect ammonia in healthy individuals, was at a sufficiently sensitive level to detect the early stages of renal failure. However, such techniques are very far from being implemented outside a laser laboratory.

1.3 Acetone

Acetone is the most abundant VOC found in human breath, and coupled with its strong link with diabetes, it represents a particularly interesting molecule to study with regard to breath analysis. It is essentially produced when the body turns to its fat deposits as a source of energy in the absence of glycogen stores [78]. The triglyceride molecules are cleaved via lypolysis to produce a glycerol molecule and 3 fatty acid chains. The fatty acid chains are then utilised in β -oxidation to generate energy and produce the acetyl co-enzyme A (Acetyl CoA) required for the Krebs Cycle, which is then used to generate more energy. Excess acetyl CoA is converted into acetoacetate and D- β -hydroxybutyrate in the liver, from which the carboxylation of the former results in the production of acetone [79], as illustrated in Figure 1.2. These ketone bodies then diffuse into the bloodstream and are also oxidised via the Krebs Cycle in peripheral tissue to generate energy [1, 2]. However, in times of stress when fat stores are used instead of carbohydrates, the rate of ketone body production outstrips their use by the peripheral tissues and their concentration builds up in the bloodstream. In the case of acetone, which is a small, volatile compound that easily diffuses from the bloodstream and into the lungs, this results in elevated levels of acetone in exhaled breath. These periods of stress which cause fat deposits to be used as energy sources could be the result of intense exercise, dieting or a lack of insulin resulting in a reduced uptake of glucose by the liver.

Therefore, untreated diabetes would be expected to result in higher levels of acetone than normally found in breath. *Diabetes mellitus* can be split up into type 1 (T1D) and type 2 (T2D) categories. Whilst the latter tends to be developed later in life and is often associated with obesity, the former is typically juvenile-onset. T1D subjects are characterised by a lack of insulin-production, due to the destruction of β cells in the islets of Langerhans of the pancreas which produce the hormone [78]. Insulin causes the liver, muscle and fat

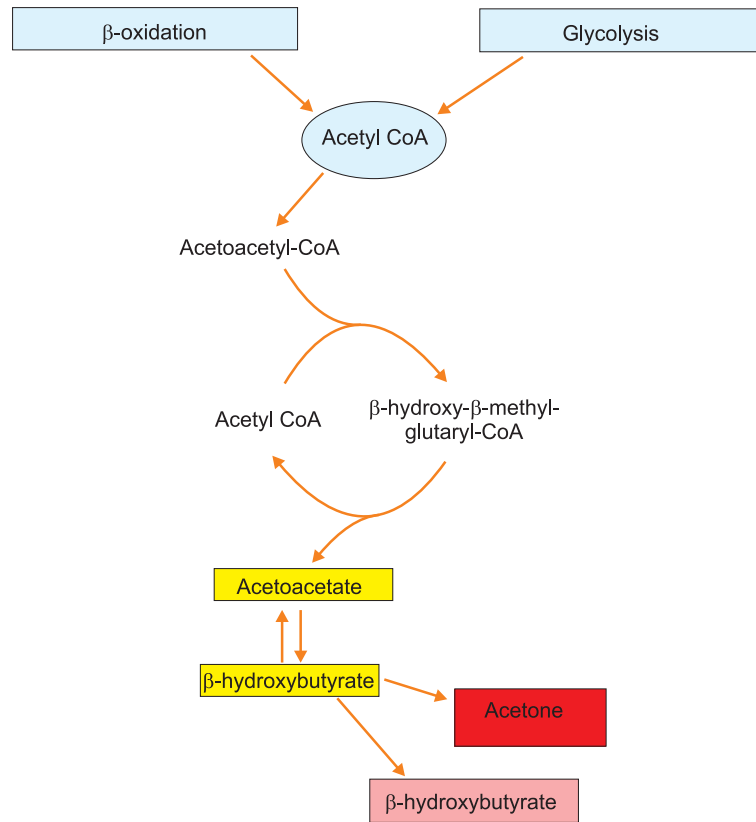


Figure 1.2: Schematic diagram illustrating the production of acetone through the decarboxylation of excess acetyl CoA; adapted from [2].

tissue to take up glucose from the blood, storing it as glycogen. It also inhibits the release of glucagon, reducing fat metabolism so that glucose is used as the primary energy source. Thus, insulin controls the level of glucose in the blood, which becomes toxic if it gets too high. When blood glucose levels become too low, the stored glycogen is broken down into glucose and utilised as the energy source. T2D is effectively a catch-all term for those suffering from diabetes that are not T1D, though it is characterised by a resistance to insulin and the decline of β cells gradually over time. This resistance, however, has a number of causes, such as a reduction in the number of hormone/transmitter receptors, insulin receptors with abnormal responses [80] and the role of glucose resistance has also been postulated as a contributing factor [81]. These different defects interact with β cell dysfunction to produce the diabetic symptoms, though the degree of insulin/glucose resistance versus impaired β cell function varies between individuals. For this reason, often studies on diabetes centre on T1D patients, where, by definition, no insulin is produced.

Although the fruity odour on breath has long been associated with diabetes, it was not until 1857 that acetone was identified as the main contributor to the smell [82], and as time progressed, methods were developed to measure the levels of acetone in both breath and blood. Initially, these centred on chemical techniques, such as passing the breath sample through an alkaline-iodine solution, followed by titration; or passing the sample through

sodium bisulphite solution and using a mercuric cyanide reagent [83–88] to determine the levels of acetone present from the resulting turbidity. Needless to say, these methods were quite tedious and not very sensitive. It was not until the 1960s that studies into breath acetone advanced, thanks to the development of GC-MS. These studies demonstrated that the measurement of acetone could be used in conjunction with blood glucose measurements to determine the exact diabetic status of the patient: for example, a high level of acetone coupled with normal blood glucose levels would reveal that the patient is not taking enough insulin or eating enough, which could then be corrected for [89–91]; indeed Crofford *et al.* [92] documented a ‘clinical use of breath acetone measurement’ in which the diet or insulin in-take would be altered depending on the particular combinations of breath acetone and blood glucose present in the patient.

However, since the development of the first blood glucose meter in the early 1980s, the measurement of breath acetone has become less prominent. The reasons for this are two-fold: it became apparent that intensive control and monitoring of diabetes was required to avoid the development of long-term complications [93], and this requirement for rigorous self-monitoring resulted in the decline in the use of breath acetone levels for diabetes monitoring as at the time there was no means to measure breath acetone out of clinical laboratories; and secondly, blood measurements of β -hydroxybutyrate can give similar information on blood ketones.

More recently, the monitoring of breath acetone has seen a resurgence as advances in mass spectrometry have allowed more detailed and comprehensive studies on VOCs in general. Turner *et al.* [94] measured the real-time breath acetone levels from type 1 diabetics and monitored their blood glucose levels, which were controlled using a hypoglycaemic clamp technique. From the small study, they demonstrated that although there was a strong positive correlation between blood glucose and breath acetone, the relationship was different for each patient. Another mass spectroscopy-based study by Minh *et al.* [95], building on work from an earlier study into the correlation between breath acetone and ethanol with glucose serum [96], has attempted to infer the blood glucose levels from breath via an indirect method employing a chemical reagent to convert the acetone into something measurable. From this study, only four molecular markers were identified as being required to determine the blood glucose levels from breath, and that acetone is by far the most significant. Wang *et al.* [97] have used a UV laser-based cavity ring-down method to measure breath acetone and attempted to show a general trend amongst T1D subjects, with a linear correlation between the average breath acetone levels and the average blood glucose levels when the patients are grouped by different blood glucose levels. In another study, Spaněl and Smith [98] demonstrate that although a predicted increase in the level of acetone is observed in the breath of those on a ketogenic diet, there is a wide variation

observed in the concentrations as a result of natural intra-individual biological and daily variability.

Therefore, it is clear from these studies and recent reviews [99–101] that more work is needed to firmly establish the exact link between blood glucose and breath acetone, as there is a certain degree of variability in the relationship between the two for different individuals. Despite this, the interest in developing a device for measuring breath acetone has grown rapidly, especially with the promise of reducing or even eradicating the need for blood testing, which is particularly painful for children.

One such device is an enzymatic electrode sensor [102] which is based on a series of enzyme reactions, which cause acetone to be converted into H_2O_2 , which is then detected by electrochemical means. On breathing into the sensor, one waits for 3 minutes before the voltage is applied across the electrodes. A detection limit of 0.25 ppm (v/v) above the background current was demonstrated, although it was acknowledged that electroactive impurities in the enzymes or from the electrode itself could cause this to vary, and that this was a subject of future work. In addition, secondary alcohols are potential interferents from the enzymatic reaction and although most are at levels too low to be significant, acetaldehyde is known to be at a level that could cause a problem, particularly after alcohol consumption. However, the sensor was successfully used in a study on the effect of diet on acetone levels, where the acetone levels reported were found to correlate well with those returned by the mass spectrometer used in the study ($R^2 \geq 0.950$).

Using a similar principle, a company called ‘positiveID’ [103] is developing a sensor which is based on the colour change associated with acetone and sodium nitroprusside, which they then claim can be used to infer blood glucose levels.

Within optical-based techniques, Bakhirkin *et al.* [104] have demonstrated the use of a quantum cascade laser at $\sim 8 \mu\text{m}$ to detect 1 ppm of acetone in air using direct absorption spectroscopy and a 1 m length sample cell. On application of wavelength modulation spectroscopy, the detecting limit was improved to 100 ppbv, and although this illustrates the potential of laser-based devices for the detection of acetone, given the size of the instrument it does not represent a practical breath analyser. Wang *et al.* have developed a UV-based CRDS sensor for detecting acetone [105–107], which was then used in their 2010 study described previously [97]. Although reporting a theoretical minimum sensitivity of 0.13 ppmv (based on the baseline stability of the instrument), the authors acknowledge the values returned represent the upper limit on the breath acetone levels (as a consequence of interfering species in the region and scattering effects), and the reported levels were not verified against a secondary device.

1.4 Overview of Thesis

This thesis will investigate a variety of absorption spectroscopy-based techniques for the potential application to breath analysis, with particular emphasis on the detection of acetone. The following chapter will firstly introduce the fundamentals of absorption spectroscopy, outlining the techniques that can be applied to increase its sensitivity before discussing the types of radiation sources utilised in this body of work. The first experimentally-based chapter, Chapter 3, introduces a preliminary study into the measurement of methane in breath using a diode laser-based cavity-enhanced detection system (CEAS) in the near-infrared (NIR) at 1.65 μm . The second half of Chapter 3 details the characterisation of a novel, widely tunable laser source, a digital supermode distributed Bragg reflector (DS-DBR), demonstrating the first application of this device to spectroscopy by probing NIR vibrational bands of CO_2 from 1.56 - 1.61 μm .

Whilst diode-based lasers are ideal for the detection of small molecules, such as CH_4 and CO_2 , which have well-resolved, narrow transitions, acetone (together with other important VOCs) has broad, congested vibrational spectra, typically spanning tens of nanometres, making it difficult to identify using such sources. Therefore, Chapter 4 describes the development of a broadband radiation-based CEAS spectrometer. Two types of broadband source are considered, a Superluminescent Light Emitting Diode (SLED) and a Supercontinuum (SC) source, whilst the use of both a dispersive monochromatic spectrometer and a Fourier transform spectrometer are demonstrated and compared. Butadiene, a molecule with a broad absorption feature spread across the spectral region of study (~ 1.6 - 1.7 μm), is used to characterise the instruments developed, before they are applied to the detection of acetone and isoprene, and finally to real breath samples. Building on this work, Chapter 5 describes the development of a prototype breath acetone analyser carried out at Oxford Medical Diagnostics Ltd. Given the requirements of a compact, commercially-viable device, a diode laser-based system is employed and the chapter deals with an investigation into negating and removing the effects of interfering species, most notably water vapour, before demonstrating and calibrating its performance against a mass spectrometer.

In the final experimental chapter, spectroscopic detection moves to the mid-infrared and 8 μm with the use of a continuous wave (cw) quantum cascade laser (QCL), allowing the stronger, fundamental transitions of acetone to be probed, providing the potential for a greater level of sensitivity to be achieved. The low effective laser linewidth is utilised to resolve rotational structure in low pressure samples of acetone and to determine the relevant absorption cross-sections. Following this, the sensitivity of the system is progressively increased using multipass cells (White and Herriott) and finally an optical cavity. Exploiting the water-removing devices developed at Oxford Medical Diagnostics Ltd., the cavity-based spectrometer is then used to detect acetone in breath samples, and cross-checked with a

mass spectrometer. Finally, the work demonstrated in this thesis is reviewed, prior to the presentation of some preliminary experimental results on the detection of acetone at 280 nm, together with a discussion on the future directions of the detection of breath acetone.

Biomarker	Physiological basis	Metabolic disorder
acetaldehyde	ethanol metabolism	alcoholism, liver-related diseases, lung cancer
acetone	decarboxylation of acetoacetate	diabetes, lung cancer, congestive heart failure, brain seizure
ammonia	protein metabolism /bacterial metabolism	renal diseases, asthma
carbon monoxide	heme catabolism catalysed by heme oxygenases	oxidative stress, respiratory infection, anaemias
carbon dioxide	respiration	isotopic labelling used to diagnose presence of <i>H. pylori</i> , oxidative stress
carbonyl sulphide	gut bacterial oxidation of reduced sulphur species	liver related diseases
ethane	lipid peroxidation	oxidative stress, vitamin E deficiency in children, peroxidation of lipids
ethanol	gut bacterial metabolism of sugars	production of gut bacteria
ethylene	lipid peroxidation	uv radiation damage of skin, peroxidation of lipids
hydrogen	carbohydrate metabolism	colonic fermentation, intestinal upset
hydrogen sulphide	anaerobic bacterial metabolism of thiol proteins	liver-related disease
isoprene	cholesterol biosynthesis	blood cholesterol
methane	gut bacterial metabolism lung diseases	intestinal problems, colonic fermentation
methanethiol	methionine metabolism	halitosis
methylamine	protein metabolism	protein metabolism in the body
nitric oxide	involved in vasodilatation, or neurotransmission; production catalysed by nitric oxide synthases	asthma, bronchiectasis, hypertension, rhinitis, lung diseases
pentane	lipid peroxidation	peroxidation of lipids, liver disease, schizophrenia, breast cancer, rheumatoid arthritis

Table 1.1: A selection of potential biomarkers found in breath, adapted from [1, 6].

Chapter 2

Absorption Spectroscopy: principles and light sources

Absorption spectroscopy represents a very powerful means for quantitative trace gas detection. In principle the energies that are absorbed are specific to each species, reflecting its unique set of internal molecular energy states (electronic and ro-vibrational), allowing a high level of selectivity to be attained. Furthermore, the technique can be quantitative via the use of the Beer-Lambert law, which allows absolute number densities of the species of interest to be straightforwardly determined from the measured absorbance. However, there is an inherent lack of sensitivity associated with direct absorption spectroscopy, as a direct consequence of measuring a small change on a large background, which must be improved upon to realise trace gas detection. This chapter initially describes the basis of absorption spectroscopy and presents a brief outline of the factors influencing its sensitivity, before discussing the specific light sources used to perform the absorption studies in this thesis.

2.1 Absorption Spectroscopy

Absorption spectroscopy is primarily based on the interaction of the electric field of light with the electric dipole moment associated with the molecular transition of interest [108] (although electric quadrupole and magnetic dipole interactions can also occur). Quantum mechanically this interaction is described in terms of the electric dipole moment operator, $\hat{\mu}$, which acts on the initial state of the atom/molecule (ψ_i) to give the new, excited final state (ψ_f^*). The degree of coupling between the initial and final states, or transition amplitude, is given by:

$$\hat{\mu}_{fi} = \int \psi_f^* \hat{\mu} \psi_i d\tau \quad (2.1)$$

and is known as the transition dipole moment. The probability of the transition occurring is then proportional to the square of the transition dipole moment. Therefore, in order for the transition to occur, the integral in 2.1 must be non-zero. The rate at which the transition occurs (per unit spectral density of the electromagnetic field) is given by the Einstein co-efficient of absorption, B_{if} [108]:

$$B_{if} = \frac{|\hat{\mu}_{fi}|^2}{6\epsilon_0 \hbar^2} \quad (2.2)$$

where ϵ_0 is the vacuum permittivity and $\hbar = h/2\pi$. Hence, the larger the transition rate, the larger the measured absorbance. The Einstein co-efficient is then related to the absorbance via:

$$B_{if} \frac{h\nu}{c} = \int_0^\infty \sigma_{if}(\nu) d\nu = \sigma_{\text{int}} \quad (2.3)$$

where $\sigma_{if}(\nu)$ is the frequency-dependent absorption cross-section for the transition $f \leftarrow i$ at frequency ν and σ_{int} is the integrated absorption cross-section. From the absorption cross-section, the familiar Beer-Lambert law [109, 110] can be derived:

$$I(\nu) = I_0 \exp(-\sigma(\nu)CL) \quad (2.4)$$

which links the frequency-dependent absorption cross-section to the intensity attenuation observed as radiation passes through a length L of a sample of concentration C , with I_0 and $I(\nu)$ the incident and transmitted radiation, respectively. Due to broadening effects, the observed absorption transition is not a δ function centred on a frequency, ν_0 , but has a well defined lineshape which is discussed below [108]. However, it is noted that the integrated absorption cross-section, σ_{int} , is independent of the environmental conditions of the molecule and is therefore a constant (at a particular temperature).

The minimum linewidth associated with all transitions is a result of natural linewidth broadening, which may be thought of as a direct consequence of the Heisenberg uncertainty principle. Given that the lifetime (τ) of the upper state of the transition is finite, an uncertainty is introduced into the corresponding energy of the transition [108]:

$$\tau \delta E \approx \frac{1}{2} \hbar \quad (2.5)$$

Thus the transition occurs over a range of frequencies, directly related to δE . However, this effect is generally very small in comparison to the broadening induced by both collisional and Doppler effects at room temperature, typically ranging from 10^{-4} Hz associated with rotational energy states, through to a few MHz in excited electronic levels, as a result of the ν^3 dependence of A_{fi} , the Einstein coefficient for spontaneous emission. Collisional, or pressure broadening arises from a decrease in the lifetime of both states involved in the transition as a result of increased collision frequency between molecules, which causes an increase in the uncertainty of the associated energy of the transition, in accordance with equation 2.5, and leads to a broadening of the absorption lineshape. Clearly, as the pressure increases, the number of collisions will increase, resulting in greater line broadening. For a given pressure, the degree to which the absorption profile broadens due to collisional effects is quantified by the pressure-broadening coefficient, γ , which in turn is dependent on the nature of the pressure broadening gas (and thus the type of collisional energy transfer processes which can take place). Lineshapes which are dominated by pressure broadening have a Lorentzian profile [110]:

$$L(\nu) = \frac{\Delta\nu_L}{2\pi\left[\left(\frac{\Delta\nu_L}{2}\right)^2 + (\nu - \nu_0)^2\right]} \quad (2.6)$$

where ν_0 is the central frequency of the absorption profile and the Lorentzian width (full width half maximum, FWHM), $\Delta\nu_L$, is related to the pressure-broadening coefficient via $\Delta\nu_L = 2\gamma p$, where p is the pressure of the broadening gas.

In the low pressure regime (where $p \sim$ few Torr or less), Doppler broadening is the dominant line broadening mechanism. The molecules in a gaseous sample at a certain temperature will exhibit a range of velocities, given by the Maxwell-Boltzmann distribution [110]. The motion of these molecules relative to the propagation direction of the radiation passing through the sample leads to a shift in the observed frequency at which each molecule absorbs. Thus, a distribution of absorption frequencies is observed, reflecting the velocity distribution of the molecules and results in a Gaussian lineshape profile:

$$G(\nu) = \frac{2}{\Delta\nu_G} \sqrt{\frac{\ln 2}{\pi}} \exp\left[-\frac{4 \ln 2 (\nu - \nu_0)^2}{\Delta\nu_G^2}\right] \quad (2.7)$$

where $\Delta\nu_G$ is the FWHM Gaussian linewidth, defined via:

$$\Delta\nu_G(T) = 2\nu_0 \sqrt{\frac{2kT \ln 2}{mc^2}} \quad (2.8)$$

where T is the temperature, k is the Boltzmann constant, m is the mass of the molecule and ν_0 is the central frequency of the transition.

At pressures where there is an observable contribution from collisional broadening as well as Doppler broadening, the absorption linewidth can be described by a convolution of the Gaussian and Lorentzian lineshapes; the Voigt profile:

$$V(\nu) = \frac{2}{\Delta\nu_G} \sqrt{\frac{\ln 2}{\pi}} \frac{y}{\pi} \int_{-\infty}^{+\infty} \frac{e^{-t^2}}{y^2 + (x-t)^2} dt \quad (2.9)$$

where x and y are given by

$$x = \sqrt{\ln 2} \frac{2(\nu - \nu_0)}{\Delta\nu_G} \quad y = \sqrt{\ln 2} \frac{\Delta\nu_L}{\Delta\nu_G} \quad (2.10)$$

Notable other, more complex, lineshape profiles include Galatry and Rautian lineshapes which are typically employed when collisional-narrowing effects take place. These processes occur when the lifetime of the upper state is large compared to the time between collisions (i.e. the mean free path of the absorber is significantly less than the wavelength of the transition being studied), so that many elastic collisions will affect the species' velocity during the absorption of a photon, leading to a reduction in the Doppler broadening of the line [111].

Sensitivity

The sensitivity of direct absorption spectroscopy is inherently low given the need to measure a small change on a large background (i.e. the decrease in signal intensity due to absorption by the molecular species is very small in comparison to the overall detected signal). However, it can be improved by increasing the absorbance or by reducing the level of noise in the system, or sometimes both (such as in Noise Immune Cavity Enhanced Optical Heterodyne Molecular Spectroscopy, NICE-OHMS [112]).

A quick glance at the Beer-Lambert law (2.4) indicates that the level of absorption can be enhanced by **(a)** increasing the concentration of the sample, C , **(b)** using a transition with a larger absorption cross-section, $\sigma(\nu)$, or **(c)** by increasing the pathlength, L . The first is not necessarily always an option, especially for trace gas detection, whilst the second can be exploited by carefully selecting the wavelength with which to probe the molecule. This could, for example, include moving to the MIR spectral region in order to probe the fundamental vibrational transitions, which have much larger absorption cross-sections than the overtone and combination bands found in the NIR. The third option, increasing the pathlength, can be achieved either by using a multipass absorption cell, such as a Herriott cell, or by using an optical cavity. Both of these techniques will be described fully later

in this thesis, with multipass cells featuring in Chapter 6 and the basis of cavity-enhanced techniques introduced in the following chapter.

A reduction in the noise associated with the measurement will also increase the level of sensitivity. There are three major types of noise in absorption spectroscopy measurements. The first is due to quantum fluctuations inherent both in the laser source and in the photocurrent of the detector. This is known as shot noise and it is a statistical phenomenon due to random emissions and the quantised nature of energy and charge carriers. The shot noise limit represents the fundamental limit of all detection methods when there are no external noise sources and is given by [113]:

$$\left(\frac{S}{N}\right)_{\text{SNL}} = \frac{\eta P_0}{2h\nu\Delta f} \quad (2.11)$$

in terms of the signal to noise ratio, where η is the quantum efficiency of the detector (i.e. the ratio of the number of carriers produced to the number of absorbed photons), P_0 is the power incident on the detector, h is Planck's constant, ν is the wavelength of the radiation and Δf is the bandwidth of the detector.

In practice, it is very rare for any measurement to approach the shot noise limit because external noise sources accumulate and cause the sensitivity to decrease. An example is noise due to environmental fluctuations in the experimental set-up. These could include mechanical instabilities in the system as a result of pressure differentials, which alter the optical alignment of the system, or thermal or acoustic fluctuations. This also covers fluctuations in the laser output itself as a result of environmental factors. This type of noise is linearly dependent on the intensity of the light, and is otherwise referred to as $1/f$ noise. White noise, so called because its power spectral density is invariant with frequency, represents the final noise source. It is associated with thermal, or Johnson-Nyquist, noise which arises from the thermal fluctuations of charge carriers, such as in the conversion of photon energy to electrical signal in a photodiode.

The level of noise in a system can be reduced using modulation spectroscopic techniques, which help to reduce the environmentally-induced noise component, the $1/f$ noise. This involves applying a high modulation to the laser output so that detection is shifted to a higher frequency, hence reducing the effects of $1/f$ noise. An overview of modulation techniques, with particular emphasis on the wavelength modulation spectroscopy variant, will be presented in Chapter 6.

There are several ways to quote the sensitivity of an absorption measurement. Throughout this thesis, the minimum detectable absorption co-efficient, α_{min} , is taken to represent the

noise equivalent absorption, in other words:

$$\alpha_{\min} = \frac{\alpha_{\text{peak}}}{\text{SNR}} \quad (2.12)$$

where SNR is the signal to noise ratio and $\alpha_{\text{peak}} = \sigma_{\text{peak}}C$, where C is the number density of the species and σ_{peak} is derived from the integrated absorption cross-section (σ_{int}):

$$\sigma_{\text{peak}} = \frac{\sigma_{\text{int}}}{\Delta\omega\Gamma} \quad (2.13)$$

where Γ is the lineshape parameter, a constant which takes into account the lineshape profile of the absorption feature and $\Delta\omega$ is the FWHM of the absorption feature [114].

In addition, to take into account the data acquisition time, a time-reduced form can also be used. This is generated simply by multiplying $\alpha_{\min}$ by $\sqrt{(n\tau)}$, where n is the number of averages and τ is the period for one scan, and has units of $\text{cm}^{-1} \text{s}^{1/2}$. A third method utilises the bandwidth-reduced sensitivity, quoted in $\text{cm}^{-1} \text{Hz}^{-1/2}$, where $\alpha_{\min}$ is normalised to the bandwidth of the detection electronics by multiplying by $\sqrt{n\pi\tau}$, where in this instance τ is the time constant of the detection system. Which form is used depends largely on the nature of the experiment: for example, sensitivities are generally quoted in the form cm^{-1} with regards to broadband radiation based experiments, whereas with diode laser-based experiments, where the laser rapidly scans over the absorption feature in a fraction of a second, the time-reduced form is often used, whilst the bandwidth-reduced form lends itself to application within modulation spectroscopy, when a lock-in detector is utilised. Given a significant degree of this thesis involves broadband spectroscopy, sensitivities will be quoted in terms of cm^{-1} throughout this work for consistency, with the duration of the measurement quoted separately.

2.2 Overview of Light Sources

Absorption spectroscopy naturally requires a source of light that is resonant with the gaps between internal energy levels to selectively probe the molecular species of interest. A wide variety of radiation sources have been used throughout this thesis, which have been divided into two categories, namely ‘narrowband’ and ‘broadband’ sources.

2.2.1 Narrowband Sources

Narrowband sources encompasses the use of lasers which allow lineshape determination of a single ro-vibrational transition in small, gas phase molecules. Two types of laser that

are capable of doing this are used predominantly in this thesis: telecommunications diode lasers and quantum cascade lasers (QCLs), which are described in the following section. These sources emit coherent, monochromatic radiation with a narrow linewidth (< 10 MHz), making them ideal for absorption spectroscopy as they allow specific energies of ro-vibration to be probed selectively.

Diode Lasers

Diode lasers are readily available, robust, compact and economical thanks to their development for use in the telecommunications industry and as such they make ideal light sources for probing the near infrared (NIR) spectral region [115]. They are based on semiconductor p-n junction diodes, which can be formed from layers of the same semiconducting material with different impurity dopants added (homojunction) [110], or from layers of different semiconducting material (heterojunction) [116]. In either case, the p-n junction consists of a side in which there is an excess of electrons in the conduction band (the n-doped side) and one in which there are electron vacancies, or holes (the p-doped side) in the valence band. In the absence of any applied potential difference, the system is at thermodynamic equilibrium and the electrons fill up to the Fermi level, as illustrated in Figure 2.1. However, the application of a forward-bias voltage causes the energy band structure of the system to change so that the two Fermi energies are at different levels. Thus, electrons will flow from the n-type side of the junction into the conduction band, whilst the positive holes traverse in the opposite direction in the valence band, inducing a population inversion such that the lasing condition, $N_u/g_u > N_l/g_l$ (where N_i is the population of state i and g_i is its degeneracy), is fulfilled. Recombination of the filled electron states and holes at the junction results in a photon emission, as illustrated in Figure 2.1.

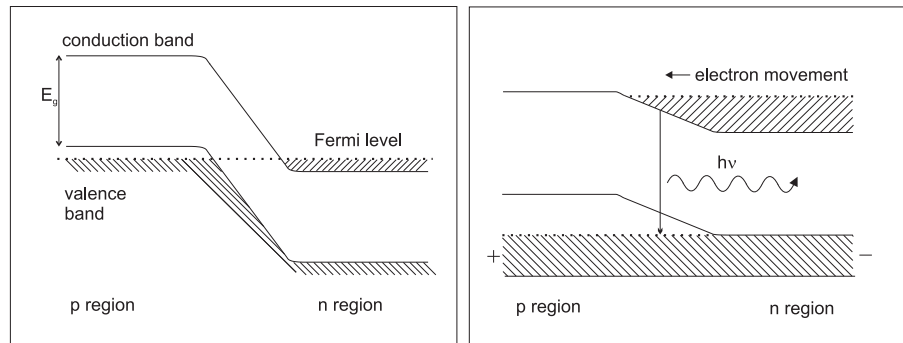


Figure 2.1: A schematic diagram illustrating the p-n junction of a DFB diode laser when **left**, no voltage is applied across the junction and **right**, when a voltage is applied and a population inversion is induced; adapted from [110].

Clearly, the wavelength of the emitted radiation depends upon the magnitude of the band gap between the conduction and valence bands when the potential difference is applied. The band gap in turn is determined by the chemical nature of the active material, and can be further subtly altered by changing the refractive index of the medium, either by tuning

the injection current or the temperature of the device. In general, the gain bandwidth is tens of nm wide and supports many lasing modes. Thus, the wavelength of the emitted radiation is reinforced whilst the other modes are suppressed using optical feedback: a grating preferentially selects a particular wavelength, which is injected back into the active medium, stimulating further emission at that particular frequency. The optical resonator can either be external or internal to the active medium; external cavity diode lasers (ECDL) are examples of the former [110], where the wavelength is selected by tuning the grating external to the active medium, whilst distributed feedback (DFB) lasers are examples of the latter [110], where a Bragg diffraction grating is written into one of the layers surrounding the active medium of the laser. A Bragg diffraction grating, also known as a Bragg reflector, consists of a series of layers of materials with differing refractive indices so that only a wavelength which satisfies $\lambda = 2\Lambda n_{\text{eff}}$ (where Λ is the period of the grating and n_{eff} is the effective refractive index) is strongly reflected. In a distributed Bragg reflector (DBR) laser, the grating is instead located at the surface of the active region [117].

Although diode lasers offer many advantages, such as the provision of single-mode operation at room temperature, a typical linewidth of a few MHz, relatively high power and tunability (~ 5 nm), they are restricted to the NIR spectral region ($\sim 500 - 2700$ nm), which limits the spectroscopist to the weak overtone and combination bands of molecules (although very recent advances in mid-IR diodes have been demonstrated by Meyer *et al.* [118], but are yet to be commercially available in single mode). In order to access the strong fundamental transitions of species, alternative laser sources are required.

Quantum Cascade Lasers (QCL)

Traditionally, the mid-infrared (MIR) spectral region has been accessed by Pb-salt, CO and CO₂ lasers, with difference frequency generation (DFG) and optical parametric oscillators (OPO) providing more recent techniques. Whilst a more detailed overview of these devices will be presented in Appendix A, the technical workings of the MIR source used in this thesis, the quantum cascade laser (QCL), will be dealt with here.

Like the diode lasers described previously, the QCL is also a semiconductor laser but rather than the emitted wavelength depending upon the recombination of electron-hole pairs, it is dictated by the discrete conduction band states within quantum wells and the subsequent electron transitions between them. These states are generated from the deposition of layers of semiconductor via molecular beam epitaxy [119]. The layers are of precisely engineered thickness approaching the De Broglie wavelength of the material, which allows the formation of quantum wells. The laser consists of three levels, as illustrated in Figure 2.2. The electron injected into the upper excited state (3) falls to the lower state (2), emitting a photon in the process at the frequency corresponding to the energy gap, before relaxing to the ground state (1). As a result of the carefully constructed layers of semiconductor, the difference

in energy between (2) and (1) is approximately equal to that of an optical phonon, which means that the relaxation of the electron from (2) to (1) occurs over a much shorter time period than the radiative transition: in other words, a population inversion is maintained between (3) and (2) [119]. Following its relaxation to (1), the electron can then tunnel to the neighbouring quantum well because the barrier thickness between the wells is < 5 nm, where it is injected into the upper excited state (3) of the next quantum well, as depicted in Figure 2.2. This energy matching occurs when a potential difference is applied across the device and it causes the electron to ‘cascade’ down through a staircase of quantum wells, each of which results in the emission of a photon at a precise frequency. This ‘recycling’ of each electron results in relatively high output powers (< 100 mW) and, as the output wavelength is governed by the engineering of layers of the device rather than the band gap properties of semiconductors, a wider range of wavelengths is possible.

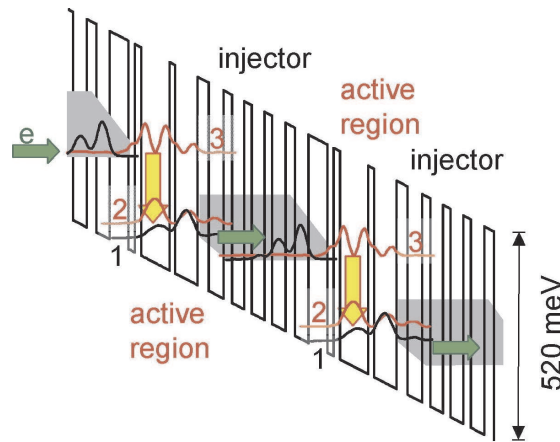


Figure 2.2: A depiction of the band structure of a quantum cascade laser, reproduced from Curl *et al.* [120]. The electron injected into level (3) falls to level (2), emitting a photon in the process, before relaxing to level (1). The electron then tunnels to the neighbouring quantum well, where it is at level (3), and so the process cascades down, with a photon emitted at each quantum well.

Since the initial proposal of the concept of semiconductor superlattices for laser gain media in the 1970s [121], QCL technology has developed rapidly [122]. Most early QCLs were pulsed, following the first demonstration of such a device in 1994 by Faist *et al.* [119], which allowed the large degree of heat that builds up within the active region of the laser to be dealt with more effectively. Improvements in coping with the excess thermal energy led to the development of continuous wave (cw) QCL sources, such as the one used in this thesis, which have a number of advantages over the pulsed systems, most notably that there is no frequency chirping observed (which eradicates the spectral broadening caused by the tuning rate of the pulsed systems). The linewidth of a QCL is generally limited by the current source, and is typically found to be around 1-5 MHz, which is of the same order of magnitude as diode lasers. However, in principle this could be much less as there

is negligible contribution from spontaneous emission because the relaxation rate is faster than the spontaneous emission rate [119].

In addition, broad tunability has been realised with the advent of external cavity (EC) QCLs, typically covering $\sim 100 - 500 \text{ cm}^{-1}$ [123, 124], as they are not limited by thermal tuning. Furthermore, broadband coverage has also recently been provided by the stacking of active regions with different optical energies which emit at several wavelengths, as demonstrated by Gmachl *et al* [125], and also from arrays of DFB-QCLs [126, 127], which offer single mode operation over 100 cm^{-1} . Although these devices are still in their infancy, they show great promise to supersede the current broadband sources available.

2.2.2 Broadband Sources

Sources of broadband radiation are desirable for the study of larger, more complex absorber molecules. These species, which will be discussed in detail in Chapter 4, do not exhibit single, well-defined transitions but rather more complex, broadened spectral features which can span over tens of nanometres. Thus broadband radiation sources allow an entire absorption feature to be probed, so that these broadband absorber molecules can be positively identified in a sample. A multitude of sources that emit over a wide spectral range are available, some of which will be discussed in Chapter 4. These include the use of Xe arc lamps and light emitting diodes (LEDs), but the work described in this thesis centres on the use of Superluminescent Light Emitting Diodes (SLED) and a Supercontinuum source (SC), both of which will now be described in the following sections.

Superluminescent Light Emitting Diodes (SLED)

A superluminescent light emitting diode (SLED) source combines the broad spectral range of light emitting diodes (LEDs) with the high optical power of a diode laser [128]. It was invented in 1986 [129] and as with a diode laser and other semiconductor devices, a SLED consists of a p-n junction across which a voltage is applied. The subsequent spontaneous recombination of positive holes and negative electrons results in the emission of radiation, which is then amplified on travelling along the waveguide in the SLED [129]. These photons will naturally have the same frequency, phase and polarisation of the initial photons, and they in turn can cause recombination, and so on, causing amplification, or gain, of the specific frequencies emitted. In a SLED, the p-n junction contains various possible energy bands, so that on recombination a range of frequencies are emitted, hence producing broadband light. In contrast to diode lasers, the waveguide is tilted and there are anti-reflection coated facets to reduce optical feedback so that lasing action and the narrowing of the optical bandwidth does not occur [129].

An increase in the injected current causes the number of electrons and holes across the p-n junction to increase, resulting in a subsequent increase in recombinations and hence optical power. Whereas with a diode laser, a specific threshold is reached and then it begins to lase, with a SLED, a more steady increase in power is seen with increasing current. Initially, the power output is dominated by spontaneous emission, but as the current increases, the SLED enters the amplified spontaneous emission regime and power increases exponentially [129]; the variation in output power as a function of drive current is illustrated in Figure 2.3 for the SLED (DenseLight Semiconductors) used in this thesis.

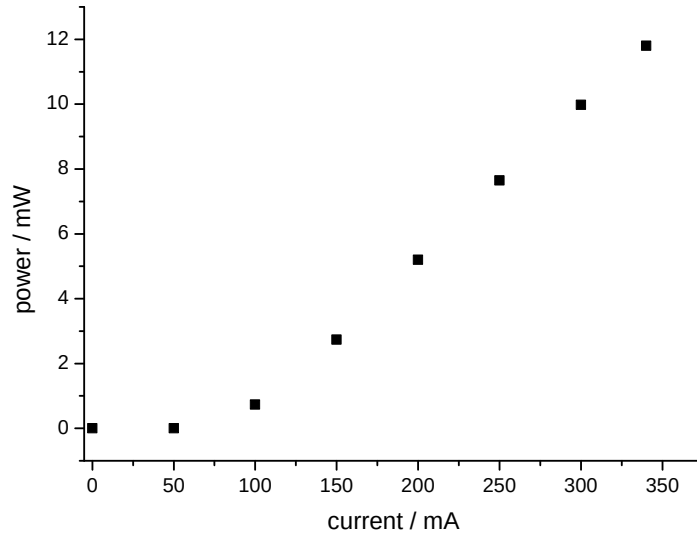


Figure 2.3: A plot of current against power for the SLED used in this thesis.

As a result of the large emission bandwidth of the SLED (~ 50 nm), it exhibits low temporal coherence. Coherence is a measure of the capability of emitted light to maintain its phase over time, and the coherence length, L_c , is determined from [128]:

$$L_c = k \frac{\nu_0^2}{\Delta\nu} \quad (2.14)$$

where $\Delta\nu$ is the bandwidth of the source output (taken as the full width half maximum), ν_0 is its central wavelength and k is a form factor which varies depending on whether the spectral output is Gaussian or Lorentzian in nature (0.66 and 0.32, respectively). The SLED used in this work has a central frequency of 1650 nm, with a bandwidth of 50 nm and an approximate Gaussian profile, indicating a coherence length of ~ 35 μm (compared to a coherence length of tens and hundreds of metres found with diode lasers). However the source, which operates over the spectral range $1.6 \mu\text{m} < \nu < 1.7 \mu\text{m}$, does have a high spatial coherence and a relatively high spectral power density of $\sim 200 \text{ mW nm}^{-1}$.

Furthermore, the high resolution spectrum of the SLED exhibits a variation in the spectral power density with respect to frequency, an effect known as the spectral ripple. This is a result of non-zero reflectivity of the SLED chip facets [128], and manifests itself chiefly around the central wavelength, where the gain is higher. An example of this, together with a figure of the total intensity output for the SLED used in this thesis, is illustrated in Figure 2.4.

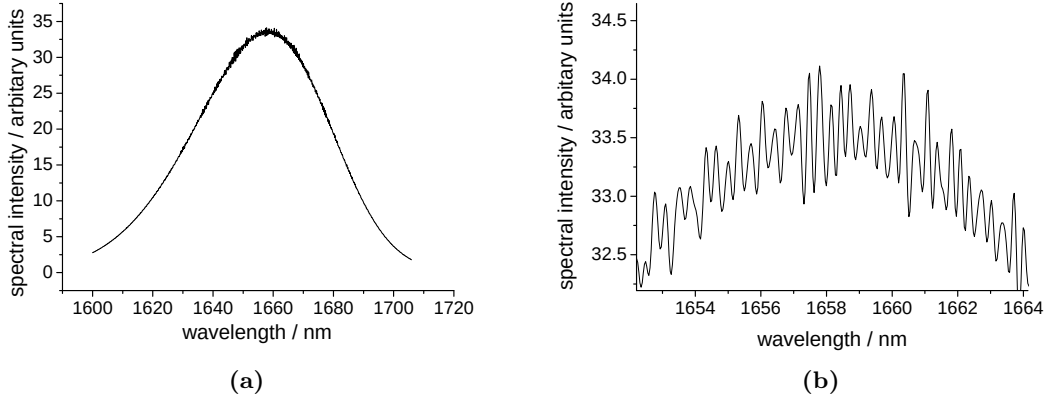


Figure 2.4: Figure (a) depicts the total intensity output of the SLED, whilst Figure (b) illustrates the spectral ripple observed with the SLED used in this thesis, taken at 0.5 cm^{-1} resolution on an FTIR spectrometer (Spectrum 100, Perkin Elmer).

Supercontinuum Source (SC)

The generation of supercontinuum radiation was first documented by Alfano and Shapiro in 1970 [130]: on focusing an intense narrow-band pulse onto various glasses, they observed that the resulting spectral output was dramatically broadened. This behaviour was attributed to a series of non-linear optic effects [131]. On interacting with light, the electron density distribution of each atom within a dielectric material will be perturbed by the oscillating electromagnetic field applied, resulting in the formation of induced electric dipole moments. In the regime of a relatively strong tuning field, such as that from a laser, the polarisation has a nonlinear response to the applied electric field and these nonlinear components lead to new frequencies being generated that are not present in the original incident radiation:

$$P = \epsilon_0(\chi^{(1)}E + \chi^{(2)}E^2 + \chi^{(3)}E^3 \dots) \quad (2.15)$$

where P is the polarisation of the material, E is the electric field strength, ϵ_0 is the vacuum permittivity, $\chi^{(1)}$ is the linear susceptibility and $\chi^{(x)}$ are the higher order susceptibility terms.

Supercontinuum generation (SCG) is based on third-order nonlinear processes and although a full description of the mechanisms behind SCG is beyond the scope of this thesis, further

information can be accessed via a number of reviews and books, such as [132, 133]. The optical Kerr effect, which is key to SCG, occurs when a high intensity wave, $I(\omega_2)$, changes the refractive index of the medium for a weak wave, itself having frequency ω_1 . This is known as cross-phase modulation and if the difference in frequencies happens to resonate with a frequency within the nonlinear medium, stimulated Raman scattering will occur. In the case where a beam modifies its own refractive index ($\omega = \omega_1 = \omega_2$), self-phase modulation is observed as a result of self-focusing [131]. Assuming that the incident beam has a Gaussian intensity profile, the refractive index will increase at the centre of the beam where the intensity is higher. Thus, the beam bends in on itself and as a result, the beam gets narrower and the intensity at the centre increases further so that the process is accelerated. If unabated, this self-focusing effect would lead to the beam collapsing, but it is counteracted by the effects of diffraction. The increase in intensity associated with self-focusing then causes a whole series of further third-order processes to occur, such as cross-phase modulation, self-steepening (when the peak of the pulse travels more slowly than its wings) and plasma formation, in addition to self-phase modulation.

Within optical fibres (as opposed to bulk media), self-focusing is absent and the spectral broadening occurs via stimulated Raman scattering and associated four-wave mixing processes, together with self- and cross-phase modulation [131]. The typical intensity output of a Supercontinuum source, reproduced from Kaminiski *et al.* [134], is depicted in Figure 2.5, next to the filtered output of the Supercontinuum (SC) used in this work (Fianium SC450-4), which utilises optical fibres together with a 1064 nm laser as the pump source to generate the Supercontinuum radiation.

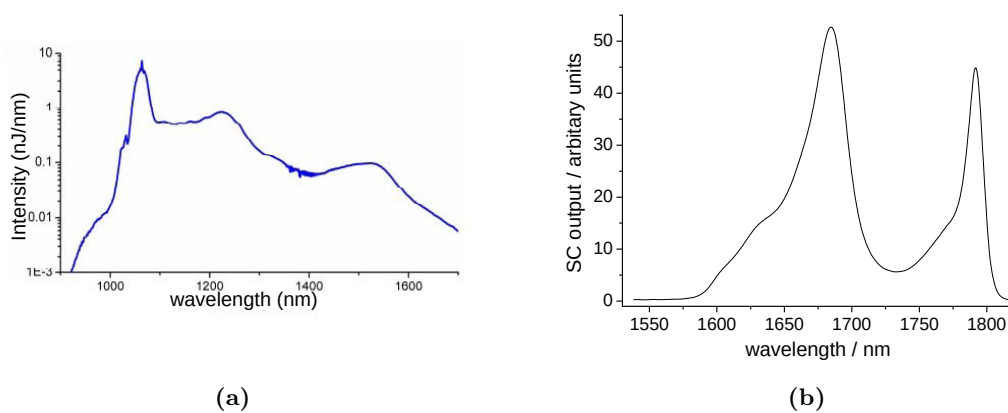


Figure 2.5: Figure (a) illustrates the typical intensity output of a Supercontinuum source, reproduced from Kaminiski *et al.* [134]; whilst Figure (b) depicts the output of the Supercontinuum source used in this thesis (Fianium SC450-4) on passing through a longpass and a bandpass filter.

This chapter has presented a brief introduction to the principles behind absorption spectroscopy, discussing the issues associated with the sensitivity of the technique and describing the diverse array of light sources applied to it throughout this thesis. The following chapter will introduce and demonstrate one way in which the sensitivity of an absorption spectroscopy measurement can be improved: by increasing the effective pathlength using an optical cavity.

Chapter 3

Application of laser-based CEAS to the detection of breath biomarkers

This chapter deals with preliminary studies investigating the feasibility of using cavity enhanced absorption spectroscopy (CEAS) for breath analysis. The first half of the chapter initially provides an introduction to CEAS-based techniques before the focus shifts to the detection of methane in breath using a DFB diode laser. The first spectroscopic application of a digital supermode distributed Bragg reflector (DS-DBR) laser, a novel, widely tunable, diode laser source, is described in the second half of the chapter with the detection of CO₂.

3.1 Cavity-Enhanced techniques

As described in Chapter 1, laser-based spectroscopic techniques offer a non-invasive and rapid means to identify species found in breath. Diode lasers in particular represent a relatively cheap, compact and readily available radiation source thanks to their ubiquitous use in the telecommunications industry. In the near-infrared (NIR) these devices enable the vibrational overtones and combination bands of many small molecules to be probed. Although the absorption cross-sections, σ , associated with these transitions are much smaller than those of the fundamental vibrations and electronic transitions in the mid-infrared (MIR) and the ultraviolet (UV) respectively, an increase in the pathlength of the radiation through the sample, L , will compensate for this and increase the sensitivity of the spectroscopic method to the molecular species. This can be achieved using multipass cells, such as the White and Herriott cells, which can provide pathlengths of up to 50 - 250 m [135] and will be described in later sections of this thesis. There are many examples of breath analysis

with diode lasers and multipass cells, a selection of which have been described in Chapter 1 [1, 6]. Moskalenko *et al.* [76] studied CO and CO₂ in breath using tunable diode laser spectroscopy in the MIR with a Herriott cell, accessing the wavelengths at 4.76 and 4.91 μm (for CO and CO₂, respectively), 10.34 μm (for NH₃) and 3.35 μm (for CH₄). Herriott cells have also been used in the detection of exhaled nitric oxide [31, 58], in the measurement of carbon isotope ratios [19, 61] and in monitoring carbonyl sulphide [75]. However, multipass cells are often limited by optical fringing and mechanical instabilities, and the large volumes often associated with such instruments (of the order of several litres, for example 16 l in [31] and 3.5 l in [57]) are not ideal for breath sampling, although the use of astigmatic mirrors can minimise the volume and the Namjou study [58] demonstrated the use of a compact Herriott cell (0.3 l) for breath analysis. However, far greater pathlengths can be obtained from utilising an optical cavity, a device consisting of two highly reflective mirrors a set distance apart, which in addition typically have much smaller volumes, making them more suitable for breath analysis.

3.1.1 Optical cavities

The simplest optical cavity is based on the Fabry-Perot interferometer used in etalons, which provide frequency calibration for spectroscopic measurements. A Fabry-Perot cavity consists of two parallel plane highly reflective mirrors, with R the geometric mean of the reflectivities [115]. The transmitted light intensity through such an arrangement, I_T , when illuminated by a perfect, coherent, white light source, [136] is given by the Airy function:

$$\frac{I_T}{I_0} = \frac{1}{1 + \frac{4R}{(1-R)^2} \sin^2 \frac{\delta}{2}} \quad (3.1)$$

where I_0 is the radiation incident on the resonator and $\delta = 2nl \cos \theta + 2\eta$, the phase difference between successive transmitted waves; η is the phase change on reflection from the mirrors of the Fabry-Perot interferometer and $2nl \cos \theta$ is the optical path difference between successive transmitted waves (with θ the angle at which the beam hits the mirrors of the resonator, l the interface spacing and n the refractive index between the interfaces).

Physically, this results in a comb-like transmission function, as depicted in Figure 3.1. The maxima in the function correspond to longitudinal cavity modes, and are the result of the constructive interference which occurs when the separation between the cavity mirrors is equal to an integer number of half wavelengths, $\frac{n\lambda}{2}$. The separation between the consecutive longitudinal modes is known as the free spectral range (FSR) and is given by [115]:

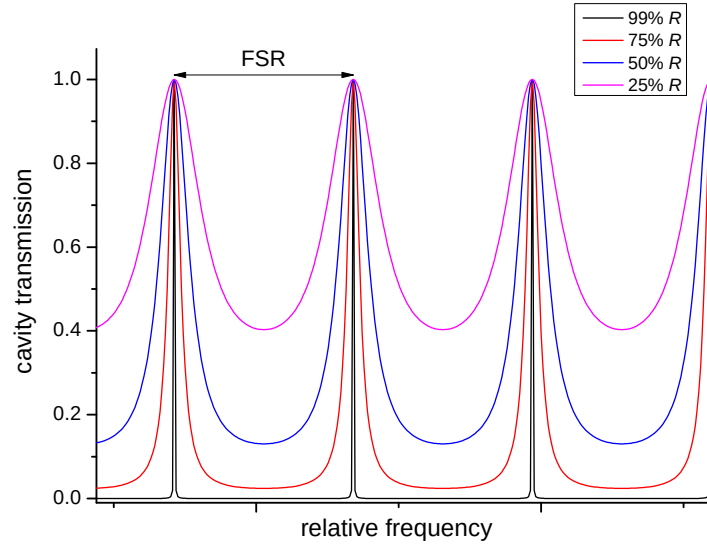


Figure 3.1: The Airy function; simulating the cavity transmission profile for various mirror reflectivity values. The frequency difference between the transmission maxima is known as the free spectral range (FSR).

$$\delta\nu_{\text{FSR}} = \frac{c}{2n_r L} \quad (3.2)$$

where c is the speed of light, n_r is the refractive index of the medium within the cavity and L is the cavity length. Figure 3.1 also illustrates that the bandwidth of the transmission function for a single mode decreases with an increase in the mirror reflectivity as a result of the subsequent increase in cavity finesse, F [115]:

$$F = \frac{\pi\sqrt{R}}{(1-R)} \quad (3.3)$$

Scanning the laser or varying the length of the cavity introduces a perturbation into the optical system, which is greater for higher reflectivity mirrors as a result of the increased effective pathlength of the radiation within the cavity. Consequently, there is a narrower frequency region in which the resonance condition holds, which manifests itself as the narrowing of the bandwidth of the cavity modes. Therefore, in addition to being a measure of the potential of the cavity to support constructive interference over a long pathlength, the finesse relates the full width half maximum (FWHM) of the modes, which have Lorentzian line profiles, to the FSR of the cavity, $\delta\nu_{\text{FWHM}} = \frac{\delta\nu_{\text{FSR}}}{F}$. The greater the finesse, the greater the intensity that builds up within the cavity: the maximum possible circulating power is given by the product of the finesse and the power incident on the cavity [115].

Another consideration is the stability of the optical cavity: in other words, the ability of the cavity to trap the radiation within it (i.e. to support cavity modes), whilst keeping diffraction losses low. There are well defined cavity stability criteria which ensure that an injected light beam is re-entrant. These are determined from the geometry of the optical cavity, namely the radii of curvature of the mirrors and the distance between them, and are quantified using g -parameters, assuming the trapped beam in question is Gaussian shaped (as is the idealised case with radiation from a laser) [115]:

$$g_i = 1 - \frac{L}{r_i} \quad (3.4)$$

where L is the distance between the cavity mirrors and r_i is the radius of curvature of mirror i . A cavity is then stable if $0 \leq g_1 g_2 \leq 1$, which reduces to $0 < L < r$ and $r < L < 2r$ if the two mirrors are identical [137]. In the situation whereby $L = r_1 = r_2$, the cavity is said to be symmetric confocal, and along with concentric ($r_1 = r_2 = L/2$) and plain parallel arrangements ($r_2 = r_2 = \infty$), they represent configurations which are on the boundary of the stability curve.

As with the Fabry-Perot interferometer described earlier, it is the geometry of the optical cavity which then determines its modal properties. Whereas the Fabry-Perot resonator consists of two parallel plane mirrors, in this instance the mirrors are spherical, with g -parameters, g_1 and g_2 , and the frequencies of the resonant Gaussian modes within the cavity are given by [137, 138]:

$$\nu_{qmn} = \frac{c}{2L} \left[q + \frac{n+m+1}{\pi} \arccos(\sqrt{g_1 g_2}) \right] \quad (3.5)$$

where q is an integer that describes the longitudinal characteristics of the mode (i.e. determines its gross frequency), whilst integers m and n represent its transverse characteristics, or the variation seen in the spatial geometry of the mode as a result of the spatial variations in the electromagnetic field in the plane perpendicular to the cavity axis. For each longitudinal mode, q , there are associated transverse modes, described by TEM _{mn} notation, which have different values for m and n [136, 138]. The frequency difference between two longitudinal modes where m and n are the same is the free spectral range, as illustrated in Figure 3.1. In addition, Figure 3.2 illustrates the cavity output imaged by an infrared camera for a selection of TEM _{mn} modes.

The laser beam is focused into the cavity in such a way that the size of the beam incident on the cavity mirrors is much smaller than their diameter, which minimises the losses due to diffraction. The beam size is governed by the degree to which the transverse modes extend

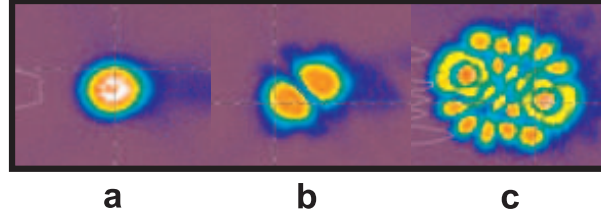


Figure 3.2: **a** TEM₀₀; **b** TEM₁₀; **c** a high order mode; note, some non-Gaussian behaviour can be seen in **c**, possibly due to some Laguerre characteristics of the beam.

into space, which is dependent on how the intensity of the beam profile varies throughout the cavity. Other transverse modes are possible and are well represented by Laguerre polynomials or a Hermite-Laguerre combination [136], however the simplest solution is the Hermite-Gaussian mode-set, represented by the aforementioned TEM_{mn} notation. Figure 3.3 illustrates how such a Gaussian beam is trapped within an optical cavity, where the spot size at the beam waist (the focal point of the cavity) is given by [136]:

$$w_0^2 = \frac{L\lambda}{\pi} \sqrt{\frac{1+g}{4(1-g)}} \quad (3.6)$$

and at the mirrors [136]:

$$w_{1,2}^2 = \frac{L\lambda}{\pi} \sqrt{\frac{1}{1-g^2}} \quad (3.7)$$

where the two mirrors are identical and L is the distance between the two mirrors.

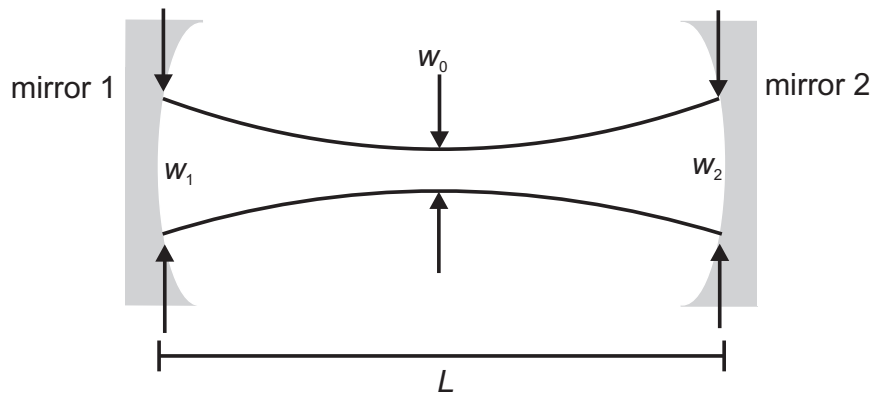


Figure 3.3: A schematic diagram of a trapped Gaussian beam within an optical cavity, illustrating the beam waist at the centre (w_0), and at the two mirrors ($w_{1,2}$).

Knowledge of these spot sizes is important for mode-matching the laser output with the cavity modes, which is often necessary for cavity-based detection schemes. Clearly, in order for light from a radiation source to couple into the cavity, its modal frequencies must

resonate with those of the cavity. In addition, the greater the spatial overlap between the laser and cavity modes, the greater the efficiency of the system and the greater the build up of intensity within the cavity. In such a system, the mode-matching optics are chosen so that the beam size and divergence of the incident radiation matches that of the TEM₀₀ mode supported by the cavity geometry so that it is preferentially excited. This is essential for mode-locked techniques, such as Optical Feedback Cavity Enhanced Absorption Spectroscopy (OF-CEAS) [139], and for Cavity Ring-Down Spectroscopy (CRDS) [140]. Cavity Enhanced Absorption Spectroscopy (CEAS) [141, 142], on the other hand, which forms the basis of the work in this chapter, has less stringent requirements and is described in section 3.1.3.

3.1.2 Cavity Ring-Down Spectroscopy (CRDS)

In Cavity Ring-Down Spectroscopy (CRDS), after power is allowed to build up within the cavity the light injected into it is rapidly switched off, such that the temporal evolution of the light intensity which leaks out can be measured. The following description is based on that detailed in Engeln and Berden [136]. The early work on CRDS [140] used ns pulsed lasers, where the pulse provides a well-defined point in time from which to monitor the ‘ring-down’ time of the cavity. On injection of a pulse into the cavity, and assuming the duration of the pulse is shorter than that of the round-trip time within it, the first pass will result in a detected intensity given by [143]:

$$I_0 = I_{\text{in}} T^2 \exp(-\alpha d) \quad (3.8)$$

where T is the transmission of the mirrors (i.e. $T = 1 - R$, where R is the geometric mean of the reflectivity of the mirrors) and $\exp(-\alpha d)$ is the reduction in the initial intensity due to the absorption or scattering loss of the sample (with α the absorption coefficient and d the length of the sample within the cavity). On the second pass, the intensity detected will be further reduced by a factor of $R^2 \exp(-2\alpha d)$, on account of the light reflecting off both of the mirrors and passing through the sample twice before reaching the detector. Therefore for n round trips the intensity is:

$$I_n = I_0 R^{2n} \exp(-2n\alpha d) \quad (3.9)$$

Normally, the detector is not fast enough to detect each discrete pulse, so it records a time-smoothed exponential decay in intensity. This allows the number of round trips, a discrete variable, to be converted into a continuous time variable via $t = \frac{2Ln}{c}$, where L is the length of the cavity. Equation 3.9 then becomes:

$$I(t) = I_0 \exp\left[-\frac{tc}{L}(-\ln R + \alpha d)\right] \quad (3.10)$$

The time constant for the decaying light intensity, known as the ring-down time of the cavity, is thus:

$$\tau = \frac{L}{c(-\ln R + \alpha d)} \quad (3.11)$$

In the situation where the mirrors are of high-reflectivity ($R \rightarrow 1$), this reduces to

$$\tau = \frac{L}{c[(1 - R) + \alpha d]} \quad (3.12)$$

as $\ln R \approx -(1 - R)$. Typical ringdown times are of the order of μs for mirrors of $R \sim 0.9999$, whilst sensitivities are regularly $\sim \times 10^{-9} \text{ cm}^{-1}\text{Hz}^{-\frac{1}{2}}$. Equation 3.12 highlights an important characteristic of CRDS, in that the ring-down time is independent of any intensity fluctuations of the laser. Furthermore, knowledge of the ring-down times when an absorber is present and absent, τ and τ_0 respectively, can be used to determine the absolute concentration of the absorber if the absorption cross-section for that particular frequency is known.

$$\alpha(\omega) = \sigma(\omega)C \quad (3.13)$$

$$= \frac{\Delta\tau}{\tau_0\tau} \frac{L}{cd} \quad (3.14)$$

where $\alpha(\omega)$ is the absorption coefficient, $\sigma(\omega)$ is the absorption cross-section, C is the species concentration, $\Delta\tau$ is $\tau_0 - \tau$, c is the speed of light, L is the length of the cavity and d is the length of the sample within L .

Although pulsed lasers have high powers, they generally have high bandwidths (resulting in low spectral resolution) and have relatively poor spatial properties such that they often excite more than one cavity mode, which results in a multi-exponential decay as each mode excited will decay at a slightly different rate. More recently, continuous wave (cw) lasers have been used with CRDS, which allow far greater spatial resolution but do require the laser radiation incident on the cavity to be rapidly switched off. This is usually achieved using an acoustic-optic modulator (AOM): as light is injected into the cavity, a power build up will only occur when the laser and cavity modes are in resonance; therefore, by monitoring the intensity output a trigger can then be sent to the AOM the moment that this occurs, which will then cause the laser beam to be deflected from the cavity and the resultant ring-down trace can be recorded.

CRDS provides a sensitive technique for detecting absolute concentrations of a species, but it has quite strict experimental requirements, including the necessity of exciting purely one cavity mode and the use of fast optical switches and detectors (the latter of which must be able to respond in a time shorter than τ in order for the exponential decay to be recorded). A more robust technique is the closely related Cavity Enhanced Absorption Spectroscopy (CEAS) technique, which will be described next.

3.1.3 Cavity Enhanced Absorption Spectroscopy (CEAS)

Cavity Enhanced Absorption Spectroscopy (CEAS), also known as Integrated Cavity Output Spectroscopy (ICOS) and variants, involves the continuous injection of light into an optical cavity and the simultaneous detection of the time-integrated light intensity as it leaks out of the cavity [141, 142, 144]; i.e. light is trapped between the high reflectivity mirrors, but a small fraction of it will leak out and represents well the average intensity of the light within the cavity. The technique is closely related to CRDS and it has been shown [142, 145] that the integrated intensity at any particular laser frequency is proportional to the cavity ring-down time. However, in contrast to CRDS, CEAS relies on exciting many of the higher order cavity modes, including re-entrant modes (rather than one low order mode) so that the mode density increases and a quasi-continuous transmission is detected. In order to remove the effects of individual cavity modes, the laser is scanned rapidly through more than one FSR and the cavity is aligned such that many higher order cavity modes are excited [115, 135, 146]. Thus, a multitude of modes are randomly and continuously excited as the laser sweeps repeatedly over the spectral region of interest. Often mechanical vibrations are introduced to arbitrarily change the length of the cavity so that on each sweep of the laser, the cavity mode structure will be slightly different, thus reducing the effects of mode structure in the overall detected signal. All of these influences mean that the transmission effectively becomes frequency-independent and a smoother averaged baseline is obtained, resulting in a higher signal to noise ratio.

In Mazurenka *et al.* [115], a theoretical framework is set out for CEAS, assuming that the cavity transmission is mode-less and that the cavity mirrors are identical, non-absorbing and non-scattering, such that the transmission through a mirror is given by $T = 1 - R$, where R is the mirror reflectivity. It is on this work [115] that the following description is based. The incident intensity, I_{in} on making one pass through the cavity is thus attenuated by the reflectivity of the first cavity mirror, the single-pass absorption of the sample, A , and by the reflectivity of the second cavity mirror, giving a transmitted intensity of $I_{out} = I_{in}(1 - R)^2(1 - A)$, as illustrated in Figure 3.4.

Clearly this analysis can be extended to an infinite number of passes:

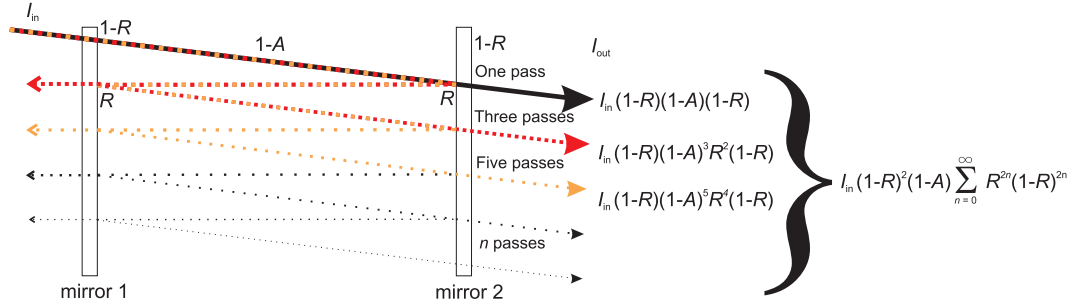


Figure 3.4: A schematic diagram illustrating the variations seen in the intensity of the radiation passing through an optical cavity.

$$I_{\text{out}} = I_{\text{in}}(1 - R)^2(1 - A) \sum_{n=0}^{\infty} R^{2n}(1 - A)^{2n} \quad (3.15)$$

When $R < 1$ and $A < 1$, this can then be expressed as a sum of a geometrical progression:

$$I_{\text{out}} = I_{\text{in}} \frac{(1 - R)^2(1 - A)}{1 - R^2(1 - A)^2} \quad (3.16)$$

In the situation where no absorber is present, the $(1 - A)$ terms will disappear and $I_{\text{out}}^0 = I_{\text{in}} \frac{1-R}{1+R}$. If the reduction in intensity due to absorption in Equation 3.16, $(1 - A)$, is then replaced with the expression from the Beer-Lambert law, $\exp(-\alpha L)$, and the ratio of the transmitted intensities when the absorber is absent and when it is present, $I_{\text{out}}^0/I_{\text{out}}$, is taken, the following is obtained:

$$\frac{I_{\text{out}}^0}{I_{\text{out}}} = \frac{1 - (R \exp(-\alpha L))^2}{(1 - R^2) \exp(-\alpha L)} \quad (3.17)$$

In the limit of low absorption, $\exp(-\alpha L)$ reduces to $1 - \alpha L$ and when high reflectivity mirrors are used ($R \rightarrow 1$), $R\alpha L$ becomes αL , so that Equation 3.17 transforms to:

$$\frac{I_{\text{out}}^0 - I_{\text{out}}}{I_{\text{out}}} = \frac{\alpha L}{(1 - R)} \quad (3.18)$$

Thus, in order to extract an absolute absorption spectrum, a knowledge of the mirror reflectivity is required. Therefore, in contrast to CRDS, CEAS is not an absolute absorption technique. The mirror reflectivity can, however, be determined from carrying out CRDS measurements on the cavity or from measuring the absorption from a sample of known pressure and absorption cross-section. With the former, the two alignments may have slightly different effective mirror reflectivities as the two types of alignment lead to different sections of the mirrors being utilised, and there may also be different losses due to diffraction. With

the latter, the accuracy of the mirror reflectivity calibration is dependent on the accuracy of the pressure reading of the sample. An additional issue with CEAS is that amplified spontaneous emission (ASE) from the laser will often constitute some of the signal, causing a broad, frequency independent background signal to contribute to the cavity enhanced signal. This can be readily removed either by filtering the output of the laser or by misaligning the cavity and taking a reading from the detector: the signal produced will be solely due to “uncoupled” light passing straight through the cavity.

Finally, CEAS centres on the recording of intensity, rather than the time-domain measurements of CRDS. This means that it is dependent on intensity fluctuations of the laser, and as such, generally is a less sensitive technique than an equivalent CRDS experiment: CRDS regularly attains sensitivities $\sim 10^{-9} \text{ cm}^{-1}\text{Hz}^{-1/2}$, whilst CEAS tends to be in the range $10^{-8} - 10^{-9} \text{ cm}^{-1}\text{Hz}^{-1/2}$ [136] (although sensitivities reaching $10^{-10} - 10^{-11} \text{ cm}^{-1}\text{Hz}^{-1/2}$ have been reported [147] using an ‘off-axis’ technique). However, for most applications the level of detection provided by CEAS is more than sufficient and coupled with the relatively straightforward and robust experimental arrangement, it is far more suitable for practical applications.

3.2 Methane in breath: an initial study

Methane is an ideal molecule to test the suitability of CEAS in breath analysis as it is present in widely varying quantities depending on whether the subject is a methane-producer or not, it has well-resolved narrow absorption transitions which are easily probed with a diode laser, and it has sufficiently large NIR absorption cross-sections which are easily detectable using standard cavity-based techniques.

3.2.1 Methane in breath

Between 30 - 50% of people in western populations are so-called CH_4 producers [148], with breath levels of CH_4 above that found naturally in the atmosphere ($\sim 1.8 \text{ ppm}$ [149]). Methane production does not occur through normal metabolic activity in the body, but may occur through the action of enteric methanogenic archaea, such as *Methanobrevibacter smithii* [150–152], in the intestinal tract. Under the anaerobic conditions found in the colon, these bacteria will reduce CO_2 , a byproduct of digestion, to CH_4 by using the H_2 produced by another set of flora, hydrogenic bacteria (like *Prevotellaceae*), which also thrive in this environment. CH_4 and H_2 from the colon are then absorbed into the bloodstream via the intestinal wall and transported to the lungs where they diffuse into the alveoli before being excreted during pulmonary respiration. As the human body does not metabolise CH_4 , if

the levels of methane in a breath sample exceed the 1.8 ppm baseline CH_4 concentration in ambient air by more than 1 ppm, it is a reasonable indicator of methane production in the intestine and that the subject is a methane producer. Thus, the ability to measure exhaled levels of CH_4 and H_2 sensitively are of significant interest as potential probes of gastro-intestinal physiology [153–157]. For example, the measurement of CH_4 and H_2 in breath following the ingestion of lactose is used in lactose intolerance testing [158–160]: in a lactose tolerant person, the lactase enzyme in their small intestine will digest the lactose ingested; however, lactose intolerant individuals lack this enzyme so the lactose will pass, undigested, into the colon where it is metabolised by anaerobic bacteria. This gives rise to elevated levels of H_2 , some of which will then be converted into CH_4 by methane-producers. Therefore, the monitoring of the concentration of both gases in breath is required for accurate interpretation of the results [161]. Similar tests can also be performed to determine malabsorption of other sugars [162, 163].

The small intestine, by contrast, is normally substantially free from bacterial colonisation, but an increase in the number of bacteria (above 10^5 CFU/ml, where CFU is colony forming units) leads to Small Intestinal Bacterial Overgrowth (SIBO) [164]. There is growing evidence that SIBO is a contributing factor in a large number of patients suffering from Irritable Bowel Syndrome (IBS). Lactulose (or glucose) can be used as a test for SIBO; if the sugar is metabolised by bacteria in the small intestine, there will be an early onset of elevated breath H_2 following ingestion [165, 166]. As with lactose intolerance testing, simultaneous measurement of CH_4 is desirable to identify when the sugar has rapidly traversed to the colon: this will result in the metabolism of lactulose by methanogens. In addition, Hwang *et al.* have established that elevated breath methane is strongly correlated with constipation-predominant IBS [167], and as such CH_4 may have use as a diagnostic marker to guide appropriate therapy. Finally, recent studies have shown a higher prevalence of intestinal methanogens amongst obese individuals as compared to people of normal weight, or those having undergone a gastric-bypass [168, 169], perhaps suggesting a possible role for breath methane monitoring as a tool in treating obesity.

Therefore, laser spectroscopy could be used as a non-intrusive way to obtain quick and reliable identification of methane in a breath sample, the presence of which could then provide diagnosis of gastrointestinal conditions.

3.2.2 The Spectroscopy of Methane

Breath methane concentrations were monitored using the absorption feature at ~ 6057.09 cm^{-1} , which consists of 4 ro-vibrational transitions within the $2\nu_3$ vibrational overtone. The degeneracy of the vibrational band is lifted by Coriolis forces, which describe the interaction

between vibrational and rotational modes of the molecule, resulting in multiple components for each rotational transition [108]. Therefore, the initial and final state for each transition is described by $(J', C', \alpha') \leftarrow (J'', C'', \alpha'')$, where J is the total angular momentum quantum number excluding nuclear spin, C is the ro-vibrational symmetry of the state and α is a counting integer for levels of the same J and C , incremented in order of increasing energy [170]. Using this nomenclature, the 4 ro-vibrational transitions studied in this work are: $6057.0778 \text{ cm}^{-1}$, $(5, E, 1) \leftarrow (4, E, 1)$; $6057.0861 \text{ cm}^{-1}$, $(5, A_2, 1) \leftarrow (4, A_1, 1)$; $6057.0998 \text{ cm}^{-1}$, $(5, F_2, 1) \leftarrow (4, F_1, 1)$; $6057.1273 \text{ cm}^{-1}$, $(5, F_1, 1) \leftarrow (4, F_2, 1)$. In order to resolve the individual transitions the sample would have to be cooled; at room temperature and pressure the transitions are unresolved and appear as one absorption feature, as illustrated in Figure 3.5.

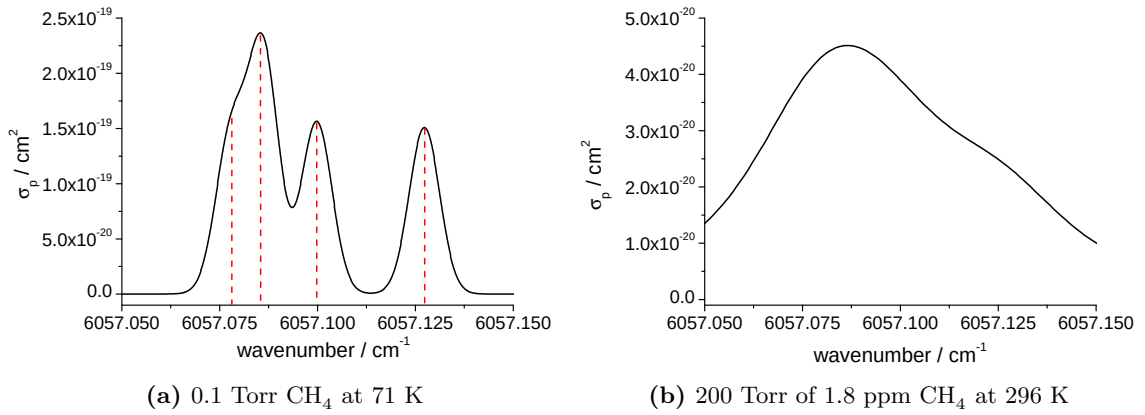


Figure 3.5: Graph (a) shows the simulated peak absorption cross-sections of pure CH_4 under the conditions of 0.1 Torr and 71 K. Three of the ro-vibrational transitions can be identified, whilst the largest feature exhibits a shoulder due to the fourth. Graph (b) depicts a simulation of 1.8 ppm CH_4 in N_2 at 200 Torr and at a temperature of 296 K [171]. This clearly illustrates the broadening of the spectral features that takes place at higher total pressures and temperatures.

This feature was chosen because it is well separated from CO_2 and H_2O absorptions, as illustrated in Figure 3.6, which would otherwise cause interferences with the methane absorption recorded.

3.2.3 Experimental

Light from a 1.65 μm semiconductor diode laser (NTT Electronics, NLK1U5E1AA, butterfly package, 10 mW) with a quoted bandwidth of 2 MHz was injected into an optical cavity, which consisted of two high reflectivity mirrors (Laseroptik, quoted $R \sim 99.95\%$, 1 m radii of curvature) 34.5 cm apart. The mirrors were held in adjustable mounts attached

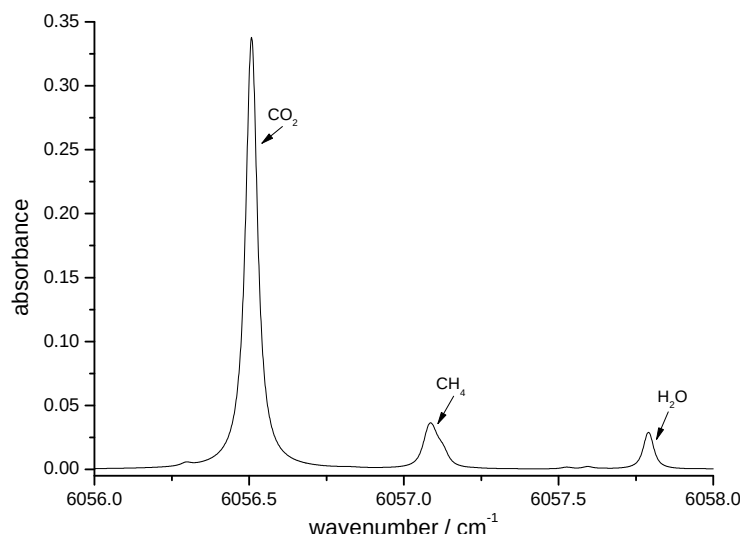


Figure 3.6: Simulation illustrating the absorbance seen in a 200 Torr sample of typical breath (5% H₂O, 4% CO₂, 1.8 ppm CH₄), in a cavity with $R = 99.95\%$ [171].

to bellows which allow small movements in the mirror positions whilst maintaining a vacuum seal. The two bellows were connected with a steel tube, with an internal diameter of 20 mm, to form the absorption cell. This is illustrated in Figure 3.7. The cell was evacuated using an oil-free diaphragm vacuum pump (KNF) and the internal pressure was measured using a capacitance manometer (Leybold Ceravac, 0-1000 Torr). Initially, the optical cavity was aligned in such a way that only low order modes were excited, before the angle of incidence was increased to invoke an off-axis alignment and the cavity misaligned so that many higher order modes were excited to produce a uniform cavity transmission.

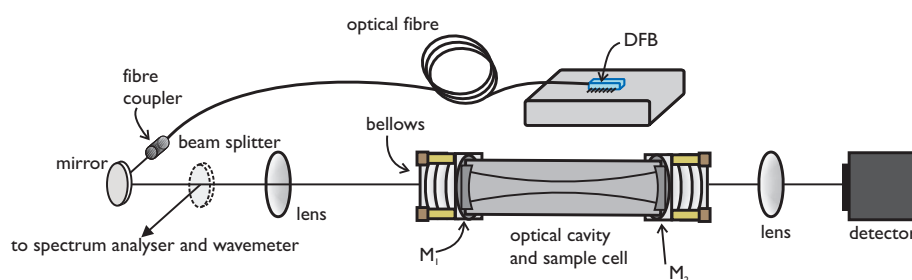


Figure 3.7: Illustration of the experimental set-up: the output from a DFB diode laser was directed via a series of mirrors and lenses into an optical cavity of length 34.5 cm, the output from which was focused into a photodiode detector.

The laser was tuned to the desired spectral region using a combination of current and temperature control (Thorlabs ITC 510) and the laser was scanned across the absorption feature of interest by applying a voltage ramp from a signal generator (Thurlby Thandar Instruments TG1010A) to the current driver of the diode laser. The frequency scale was determined using a Fabry-Perot type optical spectrum analyser (Thorlabs SA200-14A, 1.5

GHz FSR) into which a partial reflection of the beam was directed. The absolute frequency scale was verified by directing the radiation via a flipper mirror into a wavemeter (Burleigh WA-1000). On passing through the cavity, the laser radiation was focused onto a photodiode (Thorlabs DET410) and the signal amplified and acquired using a digital storage oscilloscope (LeCroy WaveSurfer 424).

3.2.4 Subjects and sampling

The breath samples used in this study were collected from 48 random volunteers between the ages of 21 and 65. After written, informed consent was obtained from all the participants, they were asked to inhale normally and then exhale the initial part of the breath before exhaling the rest into a collection bag (Fischer Analysen Instrumente GmbH, F201-VP-05c, 1.5 litres), which was fitted with a mouth piece valve (Fischer Analysen Instrumente GmbH, F201-VP11). This ensured the majority of the sample was end tidal, so that the measurements undertaken would be representative of the gas exchange taking place in the alveoli and not affected by the dead space volume that makes up the rest of the respiratory tract. The samples were collected at different times during the day from the different volunteers and no attempt was made to control their fasting status. The breath sample bags were connected to the evacuated absorption cell before it was flushed with a portion of the breath sample to ensure the removal of any air from the dead space within the vacuum system. Samples of ~ 200 Torr were then introduced into the absorption cell for measurement; the measurements on the breath samples were made within 24 hours of collection.

3.2.5 Data processing and analysis

In order to determine an absorption spectrum from a CEAS experiment, the transmitted intensities through the cavity are compared in the absence (I_0) and presence (I) of an absorber. The resulting raw data are treated in accordance with Equation 3.18, as illustrated in Figure 3.8.

As $\alpha = \sigma C$, assuming the absorption cross-section of the feature is known, the concentration of the sample can be extracted once the mean mirror reflectivity has been determined. As described in the previous section, this can be deduced from measuring the absorption due to calibrated samples of known pressure. Samples from a 49.1 ppm CH_4 in N_2 mix (CK Gas) were passed into the absorption vessel at various pressures and the spectra recorded, as illustrated in Figure 3.9.

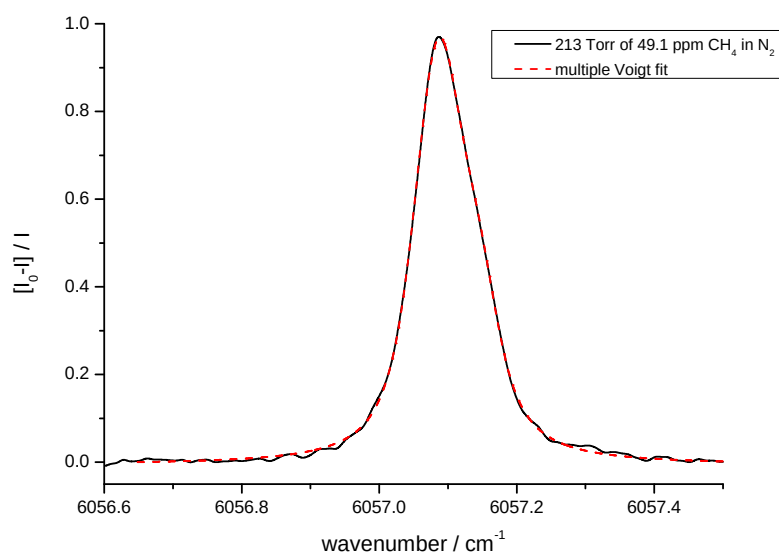


Figure 3.8: An example spectrum of 212 Torr of 49.1 ppm CH_4 in N_2 mix.

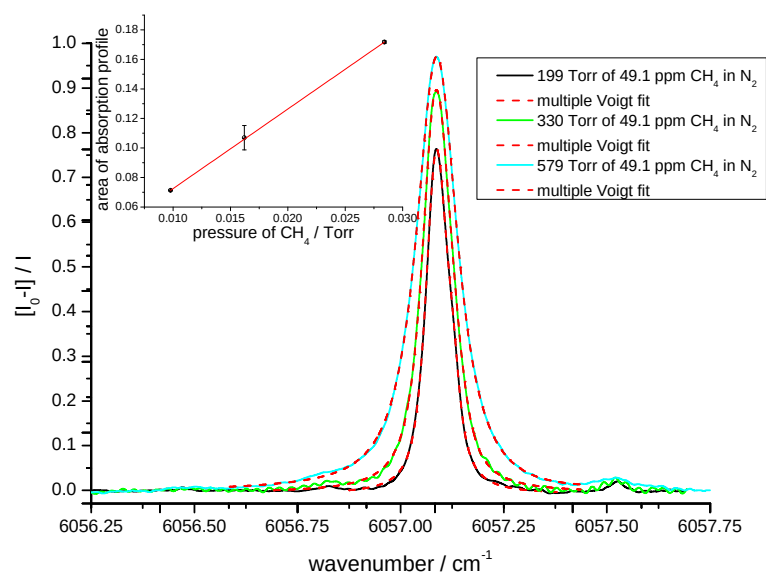


Figure 3.9: Example spectra from various pressures of a 49.1 ppm CH_4 in N_2 mix; inset, determination of the mirror reflectivity.

A multi Voigt lineshape was used to fit the data as, at the lower pressures in particular, a shoulder is still just visible due to the fact the feature is comprised of 4 overlapping methane lines. The optimised area of the fitted spectral lineshape was then plotted against the concentration of the absorption and a mean reflectivity value of $R = 99.92\%$ determined from the slope of the plot, as depicted as an inset of Figure 3.9.

The sensitivity of a CEAS measurement is described by the minimum detectable absorption coefficient, $\alpha_{\min}$. This is based upon the minimum detectable change in the output intensity in the presence of the absorbing medium, $\Delta I_{\min}$, which is taken to be one standard deviation (SD) in the signal over a region of the baseline approximately twice the full width half maximum (FWHM) of the absorption feature. On optimisation of the cavity alignment to reduce residual mode structure, the minimum detectable absorption coefficient, $\alpha_{\min}$, was found to be $6.5 \times 10^{-8} \text{ cm}^{-1}$ in a 5 s acquisition time, which is equivalent to a minimum detectable concentration at atmospheric pressure of 600 ppb of CH_4 . This is sufficiently lower than the 1.8 ppm average CH_4 concentration found in the atmosphere to allow accurate determination of the methane concentrations found in human breath.

Conventionally, the background ASE is determined by misaligning the cavity and recording the resultant signal on the detector. However, as this requires the alignment of the cavity to be altered, it is preferable for this to be avoided. An alternative approach is to compare the absorption at the line centre from the breath sample with the measured absorption signal for various calibrated CH_4 in N_2 mixes at the same pressure. This allows the absorptions from the breath samples to be calibrated without changing the cavity alignment and it also acts as a test for changes to the mirror reflectivity as a possible consequence of contamination. The two methods were compared and found to give equivalent CH_4 concentrations within the error bounds of the measurements. Consequently, the breath samples of the 48 volunteers were analysed in the more time efficient manner by referring to the calibrated signal intensities.

3.2.6 Results and Discussion

The CEAS spectra of the breath samples were analysed as described in the previous section and the methane concentration in each determined. Example spectra from the breath of both a non-methane producer and a methane-producer are illustrated in Figure 3.10.

A non-methane producer would be expected to have a breath CH_4 level of ~ 1.8 ppm, the average concentration found in the atmosphere [149], as it is generally accepted that there is no metabolic process consuming methane [172]. In line with the practice of other studies [153, 172, 173], CH_4 production is defined as 1 ppm above the baseline of 1.8 ppm, and given the ± 1 ppm uncertainty in the measurements of this study (derived from the variability in repeat measurements on the same breath sample made on different days), a 4 ppm cut-off value for the positive identification of methane production has been imposed here. Table 3.1 depicts the collated results from the measurements. The participants diagnosed with IBS or SIBO are marked with *, ^c indicates those diagnosed with Crohn's disease, ^v and ^d

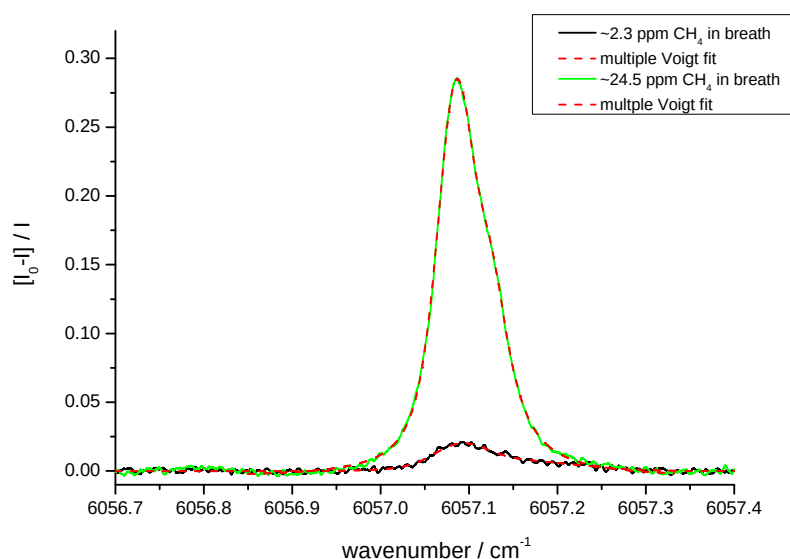


Figure 3.10: Example spectra from breath samples illustrating the absorption seen for a non-methane producer and a methane producer; the areas of the Voigt fits indicate methane concentrations of 2.3 ppm and 24.5 ppm respectively.

mark those with a vegetarian and dairy-free diets respectively. The variation seen in the breath CH_4 concentrations is illustrated graphically in Figure 3.11.

From these data, it was found that 29% of the volunteers had a significant elevation of CH_4 concentration in their breath. The average CH_4 concentration of the methane producers was 11.1 ± 5.9 ppm, where the quoted range is the standard deviation around the mean. 19% of the women tested showed elevated CH_4 levels, compared to 41% of the men, though the sample size in this study is too small to draw any conclusions from these figures. The average CH_4 concentration of methane producers separated by gender was 13.5 ± 6.5 ppm and 9.8 ± 5.5 ppm for women and men respectively. Within this study, six of the volunteers indicated that they had been diagnosed with IBS or SIBO and a further person that they had a suspected case (no attempt was made to verify this). Of these six, 2 were found to be methane producers. Additionally, two of the subjects suffer from Crohn's disease and neither of these cases were found to have raised CH_4 levels. This is in keeping with the literature in which methanogen incidence in people with inflammatory bowel diseases, such as Crohn's, is found to be reduced [153, 174, 175]. However, the number of participants in this study is small in comparison to other studies in the literature and the number of people with Crohn's disease too low to draw any conclusions from this work.

The CH_4 concentration is shown as a function of age in Figure 3.12, with men and women distinguished as filled and hollowed circles respectively. Previous studies have shown that methane production increases with age [148, 176, 177], but here, although there is a weak

Sample no.	Sex	Age	[CH ₄] (ppm)	Sample no.	Sex	Age	[CH ₄]
1	F	21	2.2	25	F	48	23.8
2	F	22	1.8	26 ^d	F	55	2.3
3	F	24	2.0	27	M	24	1.9
4	F	24	1.5	28	M	24	2.2
5	F	25	15.6	29	M	25	2.0
6	F	26	2.5	30	M	26	5.9
7	F	26	7.0	31 ^c	M	27	1.7
8	F	26	2.1	32	M	27	2.6
9	F	27	2.6	33	M	28	19.3
10	F	27	2.5	34	M	29	2.2
11	F	27	2.4	35	M	33	1.7
12	F	27	2.1	36	M	34	1.6
13*	F	29	3.5	37	M	34	1.6
14	F	29	2.9	38	M	35	4.0
15	F	30	11.1	39	M	40	6.9
16	F	31	1.7	40*	M	42	5.9
17* ^c	F	32	1.5	41	M	44	1.6
18	F	33	9.8	42	M	47	8.7
19	F	34	2.2	43	M	47	16.9
20	F	36	1.6	44	M	49	1.7
21*	F	39	2.0	45 ^v	M	49	1.9
22	F	41	1.6	46	M	56	2.2
23*	F	44	1.8	47	M	62	14.2
24	F	47	2.0	48*	M	65	6.7

Table 3.1: The levels of breath CH₄ measured in the subjects of this study.

correlation between CH₄ production and age (observed primarily in the male population of the study), it is statistically insignificant.

Finally, the prevalence of CH₄ production within a population has previously been reported to be affected by the ethnic origin of the participants [173, 176]. In this study the majority of the subjects were Caucasian and the observed abundance of CH₄ producers, 29%, is in very good agreement with the 33.6% reported by Bond *et al.* from a study conducted in a North American hospital [172], but lower than that reported both by Pitt *et al.* [173] (48%, all Caucasian) and by Fernandes *et al.* [177] (51%, mainly Caucasian).

Clearly, in order to carry out a more detailed study from which significant conclusions can be drawn, a larger sample of people is required. In addition, a simultaneous measurement of CO₂ on the breath sample would ensure that it was definitely end-tidal [178] and defining fully controlled conditions under which the participants gave breath samples (such as the time of day, the subject's diet and the time since they had last eaten) would allow a standardisation of the breath sampling [148].

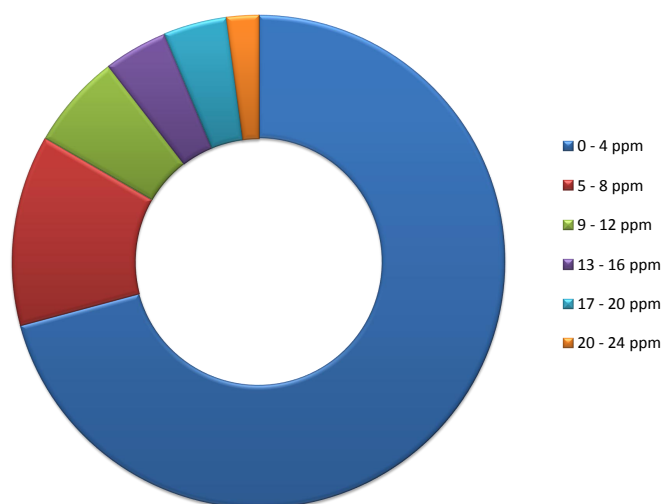


Figure 3.11: An illustration of the variation observed in breath CH₄ in the study.

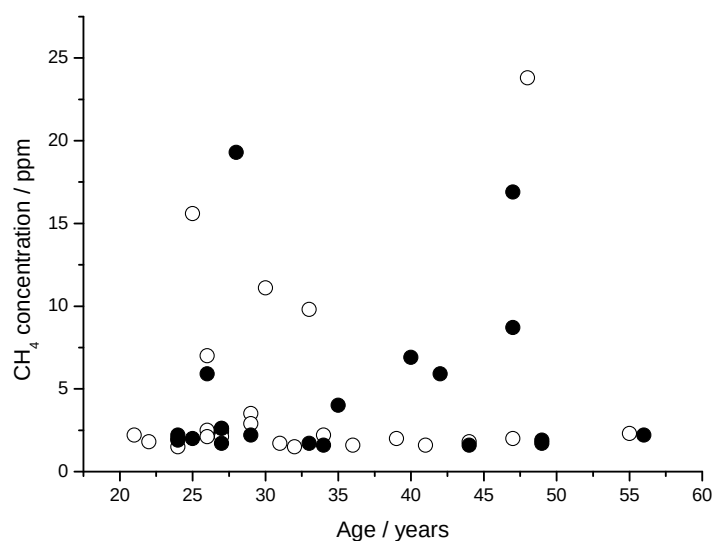


Figure 3.12: CH₄ breath concentrations in the samples studied as a function of age, with filled circles representing men and hollowed circles women.

However, the purpose of this work was not to undertake a comprehensive study into the causes and correlations involved with CH₄ and non-CH₄ production, but rather to demonstrate the suitability of CEAS as an alternative technique to the more commonly used GC-MS (gas chromatography mass spectrometry, see Section 1.2) for the detection of trace gases in human breath. By applying CEAS to the sensitive detection of methane in breath, this study has illustrated that CEAS does indeed have potential as a breath analysis tool,

and the flexibility afforded by this technique may allow its future application to other trace species found in breath that act as biomarkers for diseases, a theme pursued throughout this thesis.

3.3 Demonstration of a widely tunable laser source to spectroscopic applications

The methane study described previously utilised a DFB diode laser; however, these sources are restricted by their spectral coverage (typically a few nm), limiting the amount of information that can be gathered from a measurement [179]. Access to a greater number of transitions presents the opportunity to determine a very accurate value for parameters such as the sample temperature (via Boltzmann statistics) by measuring several transitions simultaneously, for monitoring the concentrations of several species in a mixture, or for accurate isotopic ratio measurements. In addition, the wider spectral coverage provided by such a source could allow molecules with continuous absorption spectra to be probed with some spectral recognition. The laser used in this study, a Digital Supermode Distributed Bragg Reflector (DS-DBR), is an example of a laser with extended spectral coverage which has been developed for the requirements of the telecommunications industry. It is one of a variety of similar lasers which provide an extended spectral coverage across the whole C- or L- band (terms for the regions 1530 - 1565 nm and 1565 - 1625 nm, respectively, in fibre-optic communications) that have been developed relatively recently. This has been achieved by using both external cavity arrangements [180, 181] and devices with an integrated pair of current-tuning gratings [182–184]. This section focuses on the use of one of the latter devices, where the tuning ability is based on a comb reflector with multiple reflection peaks, coupled with a second dispersive element for peak selection. These devices are monolithic and as such, their mechanical stability is maximised and they can support fast modulations. Examples of such devices include the modulated grating Y-branch (MG-Y; with the name referring to the shape of the device) and the sampled grating distributed Bragg reflector (SG-DBR), which utilise Vernier tuning between two grating combs to access a wide spectral range. Their potential application to spectroscopy, for example in detecting multiple species using a digital form of WMS [185], have recently been demonstrated [186, 187].

The DS-DBR laser used in this study was developed for optical switching and wavelength division multiplexing in telecommunication equipment but this is the first demonstration of its applicability to spectroscopy. Offering a wavelength coverage of 50 nm over the L-band, coupled with a ~ 1 MHz laser linewidth [188], the device has the potential to be a powerful spectroscopic tool, facilitating high resolution measurements of molecular species across a relatively large spectral range.

3.3.1 Digital Supermode Distributed Bragg Reflector (DS-DBR)

The Digital Supermode Distributed Bragg Reflector (DS-DBR) is an indium phosphide (InP) laser and it consists of four sections: front and rear gratings, a phase section and a gain section, with the power output boosted by an integrated semiconductor optical amplifier (SOA), as illustrated in Figure 3.13. It is based on a conventional three-section Distributed Bragg Reflector (DBR) laser: the rear grating defines the wavelength emitted, whilst the phase section allows the fine tuning of the wavelengths within the free spectral range of the laser cavity. Injecting current into the phase section adjusts the effective laser resonator length, which enables continuous tuning and allows longitudinal mode tracking, while the gain section provides the optical amplification. In contrast to a Distributed Feedback (DFB) diode laser, the grating structure of a DBR is not within this active region.

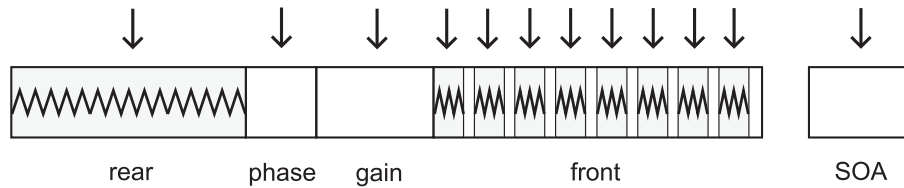


Figure 3.13: Schematic diagram of the multi-sections of the DS-DBR: the SOA is not part of the laser cavity but is found next to the front grating, whilst the gain and phase sections are bound by the rear and front gratings and the arrows represent electrical contacts; adapted from Ward *et al.* [189].

The DS-DBR essentially combines several DBR devices to provide an extended spectral coverage, with the addition of a front grating section. The rear section consists of a phase grating which, by carefully selecting (via e-beam processing) the positions of the phase shifts by varying the lengths of the constituent gratings, gives rise to a spectral response consisting of a top-hat comb of seven sharp reflection peaks [189], as illustrated in Figure 3.14. The peaks, known as supermodes, are of equal height and spacing (~ 6.8 nm), and have an excellent sidemode suppression ratio [190, 191]. The front grating consists of a series of very short (20-50 μm) holographic gratings with different pitches, each of which has its own electrical contact. As a result of the very short length of the gratings, when no current is applied the front reflector has a broad, featureless, non-selective spectral response. Coarse wavelength tuning across the ~ 50 nm range of the laser is provided quasi-digitally by applying current to a contact pair in the front section. The applied current induces a change in the refractive index of the selected pair, so that its response is blue-shifted and coincides with that of an adjacent grating, reinforcing the reflectivity at that wavelength. This reflectivity enhancement overlaps with one of the supermodes of the rear grating, as illustrated in Figure 3.14. The position of the supermode controls the lasing wavelength, and its exact position can be varied by adjusting the current applied to the rear section.

Fine control of the lasing wavelength can thus be achieved by varying the rear current in conjunction with the injected current into the phase section. Each supermode contains several longitudinal modes (the spectral separation of which is determined by the geometry of the laser cavity), and the phase current allows one of these longitudinal modes to be selectively tracked. Application of current to the next front grating contact, so that the second front pair is selected, allows the next supermode to be accessed.

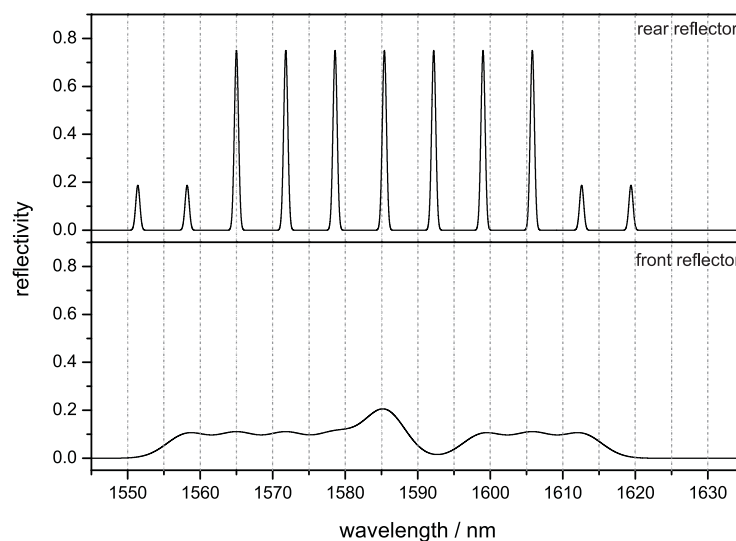


Figure 3.14: A schematic diagram adapted from Ward *et al.* [189] illustrating the responses of the rear reflector and front grating when current is injected into one of the front contacts.

In the telecommunications industry, the laser is used to rapidly switch between static frequencies [192], but for spectroscopy it is necessary to sweep the device continuously over the absorption features of the target molecule(s). Therefore, in order to explore the suitability of the DS-DBR for sensitive, high resolution spectroscopy, the laser response under such conditions must be investigated. This was undertaken by probing the well-characterised ro-vibrational absorption features of CO_2 found within this spectral region.

3.3.2 Carbon dioxide

The spectral window of the laser coincides with two ro-vibrational bands of CO_2 . These bands represent two of the four Fermi resonance components of the $3\nu_1 + \nu_3$ combination band which is mixed with other nearly degenerate bands exhibiting the same symmetry (where ν_1 represents the symmetric and ν_3 the asymmetric stretch of the normal modes of vibration; ν_2 is the doubly degenerate bend). The initial and final states of the transitions are written in the form $\nu_1 \nu_2 l_2 \nu_3 r$, where in addition to the vibrational modes, l is the angular momentum associated with the bending mode (ν_2) and r is the ranking index,

which equals unity for the highest vibrational level of a Fermi resonating group. The wide-tunability of the DS-DBR makes it very well suited to the measurement of isotopic ratios, and in particular the $\delta^{13}\text{C}$ ratio used in the urea breath test, a potential application which will be discussed later in this chapter. Initially, however, it is necessary to fully investigate the tuning characteristics of the laser.

3.3.3 Characterising the source

The DS-DBR unit (Bookham Technology PLC, now Oclaro Inc.), has an internal TEC and is packaged in a standard 26-pin butterfly pigtail module. It was placed in a commercial diode laser mount (ILX Lightwave LDM-4989), but controlled by a homemade driver unit, which allows the current levels for each section of the device to be set independently. The wavelength of the emitted radiation is controlled by the current settings applied to the different sections of the laser, as specified in the manufacturer's look-up table. A fibre-optic collimator was used to direct the beam into a multipass cell set-up, before the transmitted radiation was collected by an InGaAs photodiode (Thorlabs DET410). The fibre-coupled output power was measured as 20 mW, whilst a relative frequency scale calibration was provided by a spectrum analyser (Melles-Griot 13SAE906; free spectral range of 2 GHz), into which a fraction of the light was directed via a beam splitter positioned before the sample cell. An absolute scale was then derived for the spectral region covered by the laser from the CO_2 absorption features assigned in the HITRAN database, aided by a wavemeter (Burleigh WA-1000) into which a portion of the light was directed via a flipper mirror.

The SOA and gain sections had fixed applied currents of 120 mA and 150 mA respectively, whilst a fixed current of 5 mA was injected into the first contact of the selected front pair. The phase section, rear grating and second contact of the front pair had a sawtooth modulation of the input current applied, the amplitudes and offsets of which were determined from the manufacturer's look-up table, depending on the target wavelength. All three were modulated at the same frequency, as set by the driver unit.

Initially, the device was set to 1610.88 nm (6207.787 cm^{-1}) and the radiation directed into a White cell, a multipass cell with a maximum pathlength enhancement of 20 m (Specac, Tornado; 2 m pathlength steps; 4.7 l volume), providing the system with an increased level of sensitivity (see Section 6.4). The transition $R(15)$ at 6207.663 cm^{-1} , in the hot band $(3\ 1\ 1\ 1\ 3) \leftarrow (0\ 1\ 1\ 0\ 1)$, was probed at various pressures of CO_2 to investigate the suitability of the DS-DBR for spectroscopic measurements. With this initial measurement, the laser was operated over a relatively short wavelength region where no mode hops occur. Figure 3.15 illustrates some sample spectra at CO_2 pressures of 26 Torr, 41 Torr and 69 Torr with Voigt line profiles fitted in this regime where pressure broadening effects are observed. On

fixing the Gaussian parameter to the theoretical Doppler width for this transition (0.01156 cm^{-1}), the Lorentzian half width half maximum (HWHM) of the spectral feature can be determined for each pressure. A value for the self-broadening coefficient for this transition can then be extracted and the value deduced, $0.103 \pm 0.003 \text{ cm}^{-1}\text{atm}^{-1}$, was found to be the same as that quoted in the HITRAN database, $0.104 \text{ cm}^{-1}\text{atm}^{-1}$, within experimental uncertainty.

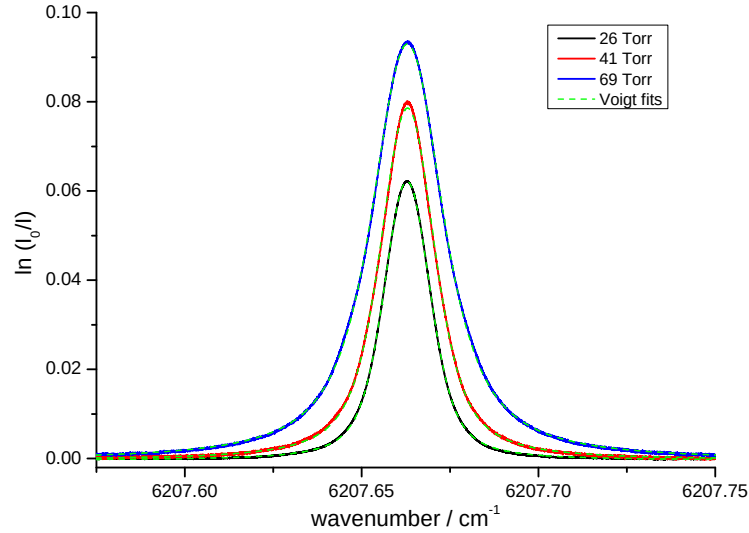


Figure 3.15: Spectra from various pressures of CO_2 ; from the Voigt fits applied, Lorentzian widths ($\Delta\nu_L$) representing pressure broadening have been determined and the HWHM used to deduce the self-broadening parameter (γ_{self}) for this transition.

Given these promising results in applying the DS-DBR to absorption spectroscopy, the next stage was to investigate fully the effects of changing the relative amplitudes of the current ramps applied to the front, rear and phase sections of the device. This work was carried out by Dr Luca Ciaffoni and Kim Whittaker in the group: the results are thus presented in Appendix B of the thesis, with the main outcome, namely a complete tuning range spectrum of CO_2 over the $1563 - 1612 \text{ cm}^{-1}$ region, shown in Figure 3.16 [193].

Therefore, it was possible to determine an absolute frequency calibration for the entire tuning range of the DS-DBR. This knowledge provides a means to perform discrete wavelength jumps to probe several absorption transitions that are spectrally quite widely separated, making it an ideal source for determining CO_2 isotope ratios.

3.3.4 CEAS

In order to achieve greater sensitivity, such as for the detection of the $^{13}\text{CO}_2$ isotopologue, an optical cavity can be utilised. In a similar arrangement to that described in the first part

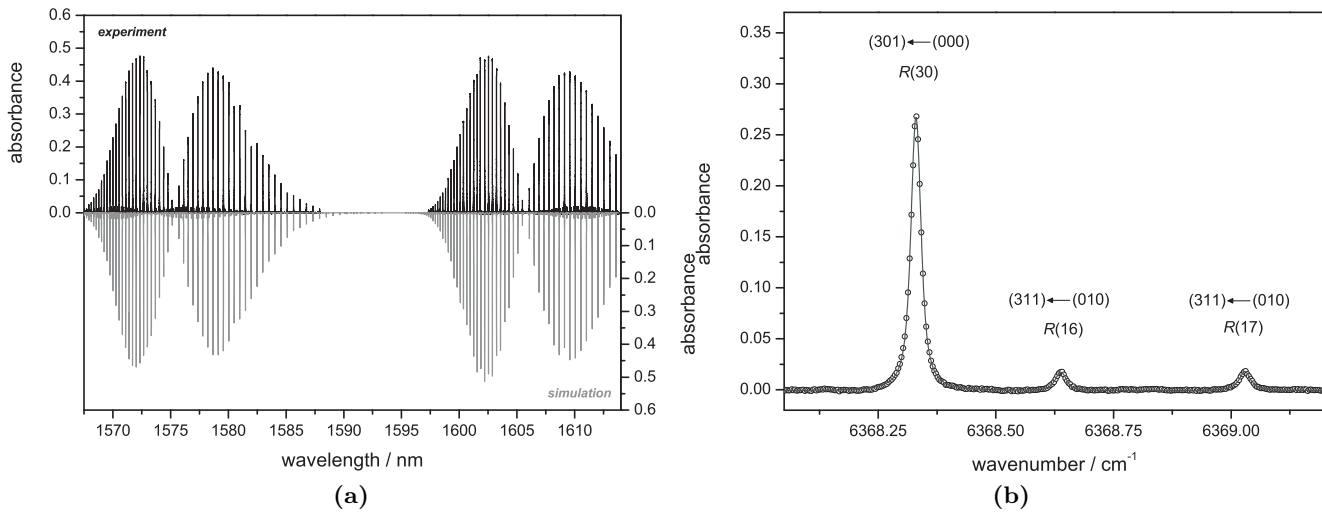


Figure 3.16: Graph (a) shows the absorption spectrum for an 85 Torr sample of CO₂ in a 4 m multipass cell in the region 1563-1612 nm (top), along with the HITRAN simulation (bottom). Graph (b) depicts an expanded spectrum over the 1570 nm region (open circles), with Voigt profiles fitted to the data (solid line) [193].

of this chapter, the optical cavity was formed from two highly reflective mirrors (Layertec, $R \sim 99.99\%$, radii of curvature 1 m), 46 cm apart. Initially a wavelength of 1595.88 nm was selected and the cavity output was directed into a Fourier transform spectrometer, so that the output of the laser over a larger spectral region could be investigated. On varying the gain and SOA current, it became clear that a secondary feature at $\sim 7000 \text{ cm}^{-1}$ was dominating the spectral output, as illustrated in Figure 3.17. In direct absorption or multipass cell measurements, this particular feature would be insignificant in comparison to the output at the selected lasing frequency. However, the presence of the cavity mirrors severely reduces the transmitted radiation at 1595.88 nm, whilst the output at $\sim 7000 \text{ cm}^{-1}$, not within the stop band of the mirrors, can pass relatively freely through without coupling to the cavity. Therefore, if not dealt with, this ASE would swamp the signal detected with a photodiode. To verify that the signal at $\sim 7000 \text{ cm}^{-1}$ was due to uncoupled light, the cavity was completely misaligned and the spectral output monitored on the FTIR. On confirmation that the feature still existed, a Thorlabs FEL1500 longpass filter was introduced which successfully cut out the unwanted emission. It was noted, however, that a very small feature was still present at $\sim 1525 \text{ nm}$, which was not fully extinguished given its wavelength falls just outside that of the filter. Therefore, despite the presence of the filter, an ASE measurement must still be taken and the level subsequently removed from the cavity data obtained.

In a similar vein to that described in Appendix B, both the rear and phase sections of the laser were ramped simultaneously, along with the second contact of the first front pair, allowing a region from $\sim 6221.61 \text{ cm}^{-1}$ to $\sim 6201.16 \text{ cm}^{-1}$ to be covered. 5 Torr of a

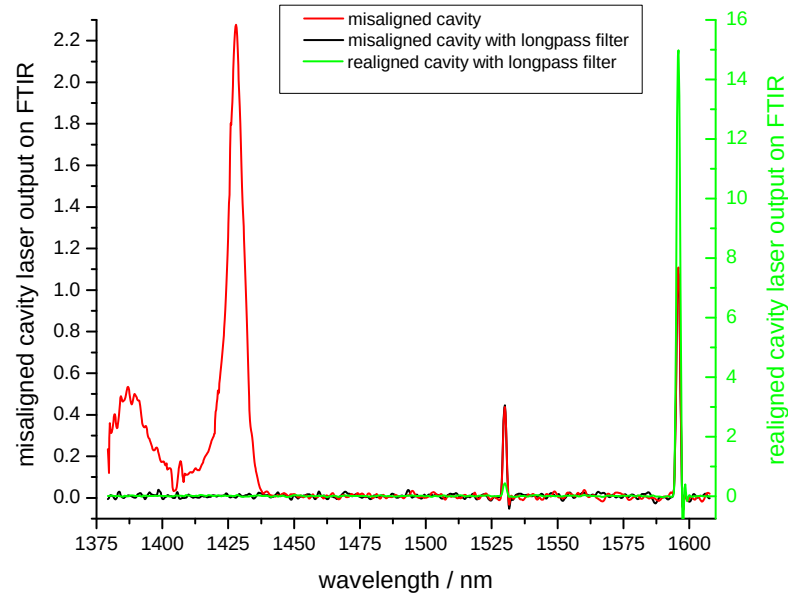


Figure 3.17: The cavity transmission on an FTIR when (a) the cavity is misaligned; when (b) the misaligned cavity is equipped with a longpass filter; and when (c) the cavity is realigned with the longpass filter in place.

1% CO₂ in N₂ mix was released into the cavity and the resulting transmission recorded, as illustrated in Figure 3.18. Numerous mode hops are observed (indicated by vertical green lines) as the rear current in particular is significantly ramped and the laser jumps from one longitudinal mode to another. There are 11 major ($\sigma_{\text{int}} \sim 10^{-23} \text{ cm}^2\text{cm}^{-1}$) CO₂ transitions in this region, as summarised in Table 3.2, but only 7 are probed due to the mode hopping behaviour of the laser (although absorption due to smaller transitions, most notably at 6212.689 cm⁻¹ and 6212.551 cm⁻¹, can just be made out within the *P*(18) section). Therefore, the total spectrum is divided into 13 sections, defined by the positions of the mode hops either side, and each with a different frequency scale.

Each low pressure absorption feature, represented by the black trace in Figure 3.18, had a Gaussian line profile fitted to yield a value for the FWHM for each transition (the *P*(10) transition is depicted in detail in Figure 3.20). The transitions found at lower wavenumber, corresponding to the region of Figure 3.18 where the mode hopping of the laser is more prevalent, returned measured Gaussian widths which were larger than those found at higher wavenumber. Given that the size of each mode hop-free section is known (they are quite similar), the effective scan rate for each section can be determined and is much greater in the lower wavenumber regions. As a result, in these regions the laser is scanning over the absorption feature more rapidly, in such a way that the detection system cannot respond quickly enough (i.e. the bandwidth of the detector is too low), and the consequent uncertainty in the transition frequency manifests itself as a broadening of the spectral feature.

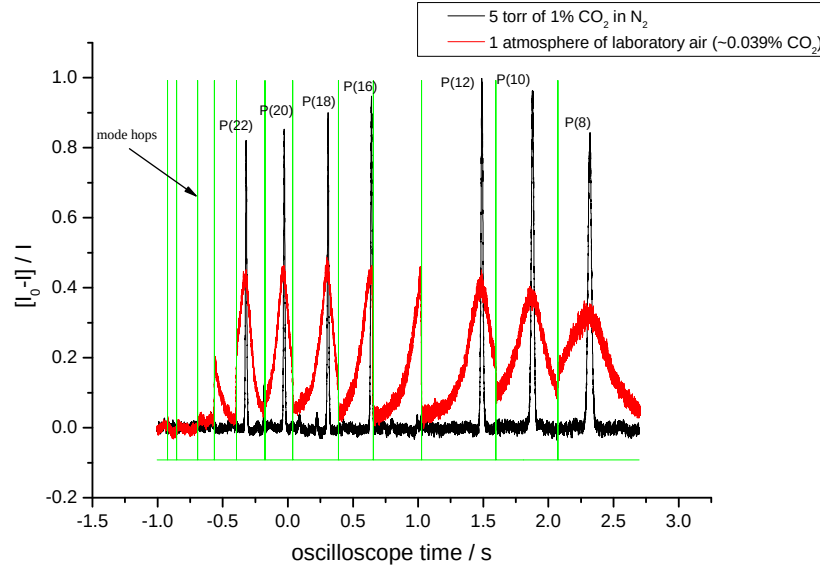


Figure 3.18: Absorption spectra of 5 Torr of 1% CO₂ in N₂ and an atmosphere of laboratory air with the transitions assigned and mode hops indicated by green vertical lines.

Frequency / cm ⁻¹	Rotational transition	σ_{int} / cm ² cm ⁻¹	γ_{air} (HWHM) / cm ⁻¹ atm ⁻¹	γ_{self} (HWHM) / cm ⁻¹ atm ⁻¹
6221.477	P(8)	1.17×10^{-23}	0.0837	0.112
6219.797	P(10)	1.36×10^{-23}	0.0816	0.108
6218.089	P(12)	1.49×10^{-23}	0.0798	0.106
6216.352	P(14)	1.57×10^{-23}	0.078	0.104
6214.588	P(16)	1.60×10^{-23}	0.0762	0.104
6212.795	P(18)	1.58×10^{-23}	0.0754	0.102
6210.974	P(20)	1.51×10^{-23}	0.0742	0.099
6209.124	P(22)	1.42×10^{-23}	0.0727	0.099
6207.246	P(24)	1.30×10^{-23}	0.072	0.097
6205.339	P(26)	1.16×10^{-23}	0.071	0.094
6203.403	P(28)	1.02×10^{-23}	0.0707	0.094

Table 3.2: The major CO₂ transitions of the $3\nu_1 + \nu_3$ band found from ~ 6221.61 cm⁻¹ to ~ 6201.16 cm⁻¹ [171].

This is clearly demonstrated in Figure 3.19, where the Gaussian width determined from the low pressure measurements is plotted against scan rate.

Although the laser was scanned at the slowest rate possible with its driver (~ 0.1 Hz), the effect of the increase in scan rate also manifested itself as a slight asymmetry observed in the transitions found in those regions of high scan rate. Although limiting the accurate determination of spectroscopic parameters from this data set to regions of lower scan rate, this artefact is not of huge concern as ultimately the laser will not be used in this way

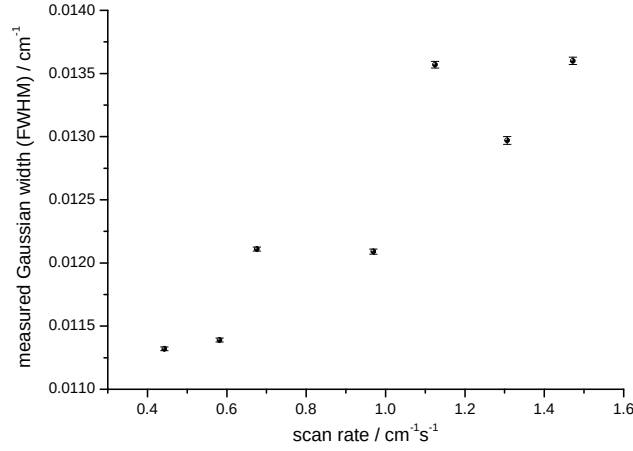


Figure 3.19: The variation seen in the measured Gaussian widths of the low pressure CO₂ samples as a function of effective laser scan rate; the error bars have been determined from the uncertainty in the fitted Gaussian width. The theoretical Doppler widths range from 0.01158 cm⁻¹ to 0.1156 cm⁻¹ for the transitions studied.

(but rather the wavelength required is selected using the spectral segments identified in Appendix B).

Two weeks later, the laser was set to identical settings and the same experiment conducted, but with an atmospheric sample of laboratory air. The red trace in Figure 3.18 illustrates these broadened CO₂ measurements, which then had Voigt lineshapes fitted to each profile, with the Gaussian width fixed to the experimentally determined value. Satisfyingly, the mode hops appeared at exactly the same positions, indicating that there is very little temporal variation in the wavelength regions accessible by the laser. Further, on applying the same relative frequency scale used with the low pressure sample to the atmospheric measurements, a red shift of the line centres on moving to higher pressure was observed [194]. The magnitude of the line shifts are reported in terms of the pressure shift coefficient, δ , in cm⁻¹atm⁻¹, and are compared to values from HITRAN [171], as listed in Table 3.3.

Frequency	Effective laser scan rate / cm ⁻¹ s ⁻¹	Experimentally determined pressure shift $\delta_{\text{meas}} / \text{cm}^{-1} \text{ atm}^{-1}$	HITRAN pressure shift $\delta_{\text{HITRAN}} / \text{cm}^{-1} \text{ atm}^{-1}$
6221.477	0.44	$-(4.5 \pm 0.12) \times 10^{-3}$	-5.40×10^{-3}
6219.797	0.58	$-(5.9 \pm 0.10) \times 10^{-3}$	-6.05×10^{-3}
6218.089	0.68	$-(6.4 \pm 0.11) \times 10^{-3}$	-5.95×10^{-3}
6214.588	0.97	$-(5.7 \pm 0.51) \times 10^{-3}$	-6.62×10^{-3}
6212.795	1.12	$-(1.1 \pm 0.11) \times 10^{-3}$	-6.48×10^{-3}
6210.974	1.31	$-(9.8 \pm 0.10) \times 10^{-3}$	-6.40×10^{-3}
6209.124	1.47	$-(1.1 \pm 0.12) \times 10^{-3}$	-6.78×10^{-3}

Table 3.3: A comparison of the experimentally determined pressure shifts, δ_{meas} , with the values quoted in HITRAN [171], δ_{HITRAN} .

The $P(10)$ transition is depicted in detail in Figure 3.20, with the Gaussian and Voigt profiles fitted to the low pressure and atmospheric pressure spectra respectively, illustrating the observed pressure shift, δ , which is measured to be $\delta_{\text{meas}} = -5.9 \pm 0.10 \times 10^{-3} \text{ cm}^{-1} \text{ atm}^{-1}$ and found to be in good agreement with $\delta_{\text{HITRAN}} = -6.05 \times 10^{-3} \text{ cm}^{-1} \text{ atm}^{-1}$. In addition, the area yielded by the Gaussian fit indicates a mirror reflectivity of 99.99%, consistent with the manufacturer's specifications.

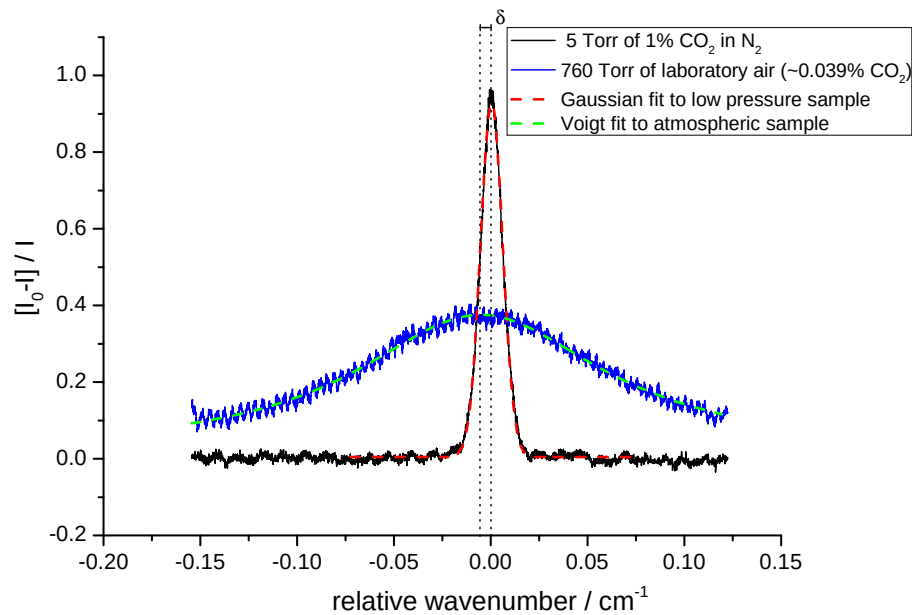


Figure 3.20: An enlarged view of the $P(10)$ transition, illustrating the pressure shift observed on an increase in the total pressure of the sample.

It is noted that the line shifts are all negative; this is expected for the case where collisionally perturbed transitions take place between an initial vibrational level whose energy is lowered by attractive interactions to a lesser extent than that for the excited level, and reflects the vibrational state-dependent changes in the polarisability and charge distribution of CO_2 . There is good general agreement between the measurements and the literature values, although it is clear that at lower wavenumber, where the effective scan rate is greater, the measured shifts start to deviate more from the values quoted in HITRAN. This is in keeping with the increased uncertainty in the transition frequency associated with higher scan rates. It follows that the measured pressure shift between two transitions under such conditions will be greater and deviate further from the published values at higher scan rates, as observed experimentally. Notwithstanding this, the fact that pressure shifts of the order of $\sim 0.006 \text{ cm}^{-1} \text{ atm}^{-1}$ can be measured between two spectra that were taken two weeks apart, illustrates the outstanding frequency reproducibility afforded by the DS-DBR, further highlighting its suitability for spectroscopic measurements.

Application to $\delta^{13}\text{C}$ ratio measurements

Figure 3.21 demonstrates another use of the DS-DBR laser: it allows transitions spectrally quite separate to be probed. The two transitions featured in Figure 3.21, $R(0)$ and $R(4)$, were selected for a preliminary investigation into using the DS-DBR as a means for determining carbon isotope ratio measurements, as they represent a compromise between minimising the temperature dependence of the ratio and ensuring that the absorption cross-sections of the transitions are similar in magnitude, as detailed in Table 3.4.

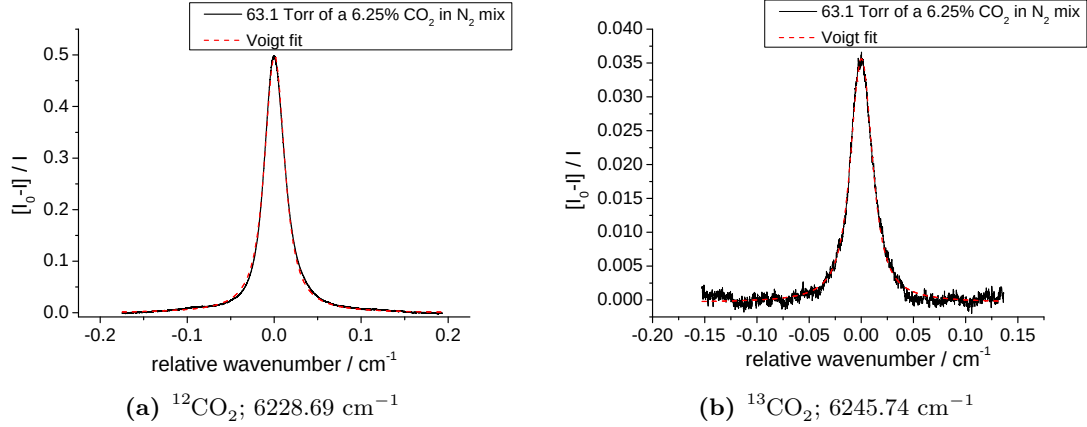


Figure 3.21: Example spectra of the two transitions probed, with Voigt line profiles fitted to the data.

Species	Frequency ν_0 / cm^{-1}	Integrated cross-section $\sigma_{\text{int}} / \text{cm}^2\text{cm}^{-1}$	Rotational transition	Ground state energy $/ \text{cm}^{-1}$
$^{12}\text{CO}_2$	6228.69	1.68×10^{-24}	R(0)	0
$^{13}\text{CO}_2$	6245.74	1.03×10^{-25}	R(4)	7.8047

Table 3.4: The spectroscopic parameters of the two transitions studied in this work.

The experimental set-up used to derive these spectra was very similar to that used in the previous section, with the exception that cavity mirrors of slightly lower reflectivity were used (REO Inc., 1.5 m radii of curvature, $R \sim 99.97\%$) in a cell that was 34.5 cm long and a 2.35 GHz spectrum analyser was used for the frequency calibration. The work was carried out in collaboration with a Part II student, Matt Kingston, working in the group. The specific data shown in Figure 3.21 were taken by Matt under my instruction and analysed by me. In the same manner as described previously, the mirror reflectivity was measured to be 99.966% from analysing various CO_2 samples of known pressure and from the signal to noise ratio of the spectra, α_{min} was calculated to be $1.2 \times 10^{-8} \text{ cm}^{-1}$, which compares well with that determined in the first section of the chapter. A sample of ~ 65 Torr of a 6.25% CO_2 in N_2 mix was leaked into the cavity before both absorption features were then repeatedly swept over by the laser, switching between the two wavelengths and allowing a settling period of ~ 2 s before each spectrum was taken. Each absorption spectrum was then fitted with a Voigt profile and the integrated area subsequently determined. Ratios can

then be calculated for each isotopic pair, and the standard deviation deduced from a linear fit to the ratios. The estimated uncertainty in the mean ratio value is then determined from weighting the standard deviation with the number of measurements taken of the isotopic pair [195]. This value, 1.85×10^{-4} , then yields an instrument sensitivity of $\delta^{13}\text{C}_{\min} = 2.98 \text{ ‰}$, which is comparable with the $\delta^{13}\text{C}_{\min}$ obtained by Kasyutich *et al.* [13] (3.7 ‰) and Ciaffoni [196] (8.0 ‰). However, as discussed in Chapter 1, for application to the urea breath test, a ‘gold standard’ precision of $<1 \text{ ‰}$ is recommended.

It is immediately obvious that a degree of uncertainty in the measurements of this study will arise from the poorly defined wings of the $^{13}\text{CO}_2$ absorption feature, which will manifest itself as an uncertainty in the determination of the integrated area of the transition. One way in which to reduce this effect could be to improve the sensitivity of the instrument by either increasing the physical pathlength of the cavity, or by using higher reflectivity mirrors. However, this could cause dynamic range problems when probing the $^{12}\text{CO}_2$ transition. Potentially, this could be overcome by selecting a pair of transitions with more comparable absorption line strengths, at the expense of a greater difference in the temperature dependence of the two transitions. The Boltzmann distribution indicates that temperature affects the absorption line strength, so in order to minimise the influence of any temperature fluctuations during measurements of the two isotopes, two transitions that were as close as possible in ground state energy (given the restrictions on the relative absorption line strengths of the two transitions) were chosen. This provided a temperature dependence of the isotopic ratio of $\sim 0.127 \text{ ‰ K}^{-1}$ at 298 K (determined via a theoretical approximation $\frac{\Delta\delta^{13}\text{C}}{\Delta T} = \frac{hc\Delta E}{k_B T^2} \times 1000$ [197], where $\frac{\Delta\delta^{13}\text{C}}{\Delta T}$ is expressed in ‰ K^{-1} at a defined temperature and ΔE is the difference in the ground state energy levels of the two transitions). Whilst this minimal temperature dependence clearly demonstrates an advantage of the DS-DBR over a laser with a more narrow spectral range (where one is restricted to transitions which are found relatively close together, which tend to suffer from a large temperature dependence as they originate from quite different J states), this leads to a required temperature stability of $< 8 \text{ K}$ in order to obtain the 1 ‰ ‘gold standard’ precision. Given the laboratory temperature can be comfortably maintained within 1 K, this would suggest that there is a certain degree of flexibility available in selecting two different transitions so that the $^{13}\text{C}/^{12}\text{C}$ absorption line strength ratio can be maximised.

Alternatively, the wide tuning range of the DS-DBR could be exploited to measure several different lines of isotopic pairs. Not only would this remove the temperature dependence issue (it would allow the sample temperature to be deduced), it would also statistically enhance the ratio determined, consequently providing a greater level of precision and a lower $\delta^{13}\text{C}_{\min}$.

3.4 Conclusions

This chapter initially illustrated the applicability of laser-based absorption spectroscopy as a viable means of breath analysis, by measuring the levels of methane in breath in a study on a small sample of people using a DFB diode laser and a CEAS detection scheme. This was followed by the characterisation of a novel, widely tunable laser source, the DS-DBR, and the first demonstration of its use in absorption spectroscopy. A preliminary investigation into its suitability for determining $\delta^{13}\text{C}$ was also conducted and although the sensitivity value determined is lower than recommended for diagnosis, it would be possible to improve it by monitoring multiple isotopic ratios.

However, sometimes the molecules of interest in breath do not have narrow transitions, but instead exhibit absorption features spanning tens of nanometres. In such situations, alternative radiation sources must be utilised and this is the subject of the next chapter.

Chapter 4

Broadband Cavity Enhanced Absorption Spectroscopy (BB-CEAS)

Many of the biomarkers identified in Chapter 1 have broad, congested absorption features which are consequently not readily discernible using narrowband laser sources. As such, this chapter deals with the application of broadband sources to cavity enhanced absorption spectroscopy for the detection of three broadband absorbers: butadiene, acetone and isoprene. Two types of broadband source, a Superluminescent Light Emitting Diode (SLED), with a spectral output covering ~ 1600 to ~ 1700 nm, and a Supercontinuum (SC) source, which emits from 450 nm to 2.5 μm , but will be used primarily in the same spectral region as the SLED, will be demonstrated in conjunction with two detection systems: dispersion using a monochromatic spectrometer and interferometry using FTIR spectroscopy. Initial work centres on the determination of the absorption cross-sections of these molecules and the subsequent detection of these species in dilute samples using BB-CEAS, before the broadband spectrometer is developed further and applied to real breath samples.

4.1 Broadband Spectroscopy

Narrowband diode lasers are ideal for studying small molecules, such as methane and carbon dioxide, as they can easily scan over the narrow absorption features. However, if the target molecule is somewhat larger, it may have quasi-continuous absorption features which span over several nanometres. In this case, the use of a broadband source can provide specificity, as is illustrated in Figure 4.1. Additionally, if it is desirable to measure several compounds

that absorb in different spectral regions simultaneously, a broadband source can provide the necessary greater spectral coverage.

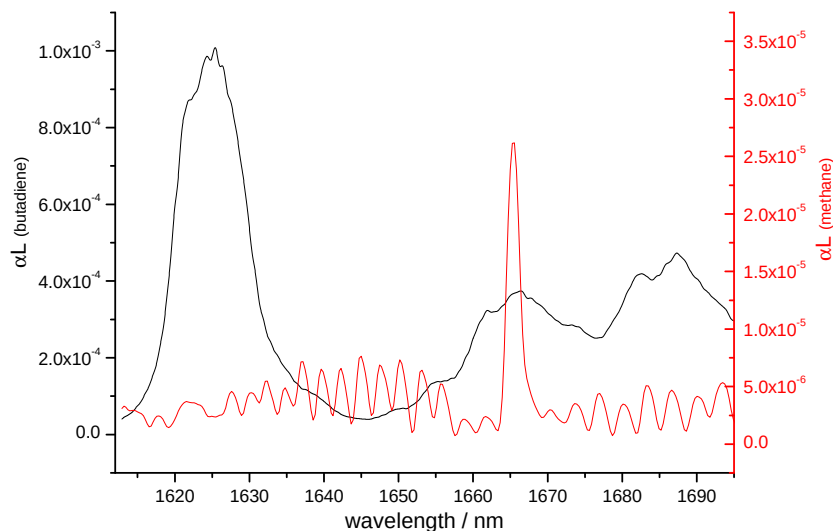


Figure 4.1: Illustration highlighting the difference between a broadband (butadiene) and a narrowband (methane) absorber; both measurements were taken at 4 cm^{-1} resolution with 1% butadiene in air and 15 ppm methane in air, respectively.

Therefore, the attractive ability to make high sensitivity measurements across a broad spectral region has led to recent developments in broadband cavity methodologies [198]. Many of the early studies in particular focused on the use of cavity ring down spectroscopy (CRDS). A significant issue with using a broadband source in a ring-down experiment is that the spectral width of the radiation is comparable with that of the absorption feature [199–201]. In order to retrieve the wavelength-dependent ring-down information, these studies employ one of three options: pre-selecting a subset of wavelengths before the detection of the resultant ring-down signals; dispersing the ring-down signals in wavelength and recording the time-integrated or time-gated signals; or an arrangement whereby the output from the ring-down cavity is dispersed in time and wavelength and then imaged onto a two-dimensional detector array [198]. Early work by Engeln and Meijer [202] utilised a Fourier Transform spectrometer to select a subset of wavelengths of the broadband dye laser used. At sequential interferometer mirror positions, the ring-down times were recorded and an absorption spectrum of oxygen determined. Similarly, in later work the interferometer was positioned before the cavity. Once again, oxygen was investigated but this time a Xe arc lamp was used as the radiation source [203]. In a similar vein, Crosson *et al.* [204] used a monochromator to resolve the cavity output into narrow wavelength ranges to avoid producing multi-exponential decays. Using a free electron laser, they recorded a water vapour absorption spectrum at $5.36 \text{ }\mu\text{m}$ by recording the ring-down decays over each

narrow range selected by the spectrometer. In studying the mid-infrared absorptions of thin films of C₆₀, Marcus and Schwettman [205] employed the same principle, with the ring-down events recorded in a time-resolved manner. The work of Czyzewski *et al.* [206] represents an example of the second option described above. In this case, the radiation from a Nd:YAG pumped dye laser was dispersed in wavelength by a spectrograph before being detected using a charge-coupled device (CCD) with a gated image intensifier. Whereas in the previous studies sections of the broadband radiation are detected in a consecutive manner over time, here the full bandwidth of the light is used for each measurement, but the temporal evolution of the cavity output has to be built up from consecutive measurements. This means that the decay trace comes from different laser shots, which necessarily introduces additional noise to the decay and greater uncertainty in the derived ring-down time.

Finally, it is possible to resolve both time and wavelength simultaneously, in so-called two-dimensional broadband cavity ring-down [198]. Ring-down spectral photography (RSP) was developed by Scherer [207], and it resolves the ring-down event both in wavelength and in time along the x - and y - co-ordinates by reflecting the cavity output off a rotating mirror and onto a diffraction grating. Hence the physical position of the radiation on the grating is determined by the time delay with respect to the laser pulse, and the dispersion from the grating is dependent on the wavelength of the radiation. Therefore, the spatial dispersion of the radiation imaged onto the CCD camera is dependent on both the time it left the cavity and its wavelength. A proof-of-principle study for this technique was carried out on propane using a narrowband dye laser [207], in which the laser was tuned in steps of 1 nm to mimic a broadband source, before the technique was applied to a broadband source, a YAG-pumped dye laser, to probe propane and oxygen [208]. Ball *et al.* [209] also achieved two-dimensional broadband cavity ring-down detection by utilising a ‘clocked’ two-dimensional CCD detector. The light leaving the cavity was projected onto the CCD via an astigmatic imaging spectrograph, which wavelength-resolved the radiation. Incident radiation on the CCD chip generated photocharge which was trapped by potential wells within each pixel. On application of voltages to the electrodes along the time axis of the CCD, the photocharge was transferred between rows of pixels in a ‘clocking sequence’. Thus, as this sequence progressed, time and wavelength resolved spectra were collected until the complete ring-down event was recorded. At this point the charge was reverse clocked so that the original arrangement was restored, ready for the next capture sequence. In addition to the NO₃ radical studied in [209], the authors later also applied this technique to molecular oxygen, NO₃ and mixtures of NO₃ and water vapour [198], highlighting a major application of sensitive broadband techniques in studying atmospheric trace species. Indeed, in a later publication from the same group [210], this instrument was actually used for field measurements to measure the levels of NO₃, N₂O₅, I₂ and OIO during the 2002 North Atlantic Marine Boundary Layer Experiment (NAMBLEX).

Cavity enhanced absorption is the time-integrated measurement of the total transmitted light intensity from a cavity which is continuously excited, and so it is not necessary to resolve the cavity output temporally. Therefore, this represents arguably a more straightforward route into sensitive broadband detection, as well as a more robust arrangement for field measurements, and the more recent developments in broadband detection have centred on its use. In their 2003 study of oxygen and azulene, Fiedler *et al.* [211] used a Xe arc lamp as their broadband source in conjunction with a monochromator to disperse the light before it was imaged on a diode array. This was followed later in the same year with a study on azulene in a supersonic jet using the same instrument [212]. Xe arc lamps have been used extensively in broadband techniques: Ruth *et al.* demonstrated the use of a Fourier Transform spectrometer with an optical cavity on the detection of oxygen and water vapour lines at ~ 688 nm [213]; Venables *et al.* [214] used a 4.5 m long cavity enclosing a reaction chamber to study atmospheric species (NO_3 , NO_2 , O_3 and H_2O) *in situ*, under realistic atmospheric conditions. Using a spectrograph with a CCD array as the detection system, they achieved a sensitivity of 4 pptv for NO_3 in a 1 minute acquisition time; Orphal *et al.* [215] recorded high resolution spectra (0.02 cm^{-1}) of CO_2 , OCS and HD^{18}O in the near-infrared using a Fourier Transform spectrometer, though it required data acquisition times of up to 540 minutes; Vaughan *et al.* also utilised a short-arc Xe lamp to probe I_2 and OIO at 525–555 nm, and IO at 420–460 nm, achieving sensitivity levels of $\sim 26\text{ pmol mol}^{-1}$, $\sim 45\text{ pmol mol}^{-1}$ and $\sim 210\text{ pmol mol}^{-1}$, respectively for these chemically short-lived, key atmospheric trace species [216]. Ruth *et al.* [217] have demonstrated that these methods can be employed in the field by monitoring NO_3 and NO_2 levels to the 2 pptv level using a 20 m length cavity at the SAPHIR atmospheric simulation chamber.

Many studies have employed visible light emitting diodes (LEDs) in the detection of atmospheric species, especially the nitrogen oxides. Early work by Ball *et al.* [218] utilised red and green LEDs in this way, leading to the development of a compact instrument that was used to measure atmospheric NO_2 *in situ* at quantitative amounts of between 3 and 32 ppb [219]. The same authors further refined the method by introducing a means to periodically measure the cavity mirror reflectivity using phase-shift cavity ring down spectroscopy (PSCRDS) with light from the same LED used in the BB-CEAS measurements, thus providing a stand-alone alternative to the usual method of using absorption spectra of samples of known pressure and cross-section to determine the mirror reflectivity [220]. A detection level sensitivity of 0.25 pptv for NO_3 over a 10 s acquisition period was attained under laboratory conditions, before the device was applied to field work and used to measure the sum of $\text{NO}_3 + \text{N}_2\text{O}_5$ concentrations in the marine boundary layer. This was followed in 2011 by the simultaneous measurement of NO_3 , N_2O_5 and NO_2 using an aircraft-based three-channel BB-CEAS spectrometer. In a similar vein, Wu *et al.* [221] used a blue LED for *in situ* measurements of NO_2 in laboratory air and, with cavity mirrors of

moderate reflectivity ($\sim 99.5\%$), achieved a detection sensitivity of 2.2 ppbv for 100 s of averaging. This was followed by the development of an open path BB-CEAS scheme for the simultaneous measurement of HONO and NO_2 in ambient air, using a 1.85 m long cavity which attained sensitivity levels of ~ 430 ppt and ~ 1 ppbv for HONO and NO_2 , respectively [222]. Similarly, Triki *et al.* [223] simultaneously measured NO_3 and NO_2 using a red LED centred at 643 nm, achieving a detection sensitivity in the pptv range with 2 minute averaging for the latter, which on extrapolation leads to a few pptv for NO_3 for a few minutes of averaging. Recently, Ventrillard-Courtillot *et al.* [224] reported a detection limit of 600 pptv and 2 pptv for the simultaneous measurement of NO_2 and NO_3 , respectively, using an LED emitting at a central wavelength of 625 nm, with a cavity of 50 cm length.

The application of Supercontinuum (SC) sources to broadband detection have also been widely reported, with the high power and wide spectral coverage afforded by these devices making them an ideal broadband source. Studies have again involved the detection of the nitrogen oxides [225], with a sensitivity of 2 pptv in 2 s reported for a cavity mirror reflectivity of 99.995 %; the study of high temperature H_2O in the NIR [226]; and the investigation of the 5th overtone of the acetylene C-H stretch using a retroreflective prism cavity [227]. SC sources have also found widespread application in the measurement of liquid and/or surface borne species using BB-CEAS [228, 229], as have LEDs (e.g. on the dyes methylene blue and Sudan black [230] and on the dyes rhodamine 6G and rhodamine B [231]) and the use of Xe arc lamps has also been reported, applied to the fifth C-H stretch overtone of benzene [232]. SC sources have also been employed in the evanescent wave cavity-enhanced technique [233, 234], as has the use of an Xe arc lamp on metallo-octaethyl porphyrins [235].

More recently, studies have involved the use of femtosecond lasers to produce frequency combs for broadband detection. Gherman *et al.* [236] used a Ti:Sapphire laser source to produce a comb-like spectral output, the central wavelength of which could be tuned between 750 - 850 nm. Via a frequency doubling crystal, this was used to produce radiation at 420 nm to probe a very high overtone transition of acetylene, resulting in a sensitivity of $\sim 1 \times 10^{-8} \text{ cm}^{-1}$. Also using a Ti:Sapphire laser, Gohle *et al.* [237], demonstrated the high resolution, frequency reproducibility and accuracy of the technique; whilst Thorpe *et al.* [77, 238], using an Er^{3+} fibre laser, have illustrated how the technique can be applied to breath analysis and the measurement of carbon isotope ratios. Finally, Bernhardt *et al.* [239] have recorded ammonia spectra at a resolution of 4.5 GHz, over 20 nm and with a noise equivalent absorption of $1 \times 10^{-8} \text{ cm}^{-1}$ in only 18 μs , with heterodyne detection using the two frequency combs replacing the use of a conventional FTIR. Clearly, these instruments show extremely good sensitivity and bandwidth, but they have quite technically demanding detection schemes and have not been used outside of a laboratory environment.

Although it has been demonstrated that high resolution is attainable [215], it comes at the cost of time and for the detection of molecules with broad absorption features, it is not always necessary. In addition, as noted above, much of the work on broadband spectroscopy has centred on detecting atmospheric species, with very little application to the broadband absorbers found in breath. Therefore, the detection of some of these medically important species forms the basis for this BB-CEAS study.

4.2 Butadiene, Acetone and Isoprene

The radiation sources used in this chapter emit from $\sim 1.5 \mu\text{m}$ to $\sim 1.7 \mu\text{m}$: whilst the SLED device primarily used emits solely from $\sim 1600\text{--}1700 \text{ nm}$ and the secondary SLED from $\sim 1500\text{--}1600 \text{ nm}$, the SC source has a much broader spectral coverage, ranging from 450 nm to $2.5 \mu\text{m}$, but in this study its output is filtered to match the band gap of the cavity mirrors used. This covers a spectral region where many hydrocarbons exhibit overtone and combination band absorptions, including butadiene, acetone and isoprene, the focus of the majority of this work. As discussed in Chapter 1, both acetone and isoprene are molecules of biological significance as markers of disease, whilst butadiene is a known human carcinogen, released from the burning of plastics and present in cigarette smoke and vehicular exhaust.

Example spectra of direct absorption measurements taken with an FTIR spectrometer for these three molecules in this region, together with inset figures of the 3D representations of each molecule, are illustrated in Figure 4.2. Butadiene, $\text{CH}_2\text{CHCHCH}_2$, has three features in this region, labelled **A**, **B** and **C**, which correspond to the $|1\rangle|1\rangle|0\rangle$, the $|2\rangle|0\rangle|0\rangle$, and the $|0\rangle|2\rangle|0\rangle$ combination and overtone transitions, respectively, where $|\nu_t\rangle|\nu_c\rangle|\nu_n\rangle$ represents the vibrational quanta in the *trans*-terminal (CH_t), *cis*-terminal (CH_c) and non-terminal (CH_n) C-H stretches [240].

Acetone, CH_3COCH_3 , has two major features which are attributed to the excitation of the in-plane (ν_3 ; CH_{ip}) and out-of-plane (ν_1, ν_2 ; CH_{op}) CH oscillators, which can be described by the notation used by Kjaergaard *et al.* [241], $|\nu_1\nu_2\rangle_\pm|\nu_3\rangle$, where $\pm$ refers to the symmetry of the two equivalent CH_{op} wave functions with respect to reflection in the skeletal plane. The single sharp feature at $\sim 1672 \text{ nm}$, apparent at higher resolution, is assigned as the $|10\rangle_\pm|1\rangle$ mode, whilst the secondary feature at $\sim 1690 \text{ nm}$ corresponds to $|11\rangle|0\rangle$.

Finally, the isoprene ($\text{CH}_2\text{C}(\text{CH}_3)\text{CHCH}_2$) features in this region are due to the first overtone of the asymmetric C-H stretch, and although an assignment does not exist in the literature, one can assume that it would be similar to butadiene, given the structural similarities between the molecules.

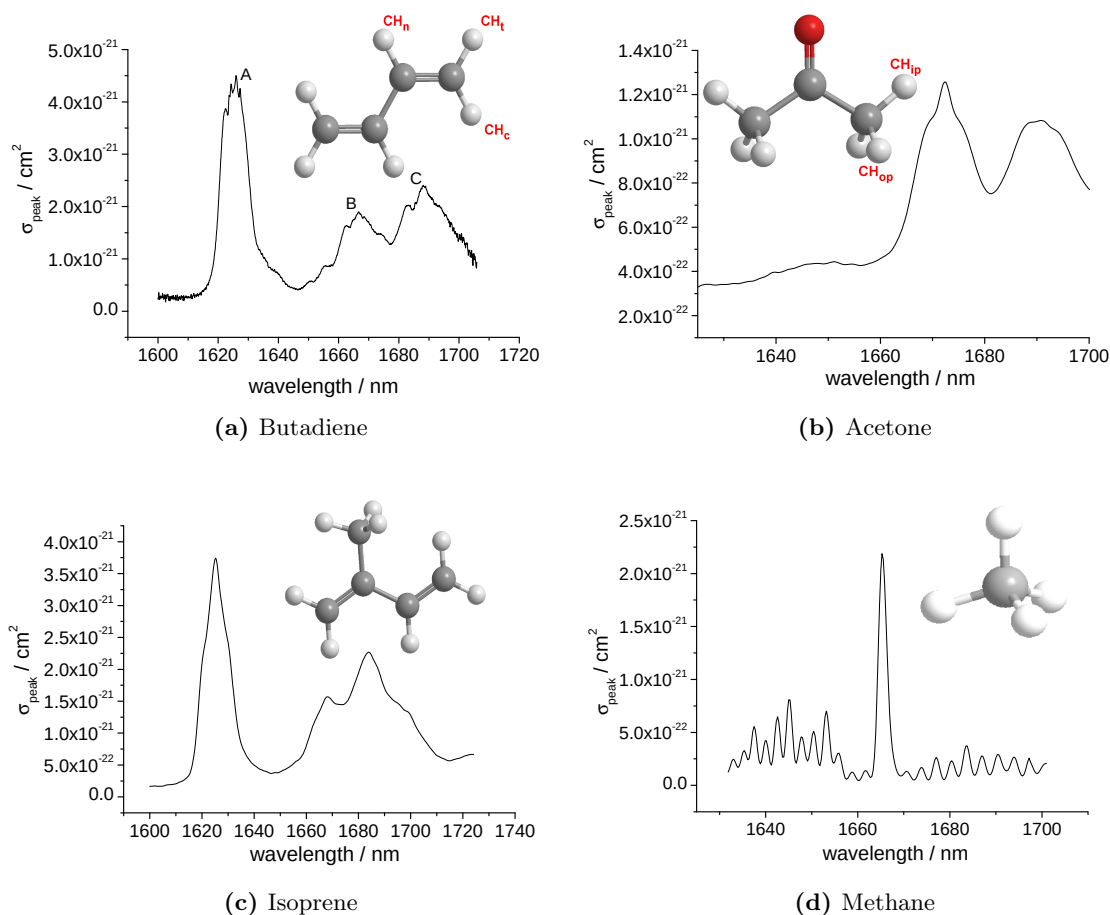


Figure 4.2: The experimentally determined absorption cross-sections for the principal species investigated in this study: Graph (a) illustrates the absorption cross-sections of butadiene with the main features labelled; Graph (b) shows the equivalent absorption cross-sections for acetone; whilst Graph (c) depicts the absorption cross-sections of isoprene; and Graph (d) is a methane spectrum, with the apparent cross-sections calculated from the HITRAN database to mimic the 4 cm^{-1} resolution of the other spectra.

In addition to these broadband absorbers, methane has also been investigated because as described in Chapter 3, it is found at enhanced levels in the breath of approximately 1 in 3 people due to the presence of methane-producing flora in the gut. The methane transitions in this region correspond to the $2\nu_3$ overtone band, with the spectrally-congested Q branch the dominating feature in the spectra.

4.3 BB-CEAS Theory

As described in Chapter 3, it is the geometry of the optical cavity alone which determines its modal properties. In order for light to couple in to the cavity, its frequency and spatial distribution must match that of the cavity modes. However, the light emitted from a

broadband source is temporally incoherent and, in contrast to that from a diode laser, cannot be described by a single Gaussian beam; it is treated as an infinite series of Gaussian beams [242] and as such, it is impossible to mode match to a single mode. However, as stated above, the existence of cavity modes is independent of the coherence length of the light source used to excite the cavity [243]. Consequently, broadband light will still couple into the cavity, but it will excite many transverse electromagnetic modes (TEM) [223], and the coupling efficiency will necessarily be lower than that achieved with a coherent diode laser source. It follows that the cavity output is a superposition of all these excited modes [242] and, given that the free spectral range is much smaller than the bandwidth of the detection system, any interference effects cannot be detected and the cavity output can be described in terms of light intensities rather than electric field strengths [243].

As with any cavity experiment, the cavity mirrors greatly reduce the intensity of the light transmitted, resulting in an intensity-dependent signal to noise ratio which is lower than that obtained with a single pass measurement. However, because the effective absorption pathlength is enhanced by a factor of $\frac{1}{(1-R)}$ by the cavity mirrors for small losses, the signal to noise ratio for the absorption coefficient will be greater than that achieved with a single pass measurement [223]. As described by Fiedler *et al.* [232], in the regime where the absorption loss, A , is $A < (1 - R)$, with mirror reflectivities R_1 and R_2 , the maximum enhancement of the signal to noise ratio, with respect to a single pass measurement, is given by:

$$\mathfrak{R}^{\text{IBBCEAS}}(\alpha) = \sqrt{\frac{(1 - R_1)(1 - R_2)(1 - A)}{[1 - R_1 R_2(1 - A)^2](1 - \sqrt{R_1 R_2})^2}} \mathfrak{R}^{\text{single}}(\alpha) \quad (4.1)$$

For very small losses, such that $A \rightarrow 0$, and assuming $1 - R^2 \approx 2(1 - R)$ for mirror reflectivities $R = R_1 \approx R_2$, this becomes:

$$\mathfrak{R}^{\text{IBBCEAS}}(\alpha) \approx \sqrt{\frac{1}{2(1 - R)}} \mathfrak{R}^{\text{single}}(\alpha) \quad (4.2)$$

$\sqrt{\frac{1}{2(1 - R)}}$ is known as the signal-to-noise (SNR) enhancement factor, and it represents the maximum enhancement by which the signal-to-noise ratio of α is improved via BB-CEAS when compared to a single pass measurement, which is less than the enhancement found in a laser-based CEAS experiment. Low spectral density, together with the difficulty in injecting an appreciable fraction of the light into the cavity (formed by relatively small diameter mirrors) and the reduced throughput of the cavity (due to high R), means that the photon intensity being detected is much reduced in comparison with studies utilizing higher power (coherent) sources. In a diode laser-based CEAS experiment (or a BB-CEAS

experiment using an intense and well collimated source, such as a Supercontinuum source), the increase in the absorption, αL , that one measures is $\frac{1}{(1-R)}$ larger than that measured in a single pass over a distance L . This result leads to the concept of an effective pathlength; it is, however, clear that this is not a physical pathlength but it instead represents the change of the average photon lifetime in the cavity caused by the presence of an absorber. It should be noted that the ratio of the minimum absorption measurable in the CEAS experiment to that in a single pass, i.e. $\alpha_{\min}(\text{CEAS})/\alpha_{\min}(\text{SINGLE PASS})$ (or the SNR enhancement factor), is not given by the same $\frac{1}{(1-R)}$ factor as the markedly reduced light intensity falling on the detector in a CEAS experiment will affect this quantity. For many of these laser-based experiments, however, there is still plenty of light incident upon the detector so that the ratio $\alpha_{\min}(\text{CEAS})/\alpha_{\min}(\text{SINGLE PASS})$ is near to $\frac{1}{(1-R)}$ and the detection sensitivity is limited by residual mode noise. However, as demonstrated by Fieldler *et al.* [211, 212, 232], with incoherent BB-CEAS the $\alpha_{\min}(\text{CEAS})/\alpha_{\min}(\text{SINGLE PASS})$ ratio can be significantly lower than the $\frac{1}{(1-R)}$ factor. In addition, equation 4.2 just takes into account the effects of quantum noise, i.e the shot noise limit, and neglects the effects of the dark noise of the detector. If the intensity of the light is reduced too much by the cavity mirrors, the dominant noise source is no longer the shot noise, or indeed the noise due to external environmental factors, but instead the intensity-independent noise associated with the detector. In this regime, the enhancement of the signal-to-noise ratio in incoherent BB-CEAS is even less than that predicted by equation 4.2. Given broadband sources necessarily have a lower spectral density to begin with, in contrast to laser-based CEAS, this scenario is one which must be considered: often as a result of this it is found that lower reflectivity mirrors produce a higher signal to noise ratio.

Fiedler *et al.* [242] also investigated a number of cavity parameters influencing the output intensity in broadband CEAS. They firstly concluded that the f number (the ratio of the focal length to the effective aperture) of the cavity should be adapted to the f number of the spectrometer used. In other words, in keeping with the general condition of dispersive grating spectroscopy, the light intensity at the entrance slit of the spectrometer should be maximised (to increase the signal to noise ratio) and the dispersive grating in the spectrometer should be fully illuminated (to maximise the spectral resolution), and then the cavity set-up adjusted accordingly to match this optical arrangement. Secondly, a focused light injection scheme should be used and a confocal cavity, if possible. The group found that a focused light injection scheme produced fewer losses in comparison to a parallel light injection, and that it was less susceptible to misalignments. The focused light injection produces an image at the centre of the cavity, whilst the very nature of the parallel light injection means that an additional reflection by the first mirror is required before the image encounters the second lens, as the image is formed after the first reflection from the second

cavity mirror. By varying the cavity length they also found that a confocal cavity arrangement produced the optimum transmitted light intensity. A confocal cavity results in the f number of the beam not changing following multiple reflections in the cavity, and hence the image of the light source in the cavity can be imaged sharply into the spectrometer by a lens after the cavity. If the cavity is non-confocal, not all the images distributed over the cavity can be imaged optimally, and this results in a mixture of blurred and sharp images on top of each other at the slit of the spectrometer, as they are imaged in different planes. In addition to the drop in light intensity, this also results in a poorly defined aperture angle. The spectral resolution is then reduced as a result of the grating only partially being illuminated, thanks to the effect of the small aperture angles of the blurred images. However, the authors note that this effect can be overcome by using an optical fibre, which generates an almost uniform angular distribution of the light intensity at the fibre exit.

With regards to the optimum aperture angle (which is limited by the diameter of the mirrors and the cavity length) for a confocal BB-CEAS cavity, it was noted that larger beam diameters result in more significant spherical aberrations of the mirrors and lenses, and the effects of small aberrations become more noticeable as the number of reflections increases, hence smaller beam diameters are preferentially utilised. Spherical aberrations increase with smaller radii of curvature (r), so mirrors with a large r and the consequent use of longer cavities should reduce these effects. Similarly, the larger the size of the image at the detector, the smaller the effect of aberration becomes compared to the image size; however, the authors note that although this suggests a large light source spot size might be desirable as a consequence, the reduction in the SNR enhancement factor is not too much on moving to a small spot size, and the higher radiance that results from the latter produces an improved single to noise ratio (SNR).

4.4 Detection Systems

An added complication when using broadband sources is the necessity to resolve the various wavelengths present in the spectral output. In the work described in this thesis, both a dispersive monochromator (SPEX Industries) with lock-in detection and a Fourier transform interferometer (Spectrum 100, Perkin Elmer) have been demonstrated and compared. The following sub-sections will present an overview of the operation of both of these instruments.

4.4.1 Dispersive Spectrometer

The basic schematic of a dispersive scanning monochromator spectrometer is illustrated in Figure 4.3. Essentially, the light source is imaged onto the entrance slit of the spectrometer

and the radiation is then directed on to a reflection grating by the first spherical mirror, M_1 . The grating disperses the radiation before the second spherical mirror, M_2 focuses it on to the exit slit. The grating is rotated, which allows different small selections of the wavelengths, $\Delta\lambda$, to go through the exit slit and be detected by the photodiode. Clearly, to optimise the alignment of the spectrometer the radiation should be imaged into the instrument so that the aperture angle (Ω) is fully used.

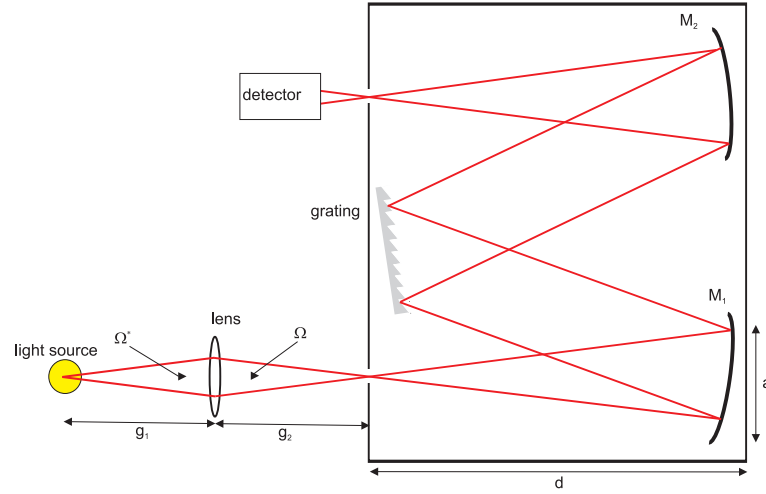


Figure 4.3: General schematic diagram of a Czerny-Turner dispersive scanning monochromatic spectrometer; adapted from Demtröder [110], where the aperture angle, Ω' , is given by $(\frac{g_2}{g_1})^2\Omega$ and $\Omega = \frac{a^2}{d^2}$.

The resolving power of the instrument is defined as:

$$\mathcal{R} \equiv \frac{\lambda}{(\Delta\lambda)_{\min}} = mN \quad (4.3)$$

where $(\Delta\lambda)_{\min}$ is the smallest wavelength difference resolvable and λ is the mean wavelength. Thus, the resolving power of the spectrometer is the product of the diffraction order, m , and the total number of illuminated grooves, N (with the diffraction order, m , given by $a(\sin\theta_i - \sin\theta_m)/\lambda$, where θ_i is the angle of incidence, θ_m is the angle of diffraction, a is the width of each groove in the grating and λ is the wavelength of the radiation) [244]. The spectrometer used in this work, SPEX Industries Inc., has a theoretical resolution of 0.1 nm.

4.4.2 Fourier Transform Interferometer

Many of the experiments carried out in this thesis utilised a Perkin-Elmer Spectrum 100, which is an example of a Michelson Interferometer. A general schematic diagram for this type of interferometer is illustrated in Figure 4.4.

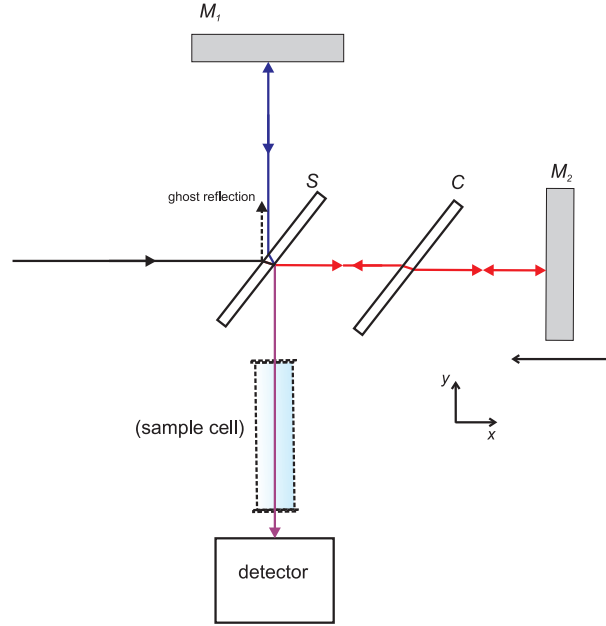


Figure 4.4: The Michelson Interferometer, where S is a beam splitter, $M_{1,2}$ are two plane mirrors and C is a compensator plate, which ensures that both beams undergo the same beam path conditions.

The incident light field is described by $E = A_0 \exp[i(\omega t - kx)]$ and on encountering the beam splitter, S , it is split into two waves, given by $E_1 = A_1 \exp[i(\omega t - kx + \phi_1)]$ and $E_2 = A_2 \exp[i(\omega t - kx + \phi_2)]$, where ϕ is the phase difference between the two waves and ω is the frequency of the monochromatic light [110]. The two beams are reflected off the plane mirrors, M_1 and M_2 , and then interfere on being recombined in the exit arm of the spectrometer. On moving mirror M_2 through Δx , the optical path difference (and consequently the phase difference, ϕ) will vary, leading to a series of peaks and troughs of detected signal intensity as the two beams constructively and destructively interfere. Consequently, the resultant intensity detected is a function of the path difference and is known as an interferogram [244]:

$$\begin{aligned}
 I &= |E_T E_T^*| \\
 &= I_0(1 + \cos \phi) \\
 &= I_0(1 + \cos(2\pi \tilde{\nu} \Delta x))
 \end{aligned} \tag{4.4}$$

where E_T is the electric field amplitude of the recombined beam, I_0 is the incident intensity, ϕ is the phase difference between the two beams, which is given by $\Delta x \frac{2\pi}{\lambda}$, where Δx is the path difference and $\frac{1}{\lambda}$ is the wavenumber of the light, $\tilde{\nu}$.

In the situation where the incoming radiation is not monochromatic, such as the case in these studies where the interferometer is used to analyse the broadband radiation from an

optical cavity, the light source has a spectral distribution, $I(\tilde{\nu})$, and the total intensity is the sum of the intensities at each wavenumber, $\tilde{\nu}$ [245]:

$$I(\Delta x) = \int_0^\infty I(\tilde{\nu})(1 + \cos(2\pi\tilde{\nu}\Delta x)) d\tilde{\nu} \quad (4.5)$$

Thus to extract $I(\tilde{\nu})$, a Fourier transform is applied to the detected interferogram, $I(\Delta x)$, so that the variation in intensity as a function of path difference is converted into the variation in intensity as a function of wavenumber: i.e. the spectral components of the incoming wave are resolved. This is illustrated in a simple case where the incident radiation consists of two frequencies, described by wavenumbers $\tilde{\nu}_1$ and $\tilde{\nu}_2$, in Figure 4.5.

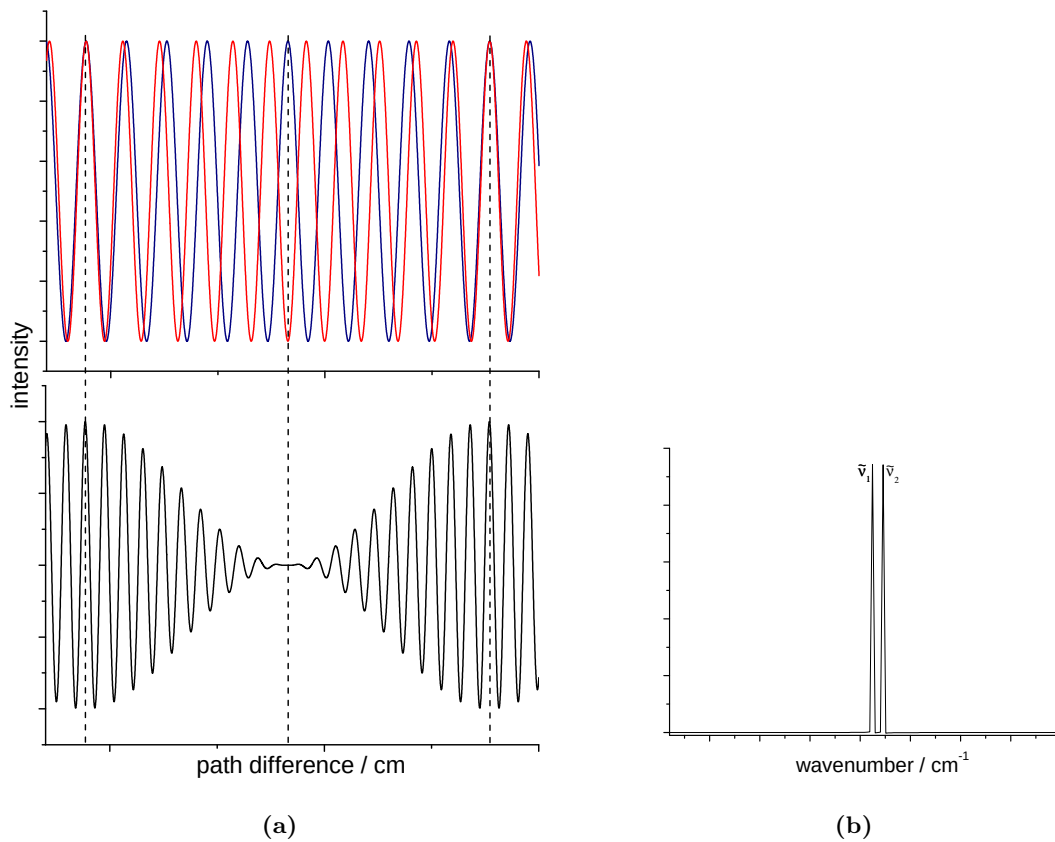


Figure 4.5: Figure (a): **top:** the intensity variation for the two frequencies of the incident light as a function of path difference, and **bottom:** the resulting interferogram; Figure (b): the Fourier transform of Figure (a), illustrating the extraction of $\tilde{\nu}_1$ and $\tilde{\nu}_2$.

Finally, the resolution of the instrument is given by the inverse of the maximum pathlength difference induced by the moving mirror (i.e. the maximum retardation). The highest resolution offered by the FTIR used in this thesis is 0.5 cm^{-1} , corresponding to a maximum retardation of 2 cm.

4.5 Development of the initial detection system: SLED with a dispersive spectrometer

The first broadband detection system developed in this study involved the use of a Superluminescent Light Emitting Diode (SLED), spanning 100 nm with a central wavelength frequency of 1650 nm, in conjunction with a dispersive spectrometer. Following the optimisation of the resolution of the instrument and the calibration of the frequency scale, direct absorption measurements on butadiene were taken in order to determine absorption cross-sections for the molecule in the spectral range of the SLED. Knowledge of these allowed a reflectivity curve for the cavity mirrors in the region 1600 - 1700 nm to be determined. Finally, the optical cavity was assembled and lock-in detection added before measurements were taken on both butadiene and acetone samples, and sensitivity values determined.

4.5.1 Experimental set-up

The fibre-coupled SLED (DenseLight Semiconductors) outputs ~ 10 mW of total power from the fibre at a driving current of 300 mA (Thorlabs current driver LDC205C) and was temperature stabilised (Thorlabs temperature controller TED200C) within a 14 pin diode laser butterfly package placed in an appropriate mount (Newport). The output initially passed through a long focal length lens (50 cm) to collimate the SLED output, before a second lens of 25 cm focal length focused the radiation into the centre of the optical cavity. The cavity consisted of two high reflectivity mirrors (REO Inc., maximum $R \sim 99.98\%$, 1.5 m radii of curvature), was 25 cm in length and enclosed within a vacuum vessel. To aid the alignment of the optical cavity, a diode laser operating at 1650 nm was co-aligned with the decoupled fibre output of the SLED. The cavity was then initially aligned with the diode laser such that low order modes dominated the cavity transmission, before the sources were exchanged and the alignment optimised for the SLED. The light exiting the cavity was then directed, via two lenses, into a 0.5 m scanning monochromator (SPEX Industries) in a Czerny-Turner configuration. The first lens collimated the cavity output beam, whilst the second focused the beam into the spectrometer so that the internal optical arrangement of the spectrometer was satisfied and that as much of the grating was illuminated as possible. At the exit slit of the spectrometer, an InGaAs detector (Thorlabs DET410), fitted with a 3 cm focal length lens, was positioned to record the intensity of the selected radiation component as the spectrometer scanned. The spectrometer was driven by a specially designed LabView programme, which also collated the data from the detector. The detector was connected to a lock-in amplifier (Stanford Research), which received a reference signal of ~ 1 kHz from the mechanical chopper controller in order to realise phase-sensitive detection. Figure 4.6 illustrates the experimental set-up.

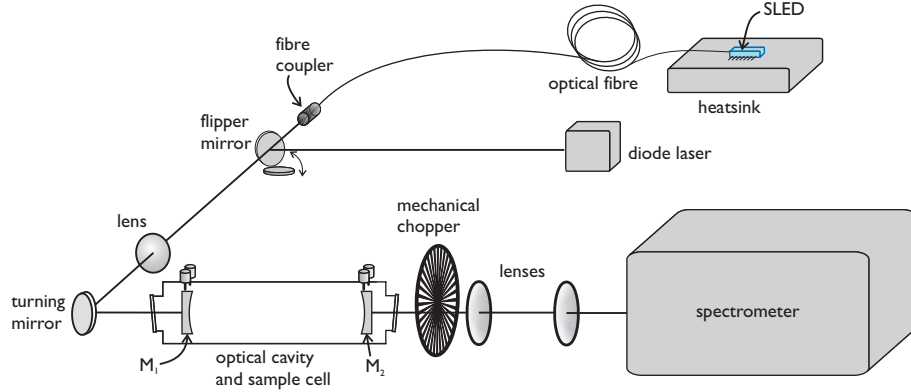


Figure 4.6: The SLED and SPEX experimental set-up.

4.5.2 Lock-in Detection

After passing through a high finesse cavity, and on dispersion by the spectrometer, the detected signal intensity is naturally very low. Therefore, a lock-in detection scheme was employed to extract this low signal from background noise. A lock-in amplifier is a phase sensitive detection system: it selects and detects only the light which has a pre-specified frequency and phase, thus rejecting all other radiation from the final detected signal.

This is achieved using an external reference signal which modulates the laser output at a frequency, ω_{ref} , and serves as the input for the lock-in, as illustrated in the top panel of Figure 4.7. In this work, the modulation is from a mechanical chopper placed in the beam path, but in other schemes it could be provided by a function generator acting on the laser, or from the internal oscillator of the lock-in amplifier itself. The resulting experimental signal (post cavity) is also directed into the lock-in amplifier. This output, illustrated in the middle panel of Figure 4.7, is given by $V_{\text{sig}} \sin(\omega_{\text{ref}} t + \theta_{\text{sig}})$, where V_{sig} is the initial signal and the sinusoidal function is the modulation applied, with θ_{sig} representing the phase shift from the reference signal. The bottom panel in Figure 4.7 illustrates the sine wave generated by the lock-in amplifier itself, $V_L \sin(\omega_L t + \theta_L)$.

This lock-in reference signal is then multiplied by the signal detected from the experiment by the lock-in amplifier to produce the phase sensitive detection (PSD) output:

$$\begin{aligned}
 V_{\text{psd}} &= V_{\text{sig}} V_L \sin(\omega_{\text{ref}} t + \theta_{\text{sig}}) \sin(\omega_L t + \theta_{\text{ref}}) \\
 &= \frac{1}{2} V_{\text{sig}} V_L \cos[(\omega_{\text{ref}} - \omega_L)t + \theta_{\text{sig}} - \theta_{\text{ref}}] - \\
 &\quad \frac{1}{2} V_{\text{sig}} V_L \cos[(\omega_{\text{ref}} + \omega_L)t + \theta_{\text{sig}} + \theta_{\text{ref}}]
 \end{aligned} \tag{4.6}$$

This results in two AC signals at $(\omega_{\text{ref}} - \omega_L)$ and $(\omega_{\text{ref}} + \omega_L)$. On passing the phase sensitive output signal through a low band pass filter the AC signals are removed, except in the case

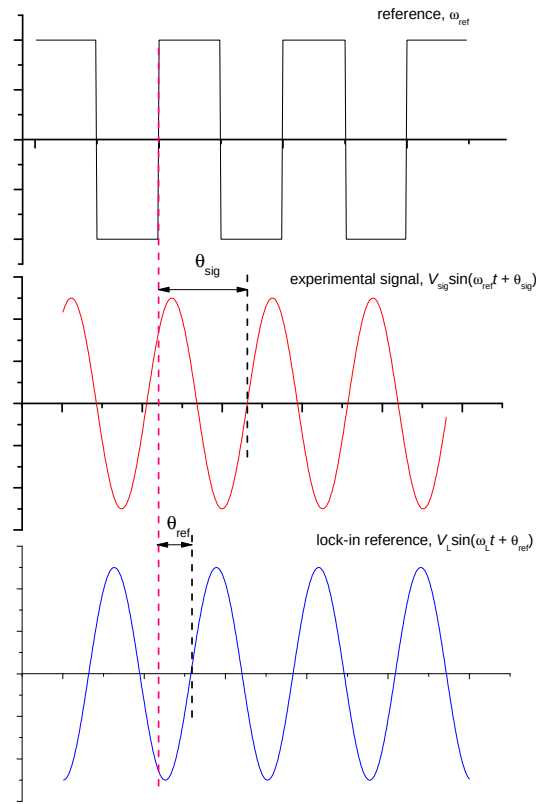


Figure 4.7: Illustration of the signals involved in lock-in detection: the top trace shows the reference signal from the chopper; the middle trace depicts the signal from the experiment after the modulation has been applied; and the bottom trace is the signal generated by the lock-in amplifier itself.

where $\omega_{\text{ref}} = \omega_L$. In this situation, the filtered output becomes:

$$V_{\text{psd}} = \frac{1}{2} V_{\text{sig}} V_L \cos(\theta_{\text{sig}} - \theta_{\text{ref}}) \quad (4.7)$$

Thus the DC signal is proportional to the signal amplitude, V_{sig} . This also highlights the necessity for $\omega_{\text{ref}} = \omega_L$ and for the phase not to change between the signals over time. This is achieved using the phase-locked-loop (PLL) in the lock-in amplifier which locks the internal reference oscillator to the external reference provided by the chopper. This also means that if the chopper slowly varies its frequency of modulation, the reference signal in the lock-in amplifier will change accordingly and the measurements taken are not affected. Thus, any signal incident on the detector not at frequency ω_{ref} will be rejected and filtered out.

4.5.3 Determination of resolution and frequency calibration

The 1650 nm diode laser, in conjunction with the Q branch of the $2\nu_3$ band of methane at 1665.6 nm, provided two fixed points of known frequency with which to calibrate the spectrometer frequency scale, as illustrated in Figure 4.8. Once a frequency scale had been established, it was possible to determine the optimum exit slit width of the spectrometer to achieve maximum resolution. The full width half maximum (FWHM) of the 1650 nm diode laser output was monitored as the slit width was varied, with the smallest FWHM representing the highest resolution attainable. With no lock-in amplification or cavity in place (i.e. for direct absorption measurements), this was found to be $\sim 1.8 \text{ cm}^{-1}$. On inclusion of the optical cavity, and optimisation of the system for broadband absorbers, the combination of the dispersion, exit slit width and time constant (10 s), yielded an approximate resolution of $\sim 15 \text{ cm}^{-1}$.

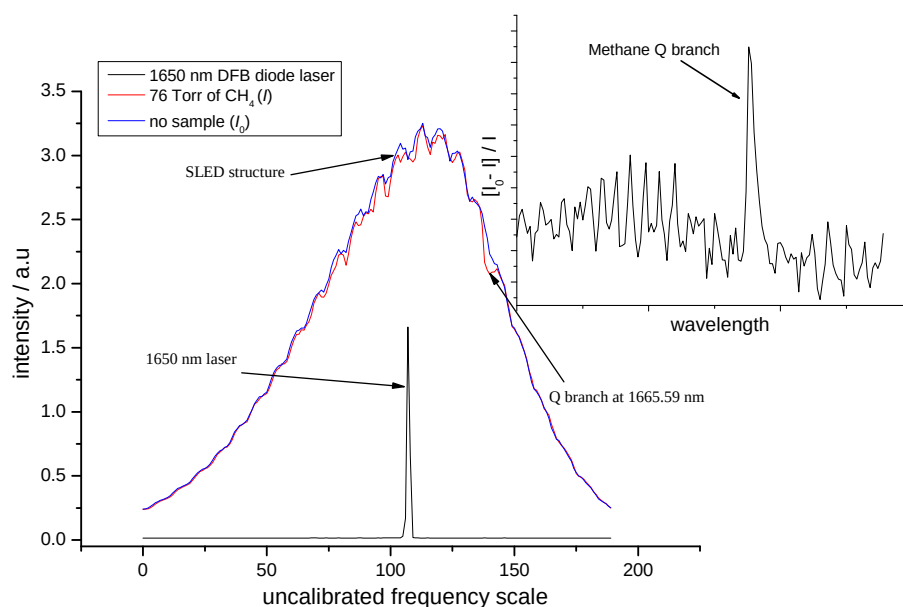


Figure 4.8: Determination of the frequency scale; inset, the $2\nu_3$ CH_4 absorption spectrum obtained.

4.5.4 Preparation of samples

In this initial study, both butadiene and acetone were investigated. The butadiene (Sigma-Aldrich 99+%) samples were used without purification and prepared by mixing a known quantity of vapour within a cell connected to a vacuum line with laboratory air for the cavity-enhanced measurements. The acetone (HPLC grade, Fisher Chemical) mixes were prepared in the same way but the liquid sample was first frozen and pumped upon for several minutes to remove dissolved air. This freeze-pump-thaw cycle was repeated three

times before the acetone vapour was collected. In addition, because of the tendency for liquid samples to condense on any surface, the pressure was allowed to equilibrate and stabilise before use.

4.5.5 Results

Determination of I_0 (the background measurement) was achieved by evacuating the cell and recording a scan, before a sample of the dilute butadiene mix (1 in 100 butadiene in air) was released into the cell and another measurement taken. A single scan of 200 points over the region of interest takes approximately 6 minutes. A range of pressures of the mix were analysed in this way, as illustrated in Figure 4.9a. From these data, a minimum detectable absorption coefficient of $\alpha_{\min} = 6.1 \times 10^{-8} \text{ cm}^{-1}$ can be extracted. This value was deduced by recording two sequential background scans (i.e. with no absorber present), and treating one dataset as I_0 and the other as I in equation 3.18, and determining the residual around the zero level. While this is a good representation of the average sensitivity over the full width half maximum of the SLED emission, clearly the sensitivity is reduced for the spectral region at the edges of the range, where the SLED has little power output. Similarly, a 1 in 81 acetone in air mix was prepared and various pressures analysed in the spectrometer, as illustrated in Figure 4.9b.

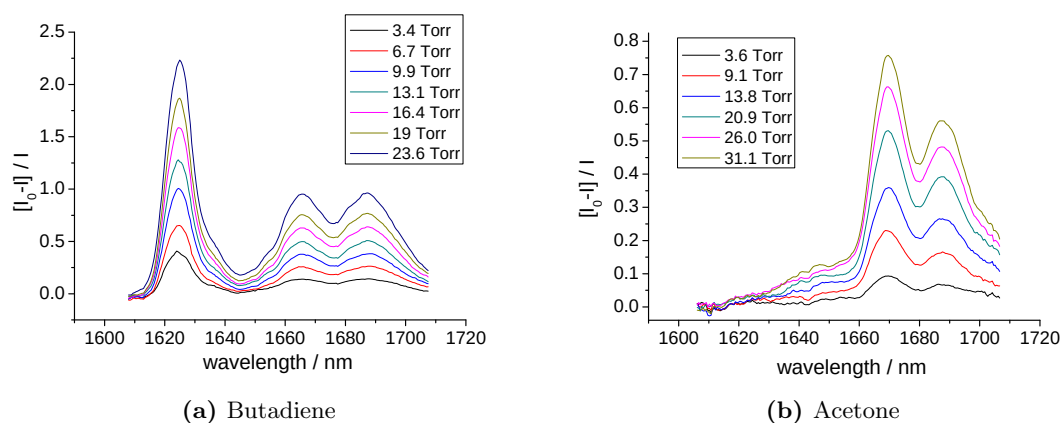


Figure 4.9: Cavity enhanced spectra of (a) various pressures of a 1 in 100 butadiene in air mix and of (b) various pressures of a 1 in 81 acetone in air mix, taken with the SLED and SPEX monochromator.

Given the large spectral region covered with these measurements ($\sim 100 \text{ nm}$), the reflectivity of the cavity mirrors will vary over this range and this needs to be corrected for in the spectra. Knowledge of the absorption cross-sections for butadiene allows the variations in the reflectivity of the cavity mirrors as a function of wavelength to be deduced. Therefore, direct

absorption measurements were recorded at various pressures of neat 1,3-butadiene (Sigma-Aldrich 99+%), within the range 5 - 70 Torr, as monitored by a capacitance manometer (Leybold Piezovac). From the absorption spectra, the peak cross-sections across the whole absorption feature can be determined. With the absorption cross-sections known, the mirror reflectivity can be deduced as a function of wavelength using the cavity-enhanced absorption equation, $\frac{I_0 - I}{I} = \frac{\sigma_p}{kT} \frac{L}{[1 - R(\lambda)]}$, from cavity-enhanced data taken. This reflectivity curve was then used to extract the absorbance (αL) from the measurements taken thereafter, thus taking into account the variations in mirror reflectivity over the spectral region of interest. These reflectivity-corrected data are shown in Figure 4.10, together with the reflectivity curve used.

4.6 SLED with Fourier Transform Interferometer

The next stage in the development of a sensitive broadband detection instrument involved replacing the scanning dispersive monochromator with a Fourier Transform infrared interferometer (FTIR). After optimisation of the settings and alignment of the interferometer, further direct absorption measurements were taken of the molecules of interest, taking advantage of the various resolutions at the disposal of the FTIR. Finally, cavity-enhanced measurements were taken on butadiene, acetone, isoprene and methane.

4.6.1 Experimental set-up and results

The experimental set-up with the FTIR (Perkin Elmer Spectrum 100) was identical to that with the monochromator, as depicted in Figure 4.6, with the exceptions that the collimated beam leaving the cavity was directed into the spectrometer with no further modification and that no lock-in detection was utilised. The same detector (Thorlabs DET410) was used to detect the resulting interferogram and the cavity signal was amplified ($10\times$) and processed on-board. Initially, the FTIR was aligned using a HeNe laser, before being switched for the SLED.

The optimum scan rate of the FTIR was deduced from comparing the output from transmissions taken at different scan rates (i.e. taking an initial background measurement at a particular scan rate, and subtracting this from a second measurement taken at the same scan rate), and was found to be 0.2 cm/s. Similarly, the optimum scan time was determined from an analysis of the baseline noise present for a variety of scan times and was found to be 4 minutes. These settings are used throughout this work, with the exception of the instances where the highest resolution (0.5 cm^{-1}) has been used: in this case, an extended time is employed as the instrument takes longer to complete one scan.

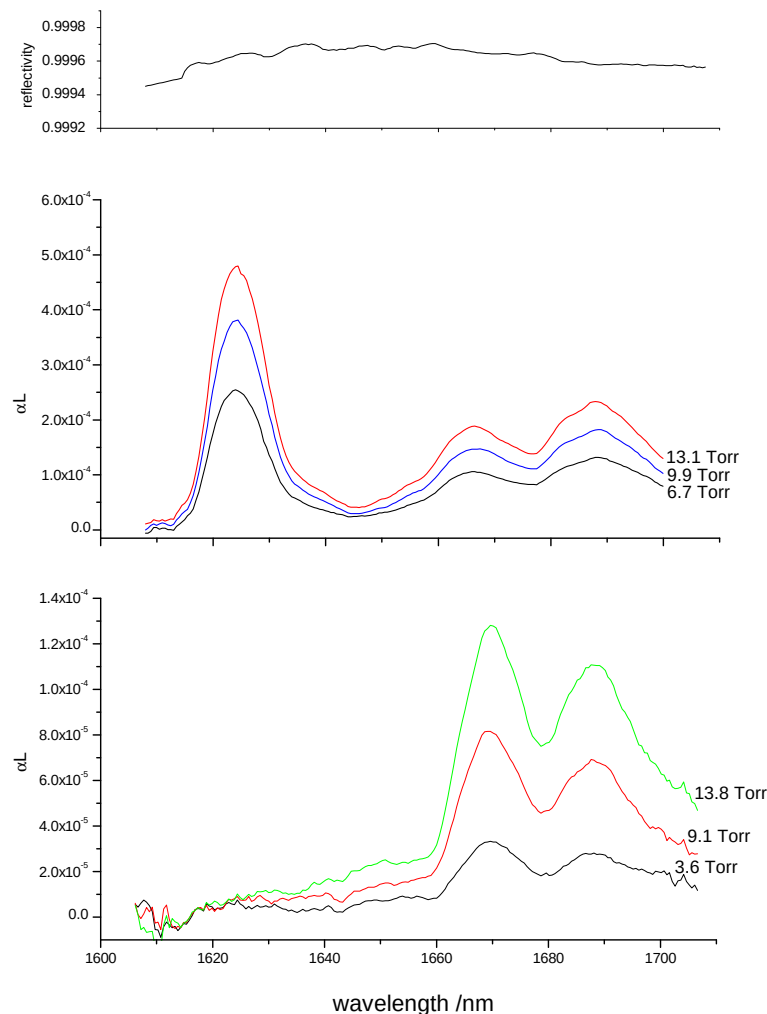


Figure 4.10: A selection of spectra obtained using the SLED with the SPEX monochromatic spectrometer; **top:** the experimentally-determined reflectivity curve of the cavity mirrors used; **middle:** selected reflectivity-corrected butadiene spectra (1 in 100 butadiene in air mix); **bottom:** selected reflectivity-corrected acetone spectra (1 in 81 acetone in air mix).

Butadiene

Initially, high resolution (0.5 cm^{-1}) direct absorption measurements were taken of various pressures of neat 1,3-butadiene (Sigma-Aldrich 99+%) using the internal detector of the FTIR. It is clear from Figure 4.11a, which illustrates these results, that some rotational structure is discernible at this resolution. Figure 4.11b shows the peak absorptions from Figure 4.11a as a function of sample pressure at 1625.98 nm and 1666.63 nm, from which absorption cross-sections can be determined. This gives $\sigma_{\text{peak}} = 4.67 \times 10^{-21} \text{ cm}^2$ and $\sigma_{\text{peak}} = 1.90 \times 10^{-21} \text{ cm}^2$ respectively, whilst a similar plot for the peak at 1688.01 nm gives $\sigma_{\text{peak}} = 2.51 \times 10^{-21} \text{ cm}^2$, with an estimated uncertainty of $\sim 5\%$. These values

compare favourably with those derived from the spectra from the PNNL database [246] ($\sigma_{\text{peak}} = 4.74 \times 10^{-21} \text{ cm}^2$, $1.92 \times 10^{-21} \text{ cm}^2$, $2.39 \times 10^{-21} \text{ cm}^2$, respectively) and the deviations observed are almost certainly due to the lower resolution used in this study. Following this (and with the Thorlabs DET410 detector replacing the internal FTIR one), direct absorption measurements were taken of ~ 62 Torr of butadiene at various resolutions to investigate the effect of resolution on the spectra obtained. A selection of the resulting spectra are shown in Figure 4.11c.

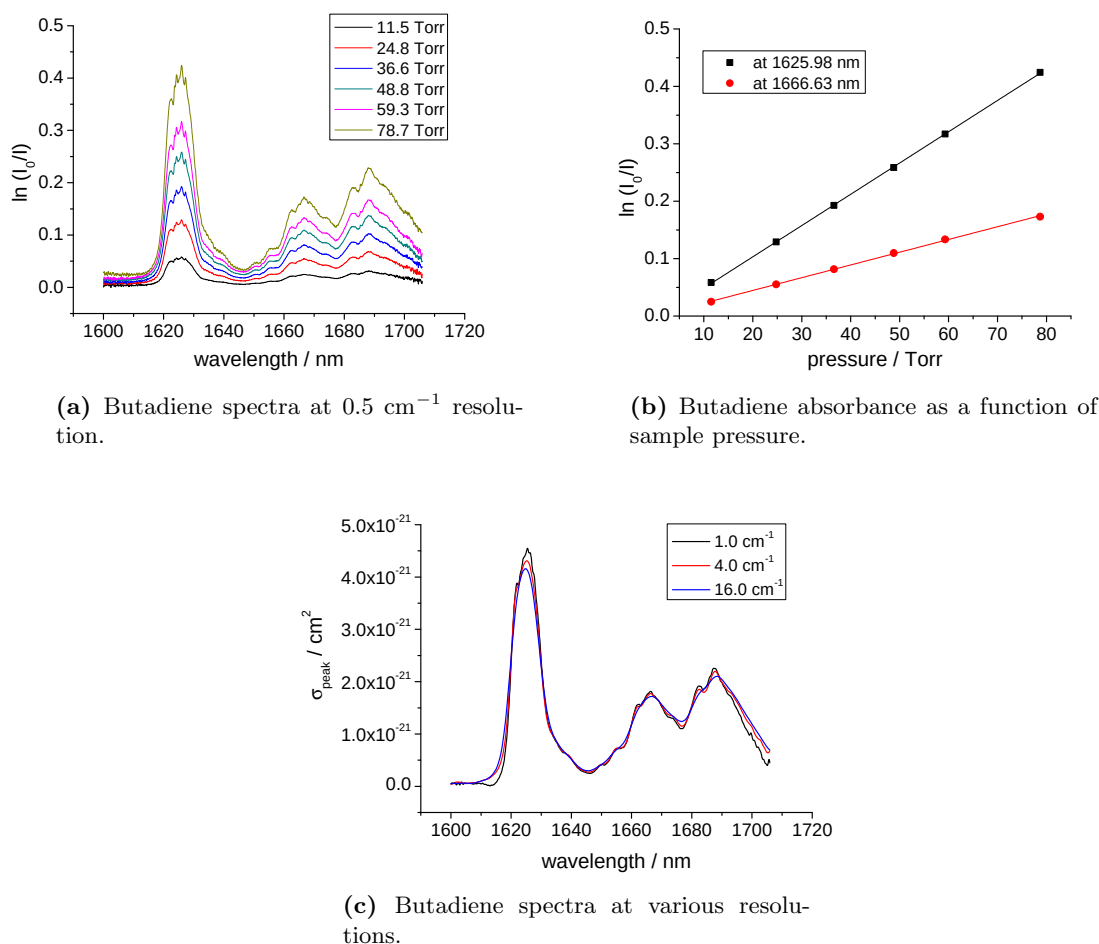


Figure 4.11: Direct absorption spectra of butadiene: Graph (a) illustrates direct absorption measurements on pure samples of butadiene taken at a resolution of 0.5 cm^{-1} over a 5 minute interval with Graph (b) depicting the peak absorbance at 1625.98 nm and 1666.63 nm as a function of pressure; whilst Graph (c) shows the subsequent determination of the absorption cross-sections for butadiene at various FTIR resolutions from direct absorption measurements on ~ 62 Torr samples of butadiene.

It is clear, both from Figure 4.11a and Figure 4.11c, that even at the higher resolutions (accessible by the FTIR), little detailed structure is observed, suggesting that there is not much to be gained from taking data at high resolution. In addition, the apparent loss in

absorption cross-section as a result of the lower resolution is not too significant ($\sim 10\%$), given the advantage gained from not requiring a lengthy scan time.

With the cavity mirrors reinserted into the vacuum vessel and the cavity realigned, a series of measurements on various pressures of a 1 in 100 butadiene mix were recorded at a resolution of 16 cm^{-1} . Whilst the majority of measurements were recorded at this resolution, the high resolution spectrum also illustrated in Figure 4.12 seems to show the detailed structure observed in the direct absorption measurements, with arguably greater definition. This could be a result of large self-broadening coefficients which potentially contribute more in the direct absorption measurements because of the higher pressures of butadiene required compared with the cavity-enhanced samples. As before, the data were reflectivity corrected to take into account reflectivity variations, with a maximum reflectivity of 99.98% recorded, equating to a pathlength enhancement of ~ 5000 relative to the 25 cm single-pass pathlength.

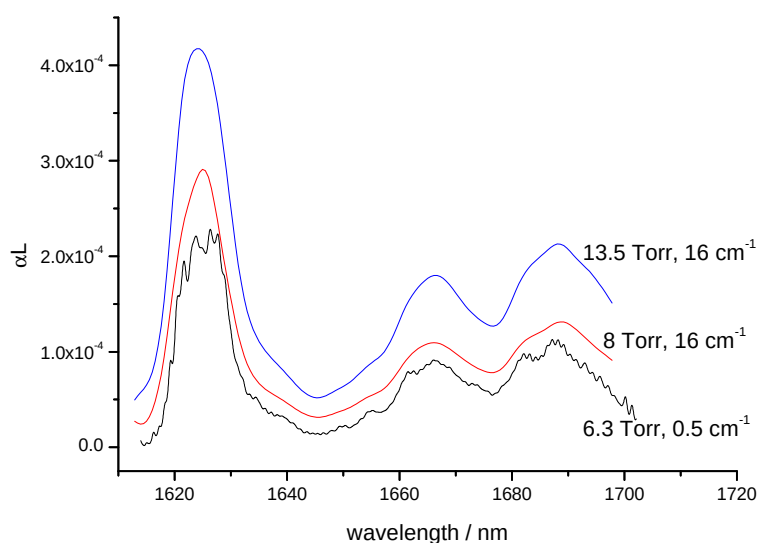


Figure 4.12: Example spectra of various pressures of a 1 in 100 butadiene in air mix at 0.5 cm^{-1} and 16 cm^{-1} resolution.

These data resulted in a minimum detectable absorption coefficient of $\alpha_{\min} = 1.5 \times 10^{-8}\text{ cm}^{-1}$, determined in the same manner as in the previous section. Assuming an average absorption cross-section of $\sim 1 \times 10^{-21}\text{ cm}^2$ for these data, this corresponds to butadiene levels of ~ 600 ppb.

Acetone

Utilising both the internal source and detector of the FTIR, direct absorption measurements were taken of pure acetone at 148 Torr at 4 cm^{-1} resolution and a typical spectrum is illustrated in Figure 4.13a. It is immediately apparent that there is some structure

centred at ~ 1672 nm which was not resolved in the previous experiment employing a monochromator. This distinctive feature was found to have a measured peak cross-section of $\sigma_{\text{peak}} = 1.3 \pm 0.1 \times 10^{-21} \text{ cm}^2$ (at 4 cm^{-1} resolution), which corroborates the value obtained by Wang *et al.* [106], $\sigma_{\text{peak}} = 1.2 \times 10^{-21} \text{ cm}^2$ (although they report this cross-section at a wavenumber of 6000 cm^{-1}) and with that derived from the PNNL database [246], $\sigma_{\text{peak}} = 1.54 \times 10^{-21} \text{ cm}^2$ at 1672 nm. Once again, the discrepancies could be a result of the relatively low resolution used in this study slightly broadening the spectral feature. In addition, there are the inherent handling difficulties associated with working with a liquid-based vapour, which has a tendency to condense on any surface. Although pressures are allowed to equilibrate and stabilise, clearly there is some uncertainty in the actual concentration of the sample within the holding cell, and further unavoidable uncertainties are introduced when any of the sample is transferred into the measuring cell.

For cavity enhanced detection, a 1 in 81 mix of acetone in air was prepared in the same manner as before: following purification of the acetone sample, the vapour was collected in a glass cell before laboratory air was used to buffer the sample. Using an empty vacuum cell as the I_0 measurement, various pressures of the acetone mix were analysed with the same methods as previously described. Taken at a resolution of 16 cm^{-1} , the sharp feature at 1672 nm observed in the direct absorption measurements of Figure 4.13a is not resolved. For these preliminary measurements using the broadband CEAS system this low resolution is sufficient, but when the spectrometer is applied to samples where there are competing species, the ability to resolve the distinctive feature will be advantageous for molecular selectivity. For this reason, acetone measurements taken latterly in this chapter will be recorded at a higher resolution of 4 cm^{-1} .

With a minimum detection sensitivity of $\alpha_{\text{min}} = 1.5 \times 10^{-8} \text{ cm}^{-1}$ for the instrument, and assuming an average absorption cross-section of $\sim 4 \times 10^{-22} \text{ cm}^2$ across the spectral range studied ($\sim 1620 - 1700$ nm for acetone), an acetone absorption of ~ 1.5 ppm should be identifiable. Given this, a calibrated mix was obtained of 10 ppm of acetone in air (BOC Special Gases) and samples were analysed at a resolution of 4 cm^{-1} , as depicted in Figure 4.14a. The figure illustrates that 10 ppm of acetone can be clearly identified with a CEAS signal of $\sim 4\%$. This confirms that the instrument is approaching the levels of sensitivity required to detect acetone in breath. Therefore, the next stage was to test the device's ability to detect acetone in a breath matrix: the 10 ppm calibrated mix was then combined in varying proportions with breath and the resulting samples analysed, as in Figure 4.14b. Absorption due to acetone can be clearly seen, as can the other main absorbers in breath, which are identified in the figure: the CO_2 band at 1645 nm ($3\nu_1 + \nu_3$, with a Fermi resonance index of 4), the CH_4 Q branch at 1665 nm ($2\nu_3$) and transitions due to water vapour at the longer wavelengths. These constituents will be discussed in more detail in a later section; suffice now to note that acetone at levels ranging from 5 - 9 ppm can be

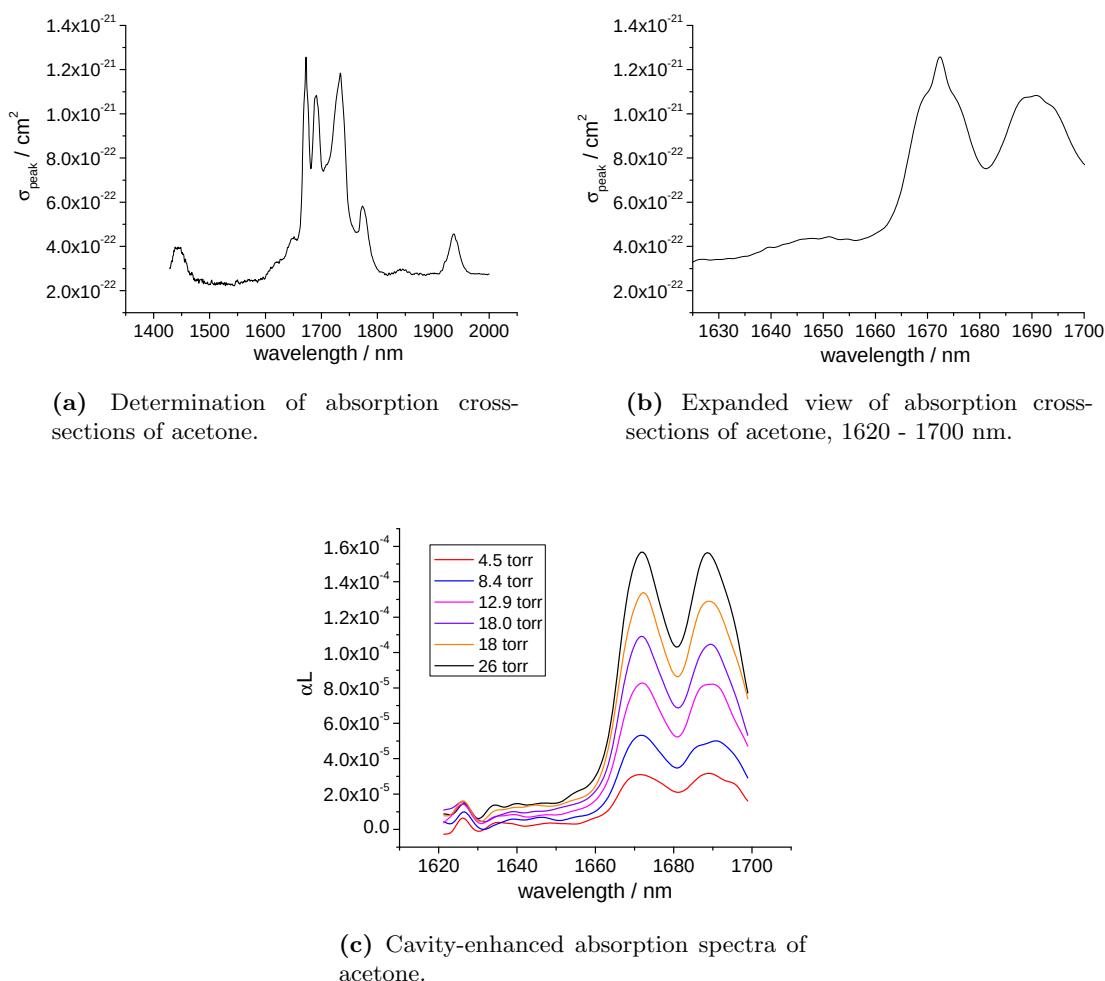


Figure 4.13: Graph (a) illustrates direct absorption measurements on 148 Torr of pure acetone, taken at a resolution of 4 cm^{-1} over a 10 minute interval with Graph (b) depicting an expanded view of the spectral region of interest, 1620 - 1700 nm; whilst Graph (c) shows cavity-enhanced absorption spectra of various pressures of a 1 in 81 acetone in air mix, taken at a 16 cm^{-1} resolution over 4 minutes.

clearly identified amongst these other species, and that the overall level of absorption across the spectral range appears to increase with increasing proportions of breath. The former suggests that detecting acetone in breath in this spectral region is a viable option, provided that a slightly higher level of sensitivity can be attained. The latter highlights a major problem when dealing with breath: the influence of water vapour and in particular the additional, broad absorption due to the water continuum, which will be discussed more fully in Chapter 5.

Isoprene

The other major broadband absorber to be studied, and another breath biomarker, is isoprene. Like acetone, isoprene is found as a liquid at room temperature (with a vapour

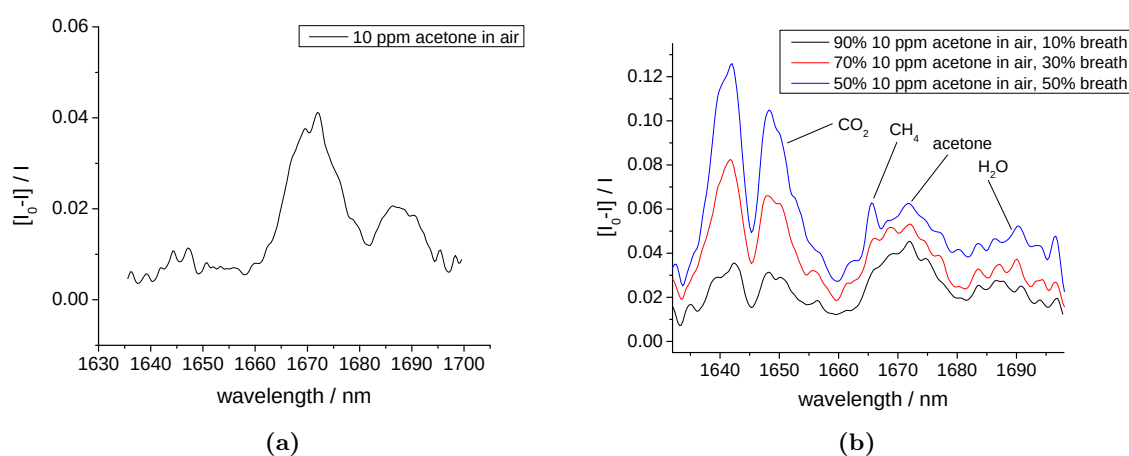


Figure 4.14: Graph (a) illustrates a CEA spectrum of 10 ppm of acetone in air; taken at 4 cm^{-1} resolution over 4 minutes; whilst Graph (b) shows CEA spectra of various proportions of breath (non-methane producer) and 10 ppm acetone in air mix; taken at 4 cm^{-1} resolution over 4 minutes.

pressure of $\sim 468 \text{ Torr}$ at 20°C , compared to $\sim 178 \text{ Torr}$ for acetone at the same temperature [247]), and isoprene samples were prepared in the same way as acetone: a liquid sample of isoprene (Sigma-Aldrich) was frozen and pumped on for several minutes to remove any dissolved gases, with the freeze-pump-thaw cycle repeated three times before the vapour was collected. Direct absorption measurements were performed on neat isoprene, whilst the samples for cavity-enhanced measurements were then buffered with laboratory air.

Figure 4.15a illustrates the cross-sections determined from direct absorption measurements at a resolution of 4 cm^{-1} and 16 cm^{-1} . As can be seen, there is very little detailed structure on the $\sim 1 \text{ cm}^{-1}$ scale, though there is a predictable reduction in peak absorption cross-section with the lower resolution. The peak cross-sections at 4 cm^{-1} resolution were found to be $3.74 \times 10^{-21} \text{ cm}^2$, $1.57 \times 10^{-21} \text{ cm}^2$ and $2.27 \times 10^{-21} \text{ cm}^2$ at 1625.22 nm, 1668.06 nm and 1683.79 nm respectively, which compare reasonably well with the equivalent values determined from the PNNL database [246], $4.74 \times 10^{-21} \text{ cm}^2$, $1.83 \times 10^{-21} \text{ cm}^2$ and $2.73 \times 10^{-21} \text{ cm}^2$. The discrepancies between the measurements are quite likely to be due to a combination of the lower resolution used here (PNNL data have a resolution of 0.112 cm^{-1}) plus the difficulties associated with handling liquid samples and the subsequent uncertainty in the actual concentration of the samples used. Cias *et al.* [248] reported a cross section of $3.24 \pm 0.16 \times 10^{-22} \text{ cm}^2$ at 1651.52 nm, whilst the PNNL database gives a much higher value of $4.44 \times 10^{-22} \text{ cm}^2$ at the same wavelength (and this work produces $4.18 \times 10^{-22} \text{ cm}^2$), perhaps highlighting the difficulty in accurately determining absorption cross-sections for molecules such as isoprene. With the optical cavity in place, an I_0 measurement of the empty cell was taken before samples from a mix of 1 in 100 isoprene in air were taken and analysed. The resulting reflectivity-corrected spectra are illustrated in Figure 4.15b.

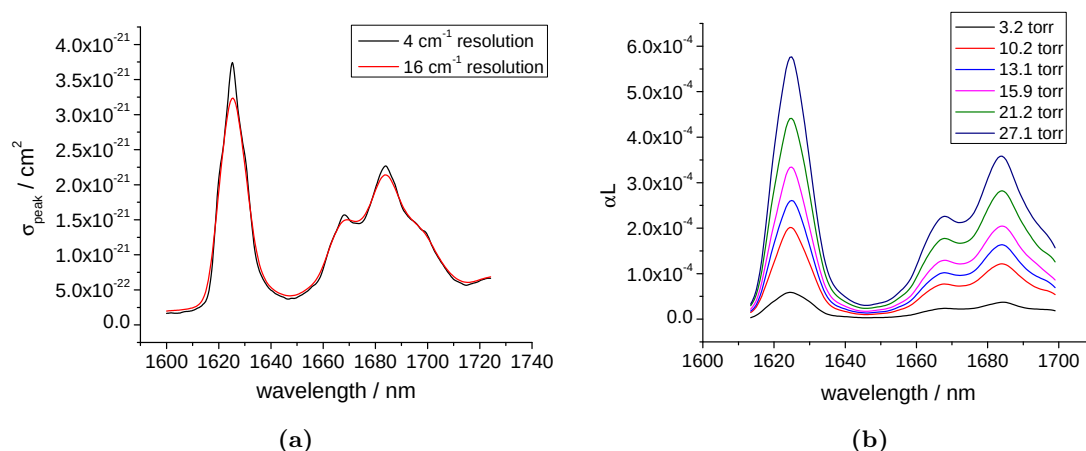


Figure 4.15: Graph (a) illustrates absorption cross-sections for isoprene determined at 4 cm^{-1} and 16 cm^{-1} resolution; taken over 4 minutes with a 290 Torr sample; whilst Graph (b) shows reflectivity-corrected CEA spectra of various pressures of a 1 in 100 isoprene in air mix; taken at 4 cm^{-1} resolution over 4 minutes.

However, it is clear from the CEA spectra of the acetone in breath matrix from Figure 4.14b that the presence of absorption due to CO_2 in breath would make it difficult to unambiguously determine breath isoprene concentrations in this spectral region, though perhaps pre-concentration or separation techniques could be employed in order to achieve this, so that CO_2 is removed (or its effects negated) from the sample and/or the levels of isoprene are increased relative to CO_2 .

Methane

As described previously, approximately one in three people are methane producers and so have elevated levels of methane in their breath. Therefore, in addition to the study of the broadband biomarker molecules present in breath, it is also important to investigate the spectroscopy of this narrowband absorber in the spectral region of interest. To this end, samples of laboratory air (approximately 1.8 ppm methane, representing the level found in the breath of non-methane producers) and two calibrated mixes of 7.5 ppm and 15 ppm (both CK gas products Ltd.) were studied. The spectra recorded are illustrated in Figure 4.16, where the P, Q and R branches of the $2\nu_3$ band of CH_4 can be observed. The sensitivity (< 1.8 ppm) of the instrument suggested by Figure 4.16 corroborates with Figure 4.14b, where it is also clear from the breath sample spectra depicted in it that breath methane is comfortably observed with the spectrometer.

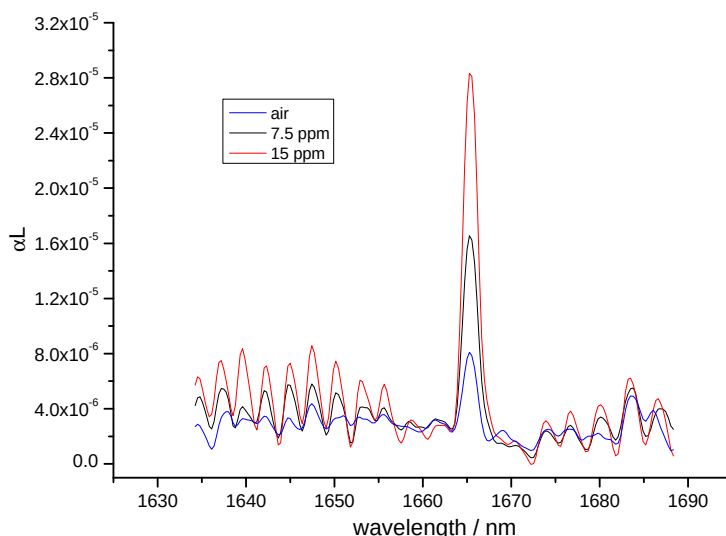


Figure 4.16: Reflectivity-corrected spectra of methane at 15 ppm, 7.5 ppm and ~ 1.8 ppm (laboratory air); taken at 4 cm^{-1} resolution, over 4 minutes.

4.6.2 Discussion and extension of the method

These early measurements utilising a SLED have employed both a dispersive monochromator and an FTIR spectrometer, thus it is instructive to compare their respective performances, with regards to minimum sensitivities attainable and their ease of use. The sensitivities achieved with the SPEX and FTIR, $\alpha_{\min} = 6.1 \times 10^{-8}\text{ cm}^{-1}$ and $\alpha_{\min} = 1.5 \times 10^{-8}\text{ cm}^{-1}$ respectively, are of the same order of magnitude over a similar acquisition time and for a similar resolution. The main advantage that the FTIR provides over the scanning monochromator is the multiplex or Fellgett advantage - i.e. that of speed. The FTIR is able to collect the information from all wavelengths simultaneously, which results in a shorter scan time for a given resolution, or a higher signal to noise ratio for a given scan time. This can be approximated by considering the ratio of the total spectral range covered (100 nm) to the effective step size of the monochromator ($\sim 0.5\text{ nm}$). This means the monochromator would take 200 times longer than the FTIR to attain the same signal to noise ratio at every point over the spectrum, leading to an increase of ~ 14 in the signal to noise ratio for the FTIR. The other typical advantage of FTIR detection over dispersive monochromator detection is the throughput, or Jacquinot advantage. With a scanning spectrometer, the light which passes through it is restricted by its entrance and exit slits, whereas with the FTIR, the diameter of the collimated beam from the source determines the throughput. However, this advantage is not accessed in this particular experiment, as the entrance slit is not utilised because the SLED output is spatially coherent and the system set up so that the beam is focused at the entrance slit.

Despite these factors, the ability of the phase-sensitive detection to mitigate noise in the scanning monochromator experiments seems to have resulted in a gain in sensitivity for that system, and this almost certainly accounts for only the modest increase in sensitivity on using the FTIR detection system. However, the major advantage with the FTIR is that of convenience: it comes as a stand-alone package, which is much easier to align optically, provides near real-time visualisation of data and does not need phase-sensitive detection. For this reason, the FTIR is used exclusively for the subsequent studies presented in this chapter.

Whilst these results look very promising with regards to the sensitivity and selectivity required to detect biomarkers in breath, it is clear that slightly higher sensitivity would be desirable. In addition, it is found that the absorption spectra resolved towards the extremities of the spectral region covered tend to suffer from a greater degree of noise, particularly for acetone (where there is little absorption) and the highest resolution data: this is due to the low power output of the SLED in these regions. Therefore, to increase the spectral coverage of the instrument, a second SLED, centred at 1550 nm (Covega), was introduced to the system. The optical field emitted by a SLED has a certain degree of polarisation, which means that when polarisation-maintaining fibre pigtails are used, this property can be exploited to combine the radiation from the 1550 nm SLED with that of the 1650 nm SLED with a polarising beam splitter, as illustrated in Figure 4.17.

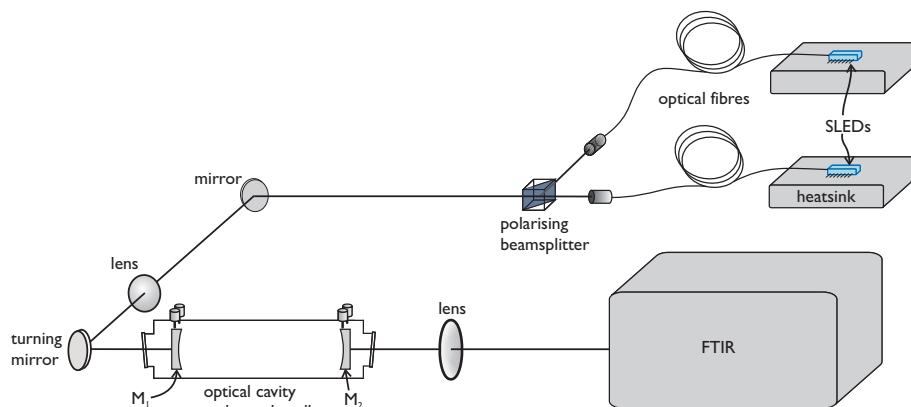


Figure 4.17: The experimental set-up for the combination of the spectral output of two SLEDs.

This arrangement allows a spectral coverage of ~ 200 nm, which conveniently approximately matches the stop band of the high reflectivity cavity mirrors used, or at least the wavelength range over which the mirrors are at their most effective, $R > 99.95\%$. This also illustrates that it would be unproductive to couple more SLEDs together to increase the spectral range unless high reflectivity mirrors with a broader response can be found. Dielectric mirrors could have their range extended with promising coating technologies, such

as sub-wavelength gratings [249], but these have yet to be demonstrated at the high reflectivity required for this work. A potential alternative could be to use prism retroreflectors based on total internal reflection [243, 250]. These promise unprecedented wavelength coverage, but require stringent specifications as a consequence of surface scattering and bulk material absorption/scattering ($10^{-3}\lambda_0/2\pi$ rms super polishing and high quality material is necessary).

The extended spectral coverage provided by both SLEDs affords the opportunity to measure and monitor the concentration of several species simultaneously. To demonstrate this capability, a mixture of 3% acetylene, 30% CO_2 and 0.5% butadiene in air was prepared (all BOC) and various pressures analysed, as illustrated in Figure 4.18. The scans were recorded over 4 minutes, with a resolution of 16 cm^{-1} . This low resolution accounts for the broad absorption features exhibited by acetylene and CO_2 , which at high resolution have relatively uncongested spectra. The inset of Figure 4.18 illustrates the spectral output of the combined SLEDs, and it is clear that where the two SLEDs join, the light intensity is very low: this leads to a large noise level in this region of the resulting spectra. Despite this, Figure 4.18 clearly shows the potential for a broadband set-up to be used to monitor multiple species. In addition, this could be simply overcome with the acquisition of SLEDs which are closer together in spectral output so that the combined density profile is more uniform.

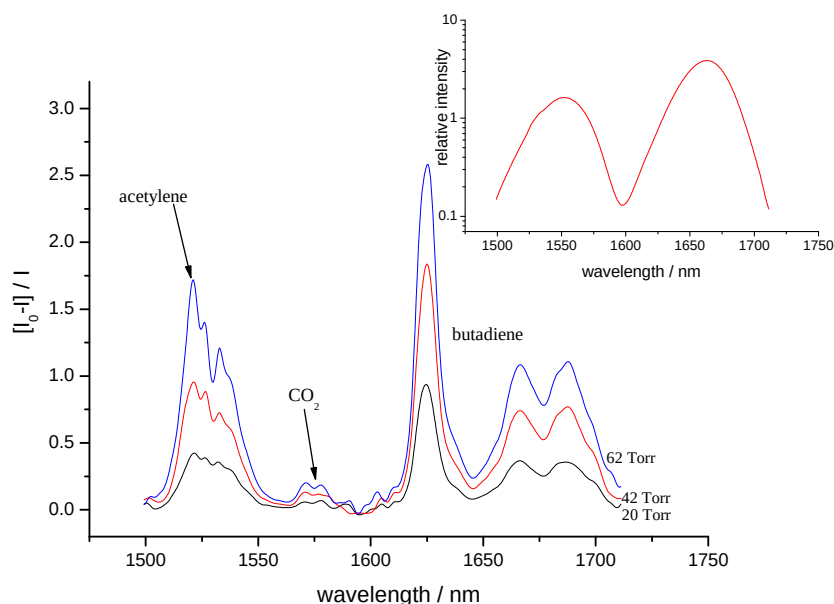


Figure 4.18: Combining the spectral output of 2 SLEDs provides the opportunity to monitor the concentration of multiple species over a 200 nm spectral range; the mixture in these spectra consisted of 3% acetylene, 30% CO_2 and 0.5 % butadiene in air; inset, the relative intensity of the two SLED sources.

4.7 Supercontinuum Source with a Fourier transform Interferometer

A second potential way to uniformly increase the spectral coverage of the broadband system is to use a Supercontinuum source (SC) in conjunction with the FTIR, replacing the SLED sources used previously. The SC source has a significantly higher output power than the SLED (~ 40 mW in the region $1.6\text{ }\mu\text{m}$ - $1.7\text{ }\mu\text{m}$, ~ 4 times that of the SLED), and broader, uniform spectral coverage so that the output intensity detected is limited solely by the stop band of the cavity mirrors used. This means a higher level of detection sensitivity should be possible with the source so that < 1 ppm acetone levels can be discerned. Initially, dilute mixes of butadiene, acetone, isoprene and methane are investigated and a minimum sensitivity value determined. Following this, a series of experiments are undertaken to further investigate the possibility of detecting biomarkers in real breath samples, taking into account the spectral interference of other species present in breath.

4.7.1 Experimental set-up

The Supercontinuum (SC) source (Fianium SC450-4 (UK)) emits 4 W of power across 450 nm to $2.5\text{ }\mu\text{m}$, so in theory it provides the possibility for cavity-enhanced absorption spectroscopy to be applied to a much larger spectral region. However, as mentioned previously, the useful operating spectral range for CEAS is limited by the stop band of the cavity mirrors used, $1.5 - 1.7\text{ }\mu\text{m}$ in this case. Everything either side of this spectral range passes straight through the cavity and so it is essential that these wavelengths are cut out, as they will otherwise saturate the detector. To this end, two filters are employed: a Thorlabs FEL1500 longpass and bk interferenzoptik bandpass bk-1655-60-B (centred around 1655 nm with a 60 nm bandwidth). On passing through these filters, the power entering the cavity is reduced to ~ 40 mW. The output of the SC is pulsed at 40 MHz, but this does not pose any problems with the experimental set-up: it is essentially treated as a continuous source as the pulse length is of the order of picoseconds. Finally, to minimise intensity fluctuations within the SC source it was operated at full power for 2-3 hours before measurements were taken.

The experimental set-up, other than the introduction of the two filters, was identical to that with the SLED: the cavity was 25 cm in length and placed within a vacuum vessel, the cavity transmission was directed through a convex lens (25 cm focal length) and straight into the FTIR and onto the detector (Thorlabs InGaAs DET410) that resided in the FTIR sample compartment.

4.7.2 Sensitivity Determination

Dilute samples of butadiene ($\sim 1\%$), acetone ($\sim 2\%$) and isoprene ($\sim 1\%$) were prepared in the same manner as previously described, whilst the calibrated methane mixes of 15 ppm and 7.5 ppm (BOC) were used together with the laboratory air to provide the methane samples for the analysis. As before, data were acquired over a 4 minute period and corrected for reflectivity variations in the cavity mirrors, allowing the absorbance, αL , to be extracted from the raw data; sample spectra are illustrated in Figure 4.19.

The sensitivity of this technique was determined with the same method as previously described: successive backgrounds, I_0 (i.e. no absorber present), were recorded over a period of 0.5 h, and alternate scans were treated as I . On application of the cavity equation (3.18), the resulting standard deviation of the linear fit to the ‘absorption’ was used to determine the minimum detectable absorption coefficient. In principle this method leads to a more realistic value, rather than just determining the noise on a particular dataset from a broadband absorber, as it also reflects the stability of the system, to a certain extent. With the SC source, $\alpha_{\min} \sim 5 \times 10^{-9} \text{ cm}^{-1}$ compared to a value of $\alpha_{\min} \sim 2 \times 10^{-8} \text{ cm}^{-1}$ for the SLED (a range from $4 - 6 \times 10^{-9} \text{ cm}^{-1}$ and $1.5 - 2.3 \times 10^{-8} \text{ cm}^{-1}$ was obtained for the SC and SLED source respectively, as a consequence of $R(\lambda)$, and that due to uncertainties in the determination of the reflectivity there is a 10% uncertainty quoted on these values). This enhancement in sensitivity cannot be attributed to the increase in power incident on the cavity alone, as after the SC has passed through the filters it has a power of 40 mW, compared to the 10 mW entering the cavity from the SLED. It has been speculated that the remaining difference is perhaps due to some residual coherence in the cw SLED that leads to interference noise, or possibly a slight susceptibility to optical feedback, leading to amplitude noise. Alternatively, it could be due to a serendipitous advantageous alignment that is difficult to optimise at this level, or an approach of the noise floor of the detection apparatus. It is felt that it is unlikely to be the former, however, as the effect seems reproducible over several misalignment-realignment operations when swapping between the SC and SLED source.

The sensitivities attained in this work are amongst the highest reported when compared to other BB-CEAS studies but direct comparison of sensitivity values reported in the literature should be undertaken with caution, as there are certain parameters that are not always easily determinable and others that are not reported, but required to make a fair comparison. Venables *et al.* [214] report the highest sensitivity to date of $5 \times 10^{-10} \text{ cm}^{-1}$ over a 60 s period, but this was achieved using an optical cavity length of 4.5 m - over 18 times longer than the one used in this study. In addition, it is noted that these type of experiments can be optimally configured for the target species: for example, increasing the

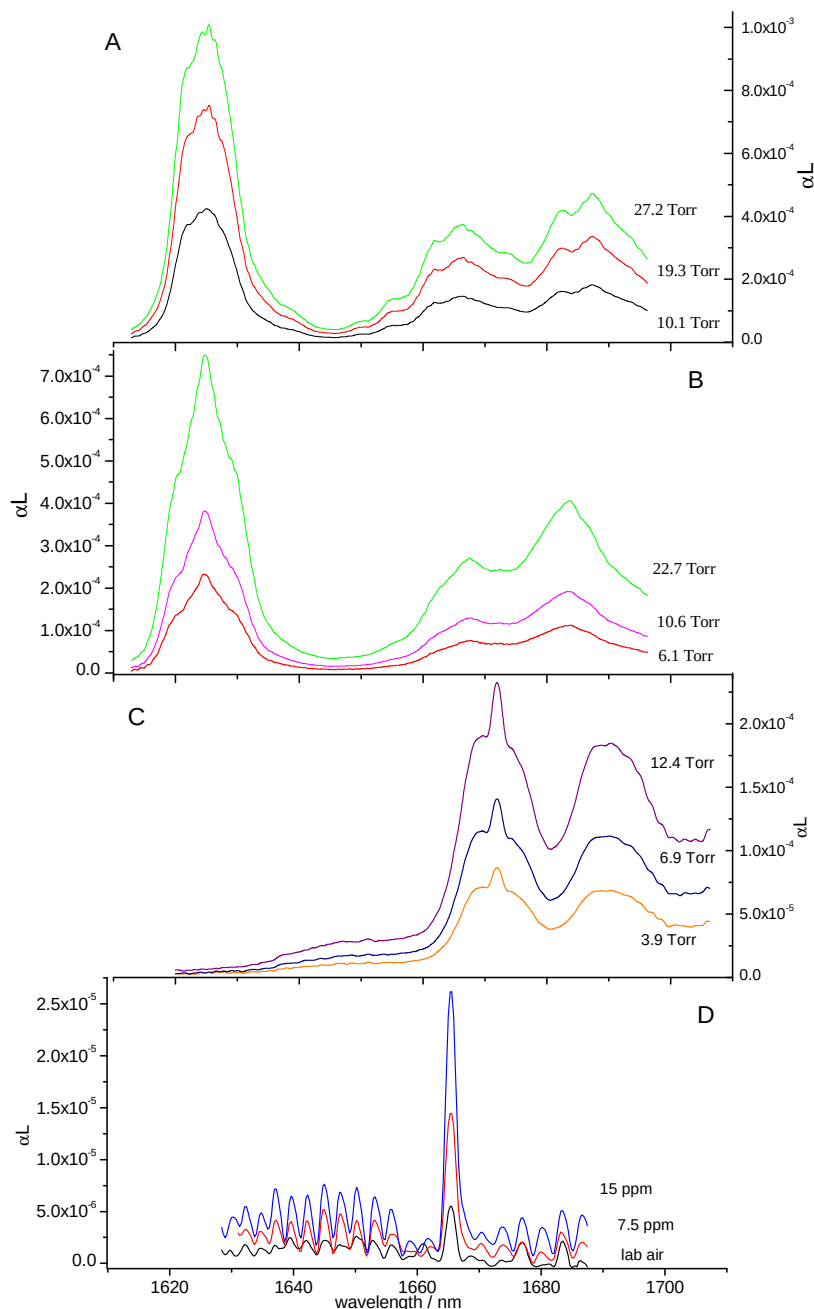


Figure 4.19: CEA spectra obtained using the SC source. From top to bottom: ~1% butadiene in air, recorded over 4 minutes at 0.5 cm^{-1} resolution; ~1% isoprene in air, recorded over 4 minutes at 0.5 cm^{-1} resolution; ~2% acetone in air, recorded over 4 minutes at 0.5 cm^{-1} resolution; various methane concentrations, recorded over 4 minutes at 4 cm^{-1} resolution.

resolution could well result in a slight increase in the signal to noise ratio (SNR) for narrow-band absorbers, but for the broadband absorbers investigated in this study the same is not true, as they are generally featureless on the 1 cm^{-1} scale and increasing the resolution will lead to a reduction in the SNR for measurements taken over equivalent acquisition times (as fewer numbers of averages are completed due to the greater length of time per scan at higher resolution).

Assuming an average absorption cross-section for acetone across the spectral region of interest of $4 \times 10^{-22} \text{ cm}^2$, as before, this level of sensitivity suggests a minimum detectable acetone concentration of ~ 400 ppb. Given the average acetone levels in human breath are ~ 500 ppb [98], this represents the kind of sensitivity required for detecting breath acetone. For butadiene and isoprene the minimum detectable concentration is ~ 200 ppb, representing approximately the upper levels of isoprene reported in human breath [37].

4.7.3 Breath

Although achieving a level of sensitivity that suggests that detecting breath acetone is a possibility, in the breath matrix there are often several absorbing species. In this instance, broadband spectroscopy comes into its own as it provides the opportunity to detect multiple species and the specificity required to identify particular absorbers of interest. In human breath, isoprene, acetone, CO_2 , CH_4 and water vapour are all present to a certain extent, and absorb radiation in roughly the same region. Levels of isoprene have been reported at 200 ppb [37], whilst those of acetone and methane can reach tens of ppm, depending upon the condition of the subject. Earlier measurements on acetone in breath matrices with the SLED, as illustrated in Figure 4.14b, have suggested that there is a ‘spectral window’ ($\sim 1667 - 1675 \text{ nm}$) from which the level of absorption due to acetone can be readily observed. Utilising the higher sensitivity afforded by the SC source, this possibility of selectively detecting and measuring the concentrations of acetone in breath was investigated further.

The measurements on breath samples, which were taken from a healthy volunteer, were obtained in exactly the same way as previously described. Nitrogen, at the same pressure as the breath sample, was used as the background measurement, to minimise any potential variation in the cavity transmission due to the effect of pressure differentials on the cavity mirrors. As before, scans were taken over 4 minutes and at 4 cm^{-1} resolution. The samples were prepared by mixing the breath samples with a 25 ppm calibrated acetone in dry air mix (BOC Special Gases) so that a range of acetone concentrations in a breath matrix could be studied. A selection of the spectra obtained are illustrated in Figure 4.20. The spectra show only relatively weak background absorption, predominantly from CO_2 , methane and water, confirming that this spectral region ($\sim 1667 - 1675 \text{ nm}$) is an attractive one for the detection of acetone. The variations seen in the absorption levels of these competing species are due to the varying pressures of breath used to make the total sample.

To investigate the influence on the spectra obtained when enhanced-methane levels are found in the breath sample, a known methane-producer’s breath was also sampled, and a resulting spectrum is depicted in Figure 4.21. The simulation illustrated uses readily available spectral information from the HITRAN database [251], which is convolved with

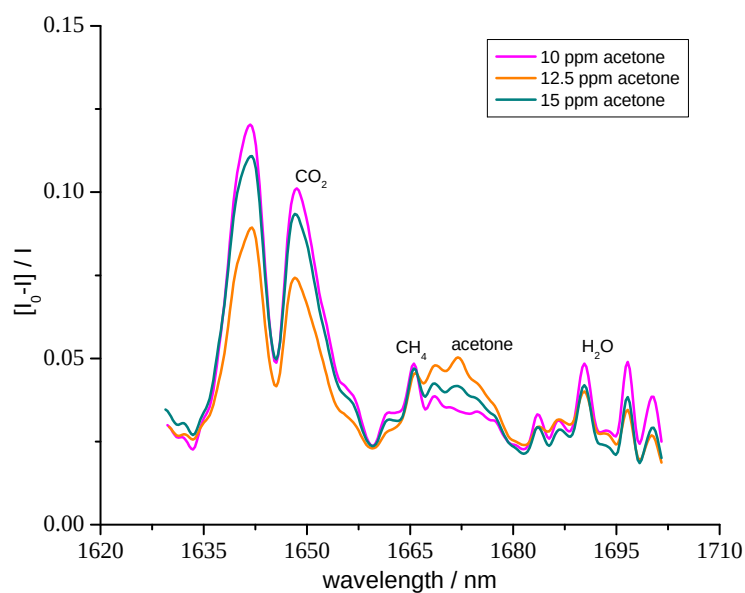


Figure 4.20: CEA of various combinations of breath from a healthy volunteer and a calibrated acetone in air mix.

a Gaussian instrument function to match the FTIR resolution of 4 cm^{-1} and a non-linear regression is applied. Whilst the effects of the elevated methane levels can be observed, there is still a spectral gap in the region where acetone absorbs ($\sim 1667 - 1675\text{ nm}$), suggesting that detecting breath acetone is a distinct possibility, even in a methane-rich environment.

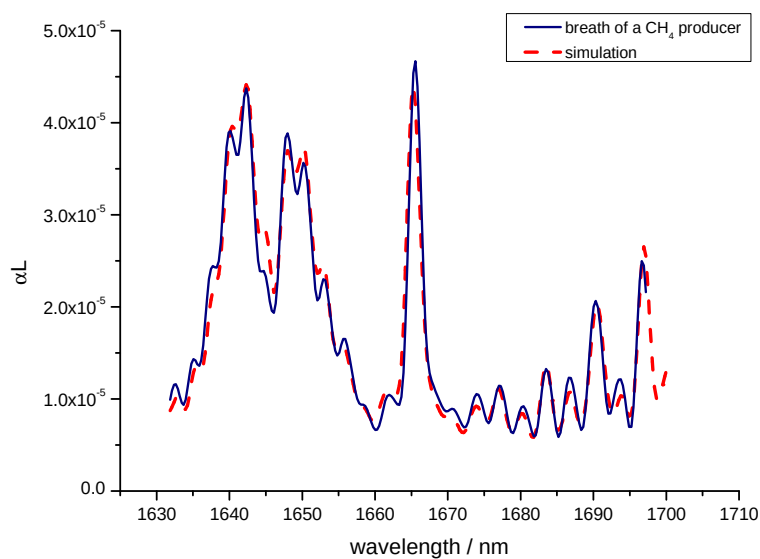


Figure 4.21: CEA spectrum of a breath sample from a methane-producing volunteer; the simulation suggests a CH_4 level of $\sim 24\text{ ppm}$.

Figure 4.22 shows a pure breath spectrum and an acetone-enriched breath spectrum offset with simulations fitted. Due to dilution, the proportion of acetone in this mixture should be ~ 10 ppm and the presence of acetone is clearly apparent. The simulation uses the acetone cross-sections previously determined in this work, and the cross-section data from the HITRAN database for the other molecules. As before, it is convolved with a Gaussian instrument function to match the FTIR resolution of 4 cm^{-1} and a non-linear regression is applied.

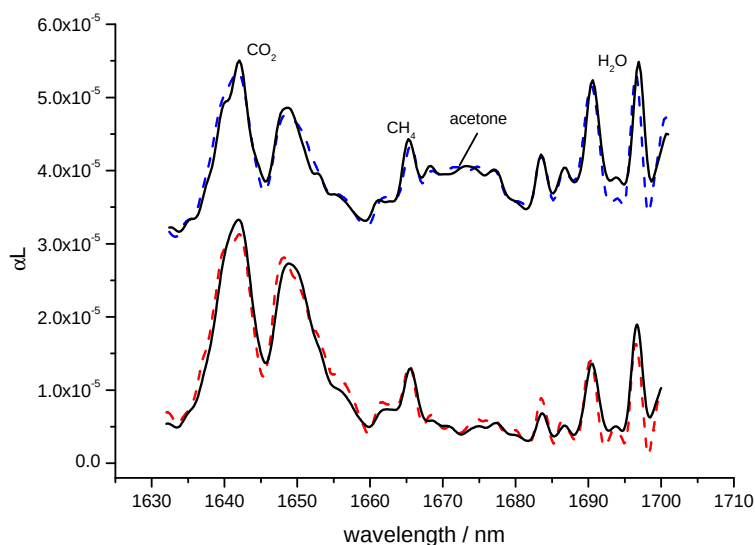


Figure 4.22: Example spectra and simulations of breath and acetone-enriched breath. **Top:** offset for clarity, the spectrum shows a sample of breath enriched with acetone; **bottom:** the absorption spectrum returned for a sample of breath from a healthy volunteer.

The simulation returns partial pressures of 28 Torr, 7 Torr and 2.4 mTorr for CO_2 , H_2O and CH_4 respectively, and for the acetone-enriched sample, an acetone level of 7.3 ± 1.3 ppm (this is the uncertainty only from the fit). The low value returned could be the result of several ambiguities, not least the amount of acetone in the original sample: a similar fit to one atmosphere of the undiluted 25 ppm acetone/air mix yielded a value of ~ 23 ppm. Part of this discrepancy could lie in the uncertainty of $R(\lambda)$ (about 2-3 ppm), but this also highlights issues when handling and producing samples containing substances such as acetone. Previous experience of handling vapour samples suggests one can anticipate to only be within 20% of expected values [252], and presumably similar uncertainties will exist in handling the pre-prepared acetone mix (both by the supplier and user).

It is also clear that there is some discrepancy between the simulation and data at longer wavelengths, where the water absorptions occur. This is due to a combination of factors: the bandpass filter begins to cut out radiation in this spectral region, reducing the signal and thus introducing greater uncertainties in measurements at these wavelengths, not least

in the reflectivity curve used to correct the data; from the manufacturer's specifications the reflectivity of the mirrors is known to drop off rapidly in this region and in conjunction with the greater uncertainty in determining the reflectivity curve at these wavelengths, this significant change in $R(\lambda)$ is not going to be accurately determined; and finally, there is an additional, broad and featureless level of absorption due to water vapour known as the water continuum that is not included in the HITRAN database as it is not attributed to specific ro-vibrational transitions of water. This latter effect will be discussed more fully in the next chapter.

At the lower concentrations of acetone, its distinctive spectral feature becomes less visible but the simulation, which takes into account the other absorbing species, allows acetone levels to still be determined. From this, it is apparent that the instrument can reach an acetone sensitivity of ppm levels in a breath matrix, which is approaching the levels required to detect acetone in healthy human breath. One way the sensitivity could be increased is by lengthening the physical cell: even from 25 cm to 50 cm would increase the effective pathlength to 2,500 m.

4.8 Conclusions

This chapter has detailed the development of three broadband radiation-based cavity enhanced systems for the detection of molecules with biological significance. Initially, the absorption cross-sections of the three broadband absorbing molecules, butadiene, acetone and isoprene, were determined from direct absorption measurements, which could then be used to deduce the sensitivities of the broadband CEAS systems developed. The first device combined a Superluminescent Light Emitting Diode (SLED) broadband source with a dispersive monochromatic spectrometer and phase-sensitive detection to undertake initial measurements on both butadiene and acetone, the former a known human carcinogen and the latter a biomarker for diabetes. The reasonable sensitivity of $6 \times 10^{-8} \text{ cm}^{-1}$ was enhanced to $1.5 \times 10^{-8} \text{ cm}^{-1}$ on moving to an FTIR detection system. The increase in sensitivity resulted in the detection of atmospheric methane and led to preliminary studies on acetone in breath samples, which demonstrated the suitability of the spectral region (with regards to interfering species) for the detection of breath acetone. A second SLED was also introduced to provide, in conjunction with the original SLED, a total spectral coverage of 200 nm, which allowed the demonstration of multiple species detection and in doing so highlighted another application of broadband detection. Finally, a Supercontinuum source (SC) was incorporated into the system and the greater power provided by this source resulted in an improved sensitivity of $\sim 5 \times 10^{-9} \text{ cm}^{-1}$, or a detection level of 400 ppb for pure acetone. Following this, the instrument was applied to breath samples enhanced with

varying amounts of acetone. Utilising a simulation to extract the absorption due to acetone alone, the device illustrated that it was capable of measuring acetone in breath at the level of a few ppm.

Although this could be improved with a cavity of longer physical length, an inherent major drawback of this arrangement is the relatively low acetone cross-sections in the near-infrared. Moving to the mid-infrared opens up the possibility of probing larger absorption cross-sections; this avenue is pursued in Chapter 6. Alternatively, one can remain in the near-infrared, taking advantage of the plethora of robust, convenient, cheap and commercially available diode lasers. These naturally have a greater power density than broadband sources, and provided the spectroscopy of the spectral region of interest is well known, can also be employed in the detection of acetone, as described in the following chapter.

Chapter 5

Development of a Device for Detecting Breath Acetone

This chapter details work carried out whilst on an industrial placement at Oxford Medical Diagnostics Ltd. (OMD) as part of my CASE Award studentship. The investigation builds on work described in the previous two chapters, utilising the narrowband DFB diode laser CEAS technique detailed in Chapter 3 but drawing on the knowledge gained from the broadband studies on acetone in Chapter 4.

As has been discussed previously in this thesis, the presence of elevated levels of acetone in breath is an indicator that the body is burning fat stores, which in turn could signify a lack of insulin and that the subject is suffering from diabetes. Being able to diagnose diabetes and monitor the condition in a non-invasive way using breath analysis would represent a significant breakthrough in the treatment of diabetes and absorption spectroscopy offers a selective, sensitive and relatively low cost technique with which to do this. The average level of acetone found in the breath of non-sufferers is ~ 500 ppb [98], whilst this can increase to several ppm in those predominantly burning fat. Therefore, a device which can comfortably detect the 500 ppb level would be desirable, as this effectively represents the baseline of breath acetone measurements.

5.1 The detection of acetone with a narrowband laser

As discussed in the previous chapter, the absorption features encountered with acetone lend themselves to detection using a broadband radiation source, given that conventional narrowband diode lasers do not have the tunability to sweep over an entire feature. However, the use of a broadband source is not particularly well-suited for application to a breath

analyser device: it necessarily requires a means to visualise the spectral output by resolving its constituent wavelengths, and this tends to involve bulky, cumbersome devices such as a Fourier Transform spectrometer. Further to this, the most powerful broadband sources, such as SC devices, represent a substantial investment and as such are not a financially viable option for use in a potential commercial product.

In contrast, diode lasers are relatively cheap thanks to their use in the telecommunications industry and they do not require very complex detection systems. In addition, diode lasers have a greater spectral density than broadband sources so that the effective pathlength enhancement is reflected in the sensitivity values determined.

In order to regain the specificity lost due to the absence of detailed structure in the absorption features of acetone, the spectral region of interest must be chosen carefully. Clearly, sweeping over a very small fraction of the acetone feature is going to result in an absorption spectrum that merely resembles an ‘offset’. Therefore, in order to use a narrowband source for the detection of acetone it is essential to select a region which has no spectral interference from other species that might be found in breath, so that the absorption seen is solely due to acetone. Secondly, it is imperative that the zero absorption level of the cavity output does not vary between the I_0 and I measurements: in contrast to measurements with narrow transitions, there is no reference zero baseline in the absorption spectrum, as the entire spectral region scanned is part of the larger absorption feature. Therefore, the reference point comes solely from a measurement taken prior to the sample measurement, so if there is any variation in the zero level between these measurements, it will manifest itself in the calculated absorption level. Finally, although a spectral region can be selected with no competing transitions within it, the influence of the water continuum cannot be ignored. This is a smooth, broad absorption due to water vapour found in addition to its narrow ro-vibrational transitions that pervades large regions of the entire electromagnetic spectrum and will be discussed later in this chapter. Its presence necessitates the reduction of the water vapour content of a breath sample before it is analysed, as it will otherwise add to the absorption level seen due to acetone. Therefore, this chapter can be split up into two sections, the first dealing with the optimisation of the device with regards to system stability and its application to ‘dry’ samples of acetone; and the second, building on those foundations with an investigation into the treatment of real breath samples (i.e. ‘wet’ acetone samples) for use with the device.

5.1.1 Acetone

Given the requirements of the breath acetone device to be compact, robust and relatively cheap to construct, the most obvious spectral region to probe is the near-infrared (NIR),

where a wide array of diode lasers are commercially available. Although the absorption cross-sections of acetone are smaller in this region in comparison to the mid-infrared (MIR) and the ultraviolet (UV) regimes, the use of an optical cavity will increase the effective path-length, L , of the radiation through the sample, negating the effect of the lower cross-sections and delivering a high level of sensitivity. The NIR absorption features of acetone were studied extensively in the previous chapter, and this can be utilised to locate the most suitable position to probe the transition with a DFB diode laser. In conjunction with readily available parameters from the HITRAN database [171, 251] on the narrowband molecules found in breath, the acetone cross-sections determined in the previous study can be used to simulate Figure 5.1, which shows the absorption expected in the spectral region that acetone absorbs in (1660 - 1700 nm) and an expanded view of the region 1685 - 1690 nm, for a typical sample of breath at 1 atmosphere with a 1 cm pathlength. The simulation assumes a 1.8 ppm CH_4 concentration in breath, but this could conceivably be a lot higher in a methane-producer and hence worth bearing in mind when considering a potential region to probe.

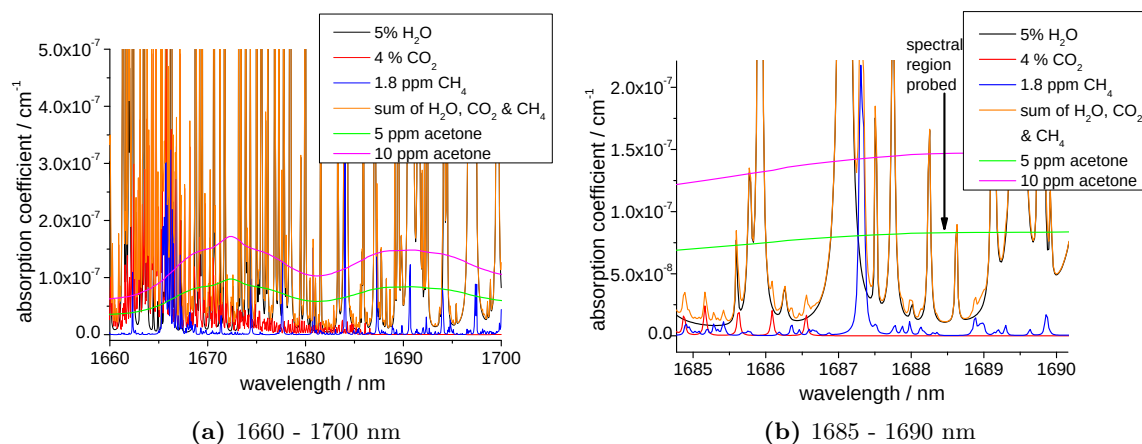


Figure 5.1: Figure (a) shows a simulation of the spectra obtained in a 1 cm absorption pathlength for the constituents found in breath at typical levels and the absorption expected due to 5 ppm and 10 ppm of acetone. Figure (b) depicts the same simulation expanded over the region 1685 - 1690 nm, with 1688.45 nm, the spectral region probed, indicated.

From this, it is clear that 1688.45 nm is optimally located as it avoids spectral interference from transitions of other molecules found in breath and acetone has a relatively large absorption cross-section of $1.07 \times 10^{-21} \text{ cm}^2$ at this wavelength. Therefore, a DFB diode laser (NEL, NLK1U5EAAA) was acquired for this spectral region and coupled into an optical cavity of length 45 cm with high reflectivity mirrors (Layertec, radii of curvature 1 m, $R \sim 99.99\%$) before being directed via a 3 cm focal length lens into a detector (Teledyne Judson, J23TE1-66C-R02M-1.9), fitted with a photodiode amplifier (ThorLabs PDA200). The output from this passed into an oscilloscope (LeCroy Wavesurfer) from which the

signal level was recorded. As described in Chapter 3, measurement of the ASE level poses problems with a device intended for frequent use by non-specialists as it requires the cavity to be misaligned. Therefore, a filter (bk Interferenoptik; 1690 nm central wavelength, 10 nm bandwidth) was inserted into the beam path to remove radiation which falls outside that of the required lasing frequency of the laser. With the probing wavelength selected and the general set-up established, the next task was to tackle the other factors outlined in the previous section that need to be overcome to realise sensitive breath acetone detection.

5.2 Initial development of a device for ‘dry’ samples

One of the major issues to be surmounted when attempting to detect a broadband absorber with a narrowband source is to ensure that there is minimal variability in the zero absorption level between measurements. There are a number of factors which could influence the level of the detected signal, ranging from drift in the laser output to contamination of the cavity mirrors from samples; each of which will now be addressed in turn.

Detection system

Prior to this work, a Thorlabs DET410 InGaAs detector had been used to monitor the signal output. However, this device has a significant level of dark current (25 nA), which adds to the signal output recorded by the detector. Dark current is the small electric current which flows through the photodiode when no photons are hitting the device, and it is caused by the random generation of electrons and holes within the diode as a result of the voltage applied across it. In addition, dark current increases with temperature, as the intrinsic charge carrier concentration in the depletion region is exponentially dependent on temperature [253]. It follows that the signal recorded by the photodiode is temperature-dependent. To investigate the extent of this effect, the DET410 was wrapped in heating tape and a thermocouple device placed by the detector itself so that the temperature applied could be monitored. The signal from the detector was then recorded on an oscilloscope (LeCroy WaveSurfer) as the temperature of the heating tape was increased. The resulting output is illustrated in Figure 5.2 and clearly shows that the signal recorded has a significant temperature dependence.

Although the laboratory temperature will never be as high as some of the temperatures studied, an increase from 21°C to 22°C induces a signal increase of 0.001 V, which represents a $\sim 5\%$ increase on the original signal. Given that the absorption level due to 1 ppm of acetone is approximately 3% at a wavelength of 1688.45 nm and with a pathlength enhancement consistent with mirrors of $R \sim 99.996\%$ for a cell 45 cm long, this potential variation in the signal due to temperature fluctuations would introduce a significant uncertainty in the recorded absorption level. Therefore, a thermoelectrically-cooled, non-bias

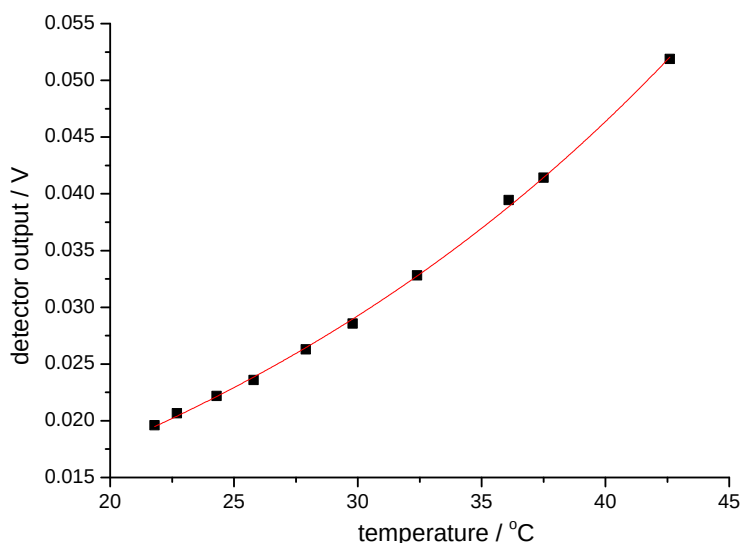


Figure 5.2: The temperature-dependent signal of the DET410 detector.

detector from Teledyne Judson (J23TE1-66C-R02M-1.9) with a dark current component $50\times$ less was installed.

Laser stability

To investigate the variability in the laser output over time, initially an I_0 measurement was taken of the empty sample cell before a 950 mbar sample of a 5 ppm acetone in air mix was released into the vessel. Consecutive measurements were then taken on the sample over a period of 30 minutes. Analysis of the raw signal detected revealed a variation of 0.23% across the course of the measurements, which suggests that the laser is reasonably stable in its output. Using the I_0 measurement taken at the beginning, absorption levels for each I measurement were determined and found to follow a normal distribution, as illustrated in Figure 5.3.

This gives a standard deviation of 0.00325, equating to a 0.115 ppm uncertainty in the absorption measurements, which represents a very satisfactory level. However, in reality the cavity cell will need to be evacuated in between different samples, so the stability of the system under such conditions needs to be investigated.

Mechanical stability

In order to monitor any slight movement of the cavity mirrors on varying the pressure within the cavity cell, a HeNe beam was reflected off the first cavity mirror and directed over several metres on to a wall. On varying the nitrogen pressure in the cell, it was noted that the position of the HeNe beam on the wall moved slightly, indicating that the pressure variations were moving the cavity mirrors. This slightly alters the cavity alignment, which

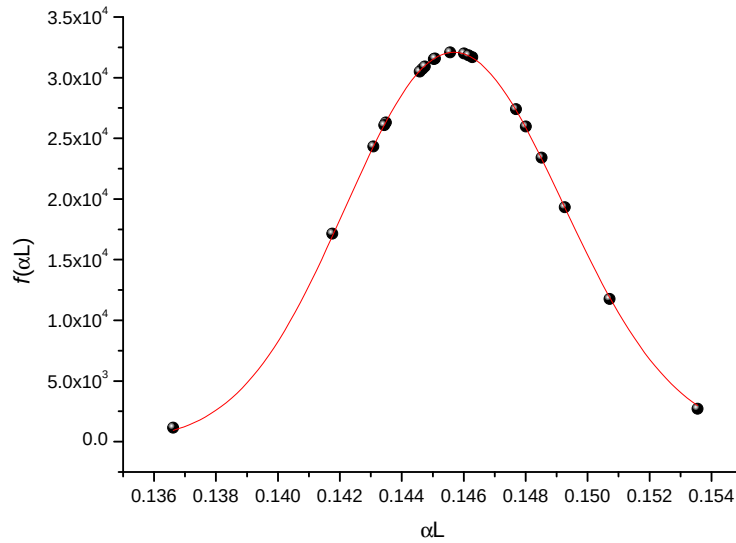


Figure 5.3: The normal distribution of absorption measurements on a 5 ppm acetone sample over a period of 30 minutes, where $f(\alpha L) = \frac{1}{\sigma\sqrt{2\pi}} \exp \frac{-(\alpha L - \mu)^2}{2\sigma^2}$ and αL (the absorbance due to 5 ppm of acetone) is the variate, μ is the mean and σ^2 is the variance.

manifests itself in the variation observed in the cavity output. Therefore, in order to reduce this effect, a fixed-mirror cavity was employed. This cavity cell has no mirror adjustments, but it means that as the cavity mirrors are fixed in place negligible movement is possible so that the cavity alignment should not change upon variations in pressure.

A similar experiment to that carried out in the previous section was undertaken, with the exception that the 950 mbar 5 ppm acetone sample was replaced for each measurement. This would allow the variability due to evacuating and refilling the cell to be investigated. A typical variability of 0.77% was observed in the transmitted intensity across the course of the measurements, a significant increase on that seen when the sample is not replaced. The vast majority of this error arises from a reduction in signal seen over time: if the standard deviation of a linear fit to the negatively correlated data is used to determine the variability, the error is reduced to 0.38%, which is much closer to the value obtained previously. Although it is possible that the drop in signal is due to mechanical variations induced by pumping down and filling the cell, it seems more likely that the drop in signal is real given the steady, linear decrease observed, and that it is either due to increasing levels of acetone in the cell or due to particulate matter from the gas tubing and cylinder contaminating the cavity mirrors. In both cases, the signal will decrease as more samples are analysed, as acetone is known to be ‘sticky’ so it is not inconceivable that residual acetone will build up as more samples are released into the cell; likewise, any particulate matter in the gas handling system will also incrementally increase as more gas is flowed through the cell. Given the drop in cavity output signal recorded, it is not surprising that

when the absorption levels for each I are determined from the initial I_0 measurement the standard deviation increases to 0.0135, which equates to an uncertainty of 0.423 ppm.

Therefore, the same experiment was conducted as before, but an empty cell I_0 measurement was recorded before every sample (I) measurement. This effectively ‘zeros’ the baseline before each data set is taken, and reduces the standard deviation of the absorption levels recorded to 0.00875, or a 0.364 ppm uncertainty.

Clearly, however, a greater control over the sample itself will improve this further.

Sample control

In order to control the flow of gases into the cell, needle valves were introduced in addition to the Swagelok taps already in place. This greater control reduces the chances of a cavity mirror being misaligned by the sudden influx of gas into the system. In addition, the introduction of a 0.5 μm particulate filter ensured that any small particles that may be present in the gas tubing, gas cylinder, or from the breath sample, would be extracted before the gas filled the cavity cell, thus eliminating a source of potential mirror contamination. Secondly, methods to ensure that acetone was completely removed in between measurements were investigated. Initially efforts focused on heating the cell in an attempt to vapourise condensed acetone found on the surfaces so that it could then be removed via the pump. However, this method instead caused a far greater variability in the cavity output and it has been hypothesised that the heating tape in fact induced convection currents within the cell. It was discovered that the most effective way to prepare the cell for the next sample was to flush the whole device with N_2 . Using this method, it was found that the maximum variation in the I_0 measurements over a period of an hour was 0.5%, which is reduced further for consecutive measurements. Therefore, by recording an I_0 measurement both before and after the sample and taking the average value as the I_0 to be used in the determination of the sample absorption, a reasonably accurate reflection of the actual zero measurement whilst the sample spectrum is taken is obtained; it allows any subtle changes in the output over the duration of the measurements to be taken into account.

With the system attaining a level of stability that would suggest sub-1 ppm levels of acetone could be comfortably detected, the device was applied to the analysis of dry mixes of known concentration of acetone.

5.2.1 Experimental

The experimental set-up was identical to that described previously and the value taken for each measurement from the oscilloscope was the average obtained within an interval in the middle section of the scan over a total period of ~ 4 s. It was assumed that over the short

spectral range scanned (~ 0.1 nm) the acetone absorption is featureless and effectively flat, and taking the average of the central part of the scan ensured that the absorption value was extracted from the cleanest data possible and essentially recorded the absorption at 1688.45 nm exclusively.

In order to determine the mirror reflectivity, the lasing frequency was shifted to the nearest narrow absorption feature, namely the water transition at 1689.45 nm. Laboratory air was released into the optical cavity, and the level of water vapour in each sample pressure was determined from a relative humidity gauge. From the amplitude of the transition (no frequency scale is applied given the nature of the experiment), a mirror reflectivity of ~ 99.9968 % was deduced. Following this, a series of measurements were taken of various concentrations of acetone in N_2 . These were prepared from diluting samples from calibrated mixes of 5 ppm acetone in air and 25 ppm acetone in air (both BOC Special Gases) with N_2 , and the resulting plot of absorption against acetone concentration is given in Figure 5.4.

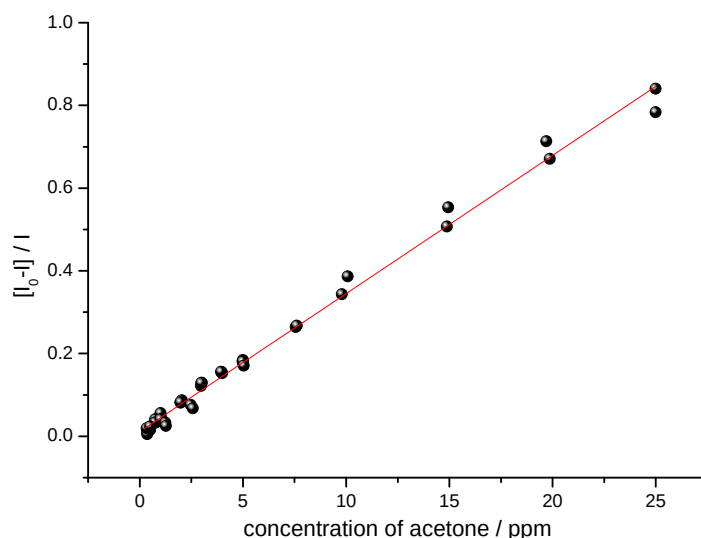


Figure 5.4: Samples of various acetone concentrations were prepared from diluting calibrated acetone mixes with nitrogen.

Using the slope of the plot, the reflectivity of the mirrors was determined to be 99.9965%, which corroborates with that deduced from the water transition at 1689.45 nm. The standard deviation of the linear fit suggests an uncertainty of 0.546 ppm, which is a little too high but could be explained by the uncertainty in the acetone pressures of each sample. The acetone concentrations were not verified by another means, i.e. if the sample was approximately 50% 5 ppm mix and 50% N_2 , it was assumed that this amounted to a 2.5 ppm sample. For greater accuracy, these samples could be verified against a mass spectrometer (AIRSENSE Compact, V&F) and indeed this is essential for the analysis of breath samples.

5.3 Applying the device to breath samples

The next stage in the development of the breath analyser device is to test it on real breath samples. As mentioned previously, in order to investigate the performance of the optical spectrometer, it is necessary to compare its response to that of a mass spectrometer.

Mass spectrometry

Mass spectrometers [254] consist of an ionising source, a mass analyser and a detector. The sample to be analysed is injected into the chamber containing the ionising source. There are many methods of effecting the ionisation: traditionally, this has involved the use of an electron ionisation (EI) source and this is still used, along with the related ion-molecule reaction (IMR) technique, for gaseous samples. The whole system must be kept at very low pressure as the ions formed are naturally very reactive. Following ionisation, the cations are accelerated into the mass analyser section where they are separated according to their mass-to-charge (m/z) ratio by electromagnetic fields. On scanning across these ratios, signal will be detected at the m/z ratios of the ions present (and consequently the presence of the parent molecule can be inferred), allowing a mass spectrum to be produced.

The mass spectrometer used in this study was an AIRSENSE Compact (V&F). It is essentially two mass spectrometers in one, utilising both an electron ionising source, and an ion-molecule reaction based source. This allows greater flexibility in the types of molecules that can be studied by the mass spectrometer: if a hard ionisation source is required, the EI source can be used, but if a soft ionisation source is desirable, such as the case with acetone, the IMR-based source can be utilised.

With an electron ionisation source the electrons are generated by electrically heating a filament and then accelerated to an energy of 70 eV by a potential difference to a trap electrode, as illustrated in Figure 5.5. At this energy, the strongest possible ionisation of the sample molecules will occur as the De Broglie wavelength of the electrons matches the typical bond lengths found in organic molecules, which can then result in fragmentation of the molecule [255].

This fragmentation can be advantageous because the fragment ions formed are also detected and can help reconstruct structural information about the original molecule. However, further fragmentation can also cause problems as the additional ions formed may have m/z ratios which interfere with those of other species in the sample being investigated. The ion-molecule reaction technique allows this fragmentation to be avoided, as the kinetic energies involved in the reactions are relatively low: the reactions are dictated solely by the enthalpy of formation of the particles involved [256]. This means that there is not enough energy available for fragmentation to occur. The initial, or primary, ions (Hg ions in the case of the AIRSENSE Compact) are produced from an electron ionisation source,

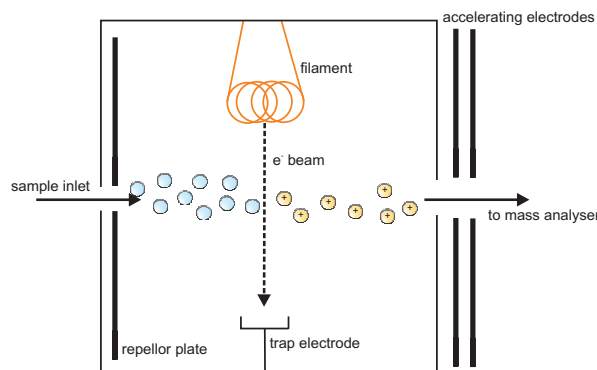


Figure 5.5: A schematic diagram illustrating the principles behind an electron ionisation source.

before they pass into a reaction chamber where they are trapped within a high frequency multipole field system. The gas sample to be analysed is introduced into this chamber and ion-molecule reactions subsequently occur, resulting in the production of ions derived from the gas sample. In order to ensure only single collisions occur, the density of gas sample is kept low. From this point, the ions are directed towards the mass analyser section.

This instrument utilises a quadrupole mass analyser to separate out the different ions produced by the ion source. The quadrupole mass filter consists of 4 metal rods that are parallel to each other, as illustrated in Figure 5.6. Oscillating electrical fields are generated within the quadrupole from the application of an AC and DC voltage. On passing through the quadrupole, only the ions with a specific mass-to-charge (m/z) ratio will be able to make it to the detector at the other end for a given set of voltages; all others are thrown out of the beam path as a result of the unstable trajectories imposed by the electrical fields [257]. This means different m/z ratios can be selected and the abundance of each recorded by the detector.

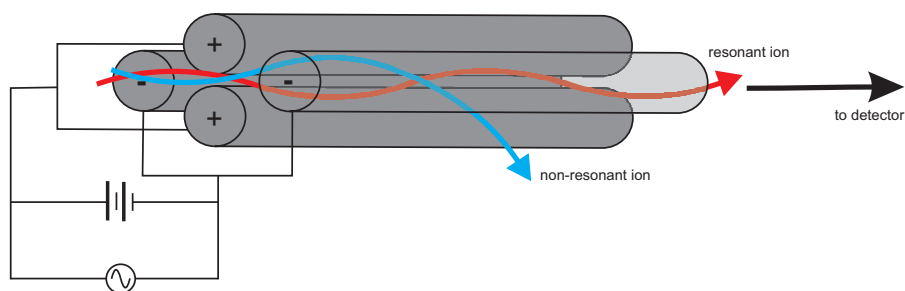


Figure 5.6: A schematic diagram illustrating the principles behind a quadrupole mass analyser: only ions with a specific m/z ratio will pass through to the detector; on varying the electrical fields, different m/z ratios can be selected. Adapted from [258].

5.3.1 Water vapour

The first experimental evidence that water vapour absorbs infrared radiation was presented by Tyndall [259], but it was not until 1918 that the first detailed water vapour spectrum over a broad spectral range was determined by Hettner [260] and not until 1938 that Elsasser noted that there was emission at wavelengths at which water did not possess rotation or ro-vibrational bands [261–263]. This broad, slowly varying component of absorption found in addition to the individual spectral lines is known as the water continuum and it is split into two components: the self-continuum and the foreign-continuum. The former is a result of the interactions between water molecules whilst the latter describes the interaction of water molecules with ‘foreign’ molecules found in the atmosphere, such as N_2 and O_2 . Since those early days, the water continuum has been the subject of countless studies because of its importance in atmospheric science whilst its physical origin has been a point of heated debate over the years, a full discussion of which is beyond the scope of this thesis but can be found in a number of reviews [264–266].

Generally, the water continuum is deduced by subtracting the contribution from the narrow absorption features from the absorption measured experimentally [267, 268]. Typically, the narrow absorptions are defined by a pressure-broadened Lorentzian lineshape (some authors use a Voigt profile [264, 267]), derived from the spectroscopic parameters found in the HITRAN database [171] with the absorption taken to extend 25 cm^{-1} from the line centre. The continuum is then assumed to be everything beyond that 25 cm^{-1} cut-off point and, in order to obtain a smoother continuum which is easier to tabulate, also the small section below the local line shape which is depicted in grey in Figure 5.7. This means, however, that some absorption due to the local line transitions is included in the continuum absorption, but this is estimated to contribute $< 5\text{--}7\%$ in the bands and $< 1\%$ in the windows of the total continuum absorption in the NIR [269].

The self-continuum is known to scale quadratically with water number density whilst the foreign-continuum is proportional to the product of the water vapour pressure and the sum of the partial pressures of the broadening gases. In addition, the self-continuum has a strong negative temperature dependence, whereas the effect is much weaker in the foreign-continuum. The former property has led to the speculation that the continuum is due to the superposition of the wings of the spectral lines [266] (the far-wing theory), as the pressure broadening of the Lorentzian lineshape is known to have a quadratic dependence on number density in its wings; whilst the latter property suggests the involvement of a dimer-based mechanism.

The Lorentzian lineshape does not fully describe what is observed experimentally, and in their semi-empirical model developed in 1989, Clough, Kneizys and Davies (CKD model)

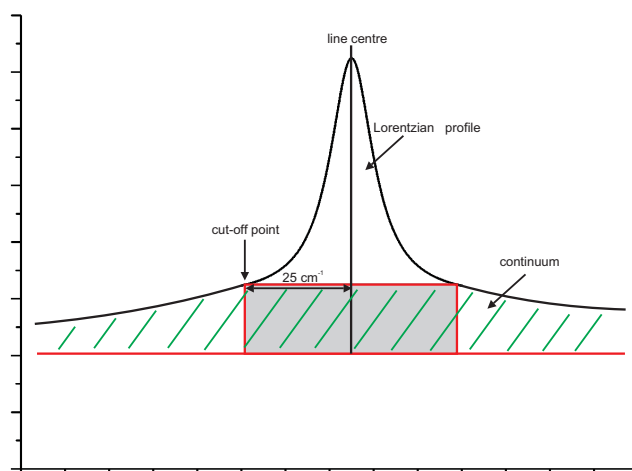


Figure 5.7: An illustration of the definition for the derivation of the water continuum, which is indicated by the green hatch; for clarity, the relative sizes of the components are not to scale; adapted from [269].

[268] invoked a super-Lorentzian lineshape near to the line centre and a sub-Lorentzian one further away in order to best fit the experimental data taken by Burch [270–272] and Burch and Alt [273]. The rationale behind this was that the Lorentzian lineshape assumes instantaneous collisions, whereas in practice they are not. However, the model did not explain the negative-temperature dependence and so the model was adjusted accordingly to match the temperature dependence observed in experiments. Despite its empirical nature, the CKD model formalised the definition of the continuum and has been widely used in the atmospheric sciences. It has been modified several times as new experimental data have come to light, until being superseded in 2005 by a major new version of the model, MT_CKD [274]. This model, developed by Mlawer, Tobin and Clough is based on a collision-induced absorption (CIA) component in addition to the line shape component used previously. The extra term was introduced to provide a physical mechanism to deal with the increased absorption seen in the intermediate wing when compared to the impact approximation and the model was fitted using newer measurement data than CKD [265]. However, it has been shown [267, 270, 275, 276] that the model's accuracy in regions or under conditions where it has not been updated or verified with experimental data [265] is severely reduced. More recently, Paynter and Ramaswamy [265] have developed a purely empirical model based on the laboratory measurements of Baranov *et al.* [277], Baranov and Lafferty [275], Paynter *et al.* [267] and Serio *et al.* [278], the so-called BPS model. These measurements have not yet been used to update the MT_CKD model, and in contrast to the MT_CKD model, the authors do not derive their continuum coefficients from a fitted analytical form, but rather

purely from measurements, which also allows uncertainty parameters to be added to the model.

Driven by the desire to formulate a more theoretical basing for the far-wing description of the continuum, a number of theories were developed [279–284]. These centred on the use of a quasi-state approximation, which assumes that the duration of collisional processes is infinite in comparison with the time frame of interest [284, 285], to derive the absorption line shapes. Ma and Tipping have demonstrated that their model fits experimental data in the MIR very well [284] and that it describes the negative temperature dependence seen experimentally. However, it does underestimate the absorption seen within bands, for which two explanations are proposed: one, that in the regime where the duration of the collisional processes are short in comparison to the time of interest, the quasistatic approximation breaks down and the original impact approximation becomes valid, (resulting in a Lorentzian line shape at the line centres) but in the intermediate region, where the two times are comparable, neither line shape can be applied; or two, a secondary mechanism is producing absorption near the line centres. With the former, a near-wing modification, combining both the far-wings and impact lineshapes, could be the solution but there is currently no theoretical basis with which to do so accurately [285]. The second was not favoured by the authors in a follow-up paper (Leforestier *et al.* [286]) who concluded, based on calculations on the temperature dependence of far-wing line shapes ($800 - 1150 \text{ cm}^{-1}$) [285], CIA ($< 1150 \text{ cm}^{-1}$) and dimer absorption ($< 600 \text{ cm}^{-1}$), that the contributions from CIA were negligible in the frequency region investigated and that dimer absorption only becomes important in the microwave and submillimetre regimes.

However, the notion of a secondary mechanism responsible for elements of the continuum absorption has found increasing popularity recently, most notably with Ptashnik and co-workers [264, 287, 288]. Following theoretical studies on the water dimer by a number of authors [289–292], the positions and strengths of the bands of the water dimer absorptions have been computed. However, the rotational structure of the bands has not been directly calculated, so assumptions about the widths and shapes of the bands have to be made, introducing a significant level of uncertainty: the band centres of the dimer absorption tend to overlap with those of the monomer, so in order to determine whether the continuum observed experimentally in the window regions is due to a degree of dimer contribution, these parameters need to be known. However, these calculations demonstrated that although the dimer absorption has a continuum nature, it also possesses broad spectral features near band centres, a result that was highlighted by Ptashnik *et al.* [287] and exploited in their study of 2004. Whilst the authors admit that uncertainties in the derived would-be dimer absorption can arise from the subtraction of the monomer absorption from the measured continuum (given the requirement of a good knowledge of the monomer spectrum), their analysis of the temperature dependence of the residuals allowed a clear distinction to be

made between the dimer and monomer contributions [266]. The results produced spectral features that agreed reasonably well with the theoretical spectra [290, 293], as did a further study in 2007 [276] and Ptashnik reviewed earlier measurements that could also be argued to demonstrate dimer absorption [288]. The new experimental data also highlighted broad spectral features that could not be explained by the dimer absorption alone and Ptashnik *et al.* [264] have recently suggested that these features could be due to shorter-lived quasi-bound dimers which were not included in the original theoretical framework. Whilst free pair states account for CIA, where the absorptions are caused by the short-lived collision-induced dipole moment that occurs during single approach collisions, and true bound dimers occur at the other extreme at low temperatures, quasi-bound dimers are formed when there is a temporary stabilisation of a pair from a multiple approach pair collision. Here the absorption arises from the temporary redistribution of part of the translational energy of the collisional monomers to rotational energy [264]. Calculations by Vigasin [294–296] and Epifanov and Vigasin [297] have indicated that these quasi-bound dimers have equal importance in atmospheric conditions and a simplified calculation by Ptashnik [264] has suggested that these extra features could be explained by quasi-bound dimers.

The physical origin of the water continuum is a contentious issue and as of yet there is no conclusive answer. Despite this, what is certain is that there is a greater level of absorption due to water than can be explained due to the impact approximation of the water monomer lines alone (i.e. a Lorentzian profile); whether this is because theoretical lineshapes are yet to fully describe the whole absorption feature of a water monomer line (centre, near-wing and far-wing regimes), or because extra absorption mechanisms are also involved, is still to be resolved.

It is clear, however, that its presence will pose a problem, adding to the absorption level recorded by the device. In order to determine an estimate for the influence of the water continuum at 1688.45 nm, a series of measurements on laboratory air were undertaken. The relative humidity of the laboratory air was determined by a humidity gauge, which also monitored the room temperature and both were noted for each sample analysed. Various pressures of laboratory air were fed into the cavity cell and the absorption signal recorded each time. The I_0 measurements were provided by samples of laboratory air, at a pressure matching that of the I measurement, which had passed through some molecular sieve to remove the water vapour. Using the pressure, temperature and humidity recorded, a value for the water vapour pressure within each sample was determined and plotted against the subsequent absorption level measured, as illustrated in Figure 5.8a. Figure 5.8b illustrates the absorption level predicted for the same region using simulations based on the HITRAN database [251], and it is clear that a greater level of absorption is seen experimentally than would otherwise be expected, which is consistent with the presence of the water continuum.

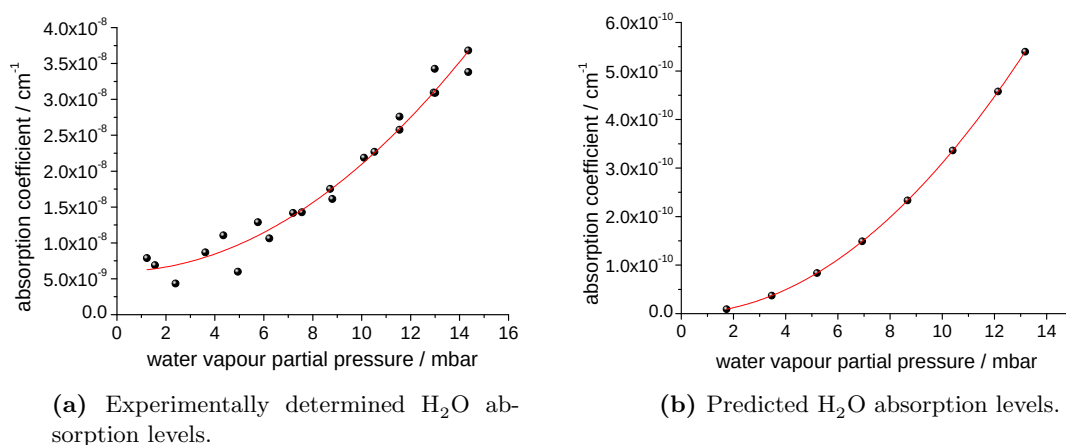


Figure 5.8: Figure (a) depicts the absorption levels due to water vapour as a function of pressures whilst Figure (b) shows the simulated levels in the same spectral region.

It is possible to determine an approximate value for the absorption cross-section for the water continuum by subtracting the contribution from the predicted absorption from that measured and normalising to the water vapour pressure present in each sample; the results of which are given in Figure 5.9. These values represent the absorption cross-sections for the *total* water continuum, as self- and foreign- components can not be extracted, though the foreign-continuum is clearly going to dominate in a laboratory air sample. Typically, the two components are determined by first measuring the absorption seen in a pure water vapour sample, and then buffering it with dry air and measuring the resultant absorption [267]. The self-continuum is then derived by subtracting the Lorentzian-shaped narrow transitions (as defined in an earlier section) from the absorption measured from the pure water vapour sample, the result of which is then subsequently subtracted from the continuum measured of the buffered water vapour sample to yield the foreign continuum. Conventionally, these values are then quoted normalised to an atmosphere of the buffering gas (i.e. water vapour in the self-continuum case and ‘foreign’ molecules, such as N₂ and O₂, with the foreign-continuum). On adjusting each component to the pressure of the buffering gas and summing the two, the total continuum cross-sections can be determined from the values given in the literature and compared to those measured in this study. Self- and foreign- continuum absorption cross-sections have been taken from Paynter *et al.* [265] and treated in such a way and are illustrated in Figure 5.9 alongside the measurements yielded in this work. In addition, more recent foreign-continuum cross-sections have been determined from data taken by the CAVIAR (Continuum Absorption at Visible and Infrared wavelengths and its Atmospheric Relevance) study [266] and these have also been used, in conjunction with the self-continuum cross-sections from Paynter *et al.* [265] (as the self-continuum measurements reported from CAVIAR [266, 269] stop short of the wavelength used in this

study) to determine secondary total continuum cross-sections for comparison.

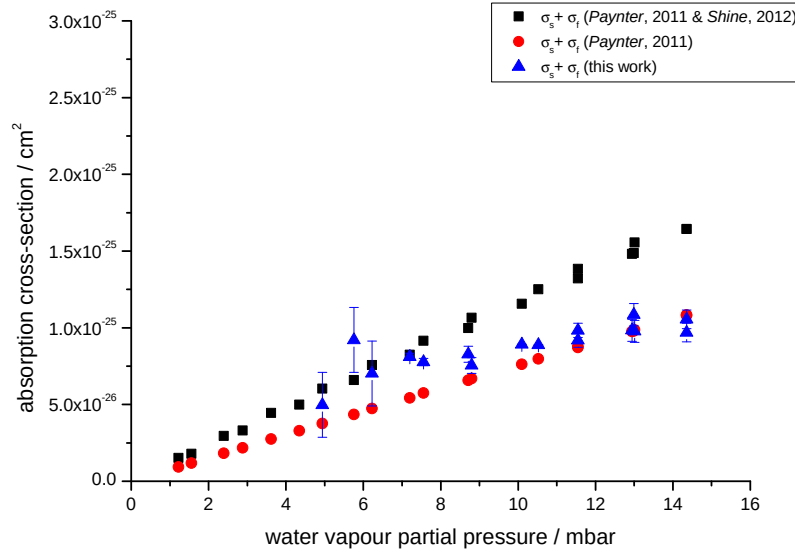


Figure 5.9: A comparison of the total water continuum absorption cross-sections of this study with values from the literature: various pressures of laboratory air were released into the optical cavity and the absorption level due to the water vapour in the sample noted; this represents the total water continuum, a combination of both the self- and foreign- continuum at that particular water vapour pressure and total pressure. In order to compare with the literature, the self- and foreign-continuum cross-sections from Shine *et al.* [266] and Paynter *et al.* [265] were adapted to take into account the partial pressure of water vapour (self-continuum contribution) and the total pressure (foreign-continuum contribution) of each sample.

Although the Shine *et al.* [266] values from CAVIAR were determined at a higher temperature (350 K) than used here, the foreign continuum has a very small, almost zero, negative temperature dependence so it should not influence the result too much. Below certain pressures the amount of water vapour was so low that the data are statistically insignificant, and as such have been excluded from Figure 5.9. The water vapour pressure in the laboratory air sample was estimated with the use of a humidity gauge (as there are no water transitions in the spectral region of interest to determine the pressure spectroscopically), and any uncertainties arising from this will be magnified in the lower pressure samples. In addition, any systematic errors in recording the absorption are also going to manifest themselves to a greater extent in the low pressure samples. The second thing to note is the variation for the total continuum absorption cross-sections in the literature values. This arises from the uncertainty in determining the foreign continuum component: as Paynter notes in [267], this is because the foreign-continuum is smaller than the self-continuum (normalised to absorber molecule and total buffering gas), which makes it more sensitive to variations between experiments.

Given these uncertainties, it is encouraging that the cross-sections determined from this work, in the main, fall within the values from the literature. However, the most significant result of this is the clear indication that water vapour must be removed from breath samples before analysis: in a typical laboratory air sample, at atmospheric pressure, an absorption of $\sim 4\%$ will be observed in a 45 cm optical cavity of $R \sim 99.996\%$. In addition, water has a tendency to condense on surfaces, which could contaminate the mirrors within the cavity. Therefore, it is essential to reduce the water vapour content in the breath sample before it is allowed to fill the cavity cell. This was achieved using a homemade Peltier-cooling [298] device.

To investigate the performance of the water-removal device, its temperature was varied and samples of laboratory air passed through it. The laser wavelength was altered to 1689.45 nm to probe the H_2O transition there so that the water vapour pressure could be determined spectroscopically. Water has three fundamental modes of vibration: ν_1 is the symmetric stretch at 3657 cm^{-1} , ν_2 is the bend at 1595 cm^{-1} and ν_3 is the asymmetric stretch at 3756 cm^{-1} , and this particular water transition represents a combination band arising as a result of motion in ν_2 and ν_3 , $|0\rangle|1\rangle|1\rangle \leftarrow |0\rangle|0\rangle|0\rangle$. Within the combination band it corresponds to the transition $(12, 3, 9) \leftarrow (11, 1, 10)$, which is written in the form $(J', K'_a, K'_c) \leftarrow (J'', K''_a, K''_c)$ where J is the total angular momentum quantum number, whilst K_a and K_c represent the angular momentum quantum numbers along the a and c axes respectively.

Samples of ~ 50 mbar of laboratory air were passed into the cavity via the water removal device, with the temperature of the chiller progressively lowered and then raised again and the resulting spectrum at each temperature recorded. As the chiller was not cleared of water in between each sample, this meant that once the lower temperatures had been reached, the following samples at higher temperatures would exhibit saturated water vapour partial pressures. The first sample at room temperature was used as a reference point for the initial partial pressure of water vapour for each sample (again using the humidity gauge to determine this) before it passed through the chiller. The peak heights obtained were converted to partial pressure of water vapour, allowing a plot of these pressures against chiller temperature to be constructed. It was found that this agreed very well with the theoretical partial pressure of saturated water vapour at those temperatures (determined using the Buck equations [299, 300]), as is illustrated in Figure 5.10.

No frequency calibration is present in the experimental set-up, given that its ultimate use in measuring an absorption level over a very short spectral range deems this unnecessary. Therefore, when studying water, the height of the absorption rather than its area is used to determine water vapour partial pressure and mirror reflectivities. However, from estimating what the FWHM of the transition should be, given the pressure of the sample and the spectroscopic parameters quoted in HITRAN, it is possible to fix a frequency scale and

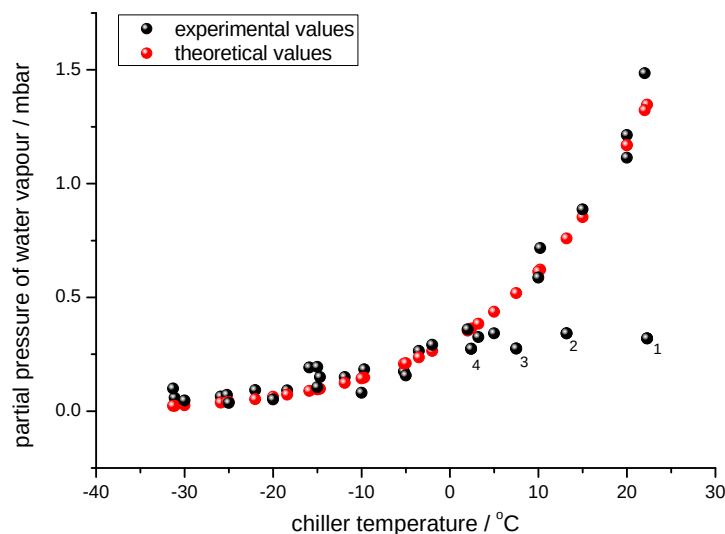


Figure 5.10: Water vapour partial pressure as a function of temperature: comparison of experiment against theory. The first 4 measurements, taken as the temperature of the chiller is reduced but before the freezing point of water is reached, are labelled and do not exhibit saturated water vapour partial pressures. Following this, the temperature is reduced to $\sim -30^\circ\text{C}$ before being increased back up to ambient temperature. As the chiller is not cleared of water in between measurements, the samples pass through a water/ice medium, which leads to saturated water vapour pressures being observed.

determine an approximate area to verify the reflectivities estimated from the peak heights. An example of this is illustrated in Figure 5.11a, alongside an illustration of the level of water reduction afforded by the water removal device at -30°C . (It should be noted that there is very little self-broadening at these water partial pressures.)

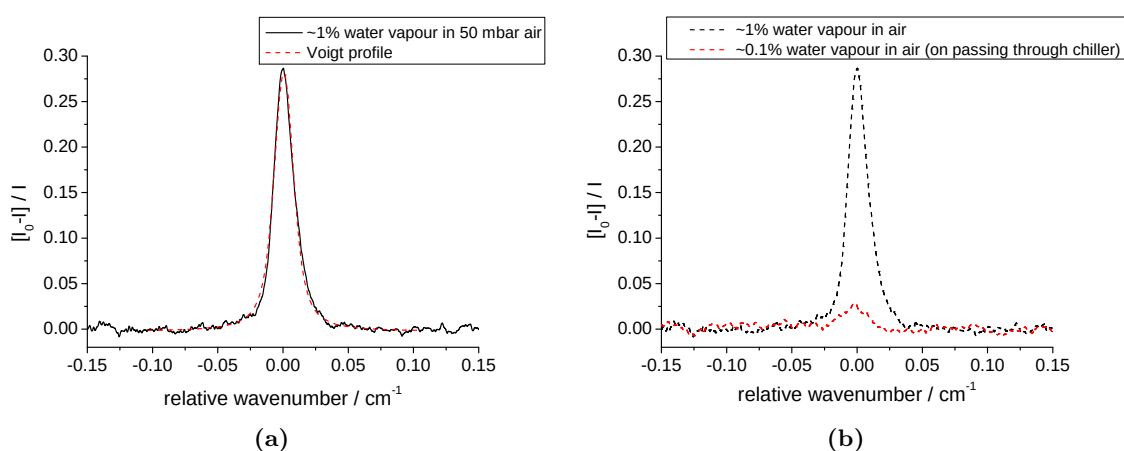


Figure 5.11: Figure (a) illustrates an absorption spectrum of ~ 50 mbar of $\sim 1\%$ water vapour in laboratory air with a Voigt profile fitted; $\sigma_{\text{int}} = 8.75 \times 10^{-25} \text{ cm}^2\text{cm}^{-1}$. Figure (b) shows the water reduction observed on passing a laboratory air sample through the chiller.

To ensure that the water removal device works equally efficiently on laboratory air and breath (in case the greater concentration of water vapour in breath poses problems) measurements were taken on multiple samples of breath and laboratory air. The spectra obtained of the water transition were found to have absorption peaks of approximately the same magnitude, indicating that the water reducing effects of the chiller are, within reason, independent of water content prior to processing. Figure 5.12 illustrates this for samples of 900 mbar taken at a chiller temperature of $-15\text{ }^{\circ}\text{C}$.

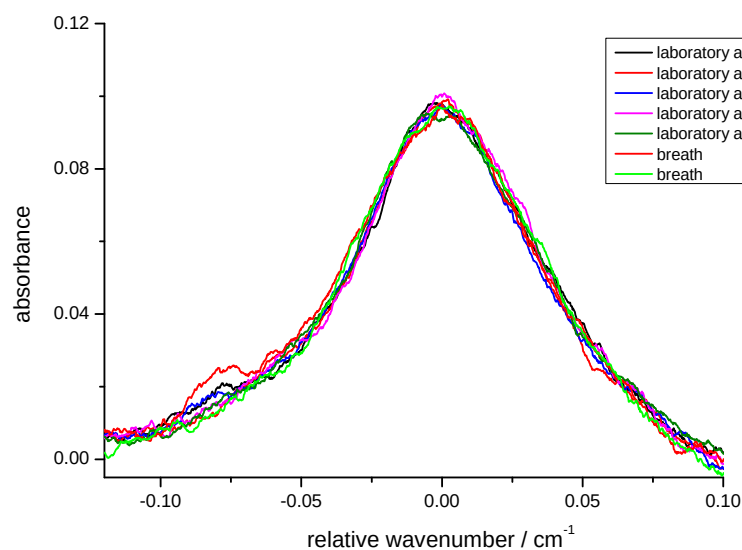


Figure 5.12: The absorption seen for the water transition at 1689.45 nm with 900 mbar samples of laboratory air and breath, on passing through the chiller at -15°C .

If samples of relatively high water content (i.e. breath, $\sim 5\%$) are used frequently, it becomes imperative that the device is cleared of water periodically, as the chiller can become blocked with ice. For consistency, this is achieved using a flush of dry N_2 through both the device and the optical cavity for a few seconds after every sample. Although this is not a viable solution for a final product as it requires pressurised nitrogen, it is adequate for development; ultimately, a pump acting on the chiller will probably be used. The height of the absorption peak suggests a partial pressure of water that is slightly higher than what would be expected for a sample at $-15\text{ }^{\circ}\text{C}$. This could be a result of treating higher pressure samples than those used previously with the chiller; it might not have been quite as efficient with the greater volume of sample this entailed. Clearly with a slower flow rate this efficiency can be improved and running the chiller at a lower temperature will also reduce the water vapour further, as illustrated in Figure 5.10. However, even assuming a partial pressure of 0.5 mbar (which is higher than that observed here), in the spectral region of interest for detecting acetone (1688.45 nm), which is between water transitions, this only yields an $\frac{I_0 - I}{I}$ value of 9×10^{-4} absorption for a 45 cm cavity of $R \sim 99.996\%$.

Furthermore, a 0.05 mbar sample, which represents a partial pressure slightly higher than would be expected for $-30\text{ }^{\circ}\text{C}$, suggests an absorption of 8.78×10^{-5} due to water, compared to an expected 1 ppm acetone absorption of 0.03 (for comparison, the vapour pressure for pure samples of water, acetone and isoprene at $-30\text{ }^{\circ}\text{C}$ are 0.38 Torr, 12.31 Torr and 54.78 Torr, respectively [247]).

Now that the device can reduce the water vapour in breath to negligible absorption levels at 1688.45 nm, it is important to investigate the effects of the chiller on the concentration of acetone in a sample. This was achieved by comparing the mass spectrometer readings on breath samples both before and after they have passed through the chiller. Both acetone and isoprene recorded a slight drop in concentration, but this only represented a loss of ~ 60 ppb in acetone and <10 ppb in isoprene.

5.3.2 Methane

The other breath constituent that may cause problems in this spectral region is methane. Although no transitions are located at 1688.45 nm, there are two small absorption features at 1688.35 nm and 1688.30 nm, the absorption wings of which do interfere marginally with the absorption levels detected. This is illustrated in Figure 5.13, which shows how the absorption level seen at 1688.45 nm increases with methane concentration [251]. Although typical levels found in breath (1.8 ppm) pose no problems, the elevated levels found in the breath of methane producers have been observed to add to the absorption measured, resulting in a higher returned ‘acetone’ concentration than would be expected from the levels signified by the mass spectrometer.

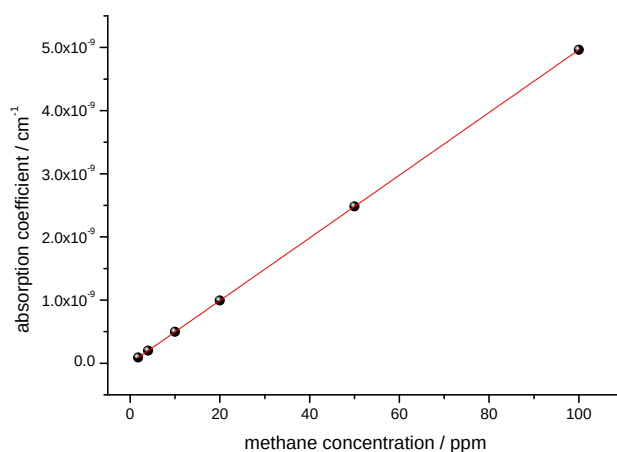


Figure 5.13: The simulated absorption seen at 1688.45 nm as a function of methane concentration, with absorption values adapted from the HITRAN database [251].

As every individual could have quite different methane levels in their breath, the ideal way to solve this issue would be to use each subject's breath as the I_0 measurement: this way, the amount of methane in the breath is negated. In order to do this, clearly it is necessary not only to remove the water vapour from the sample, but also the acetone and it was found that molecular sieve would remove both of these molecules from a sample. In this work 3A sieve (Merck Chemicals), which equates to a 3 Ångstrom pore size, was used. On passing through the sieve, only those molecules which are smaller than these diameters will be adsorbed onto the pores, and molecules of greater polarity are adsorbed preferentially [301]. The molecular sieve was placed in a section of small copper tubing, with filters at both ends preventing it from moving. This was attached to the optical device, alongside the chiller, with a tap to control when the sample flowed through it.

Samples of laboratory air (so removing any influence of other species found in breath but not air) were fed through both the chiller and the molecular sieve, to ensure that the water vapour passing through both devices is of a low enough level to form an insignificant/negligible part of the total absorption signal. Although the molecular sieve is likely to be more efficient at removing water vapour (typically down to 1 ppm levels and removing water up to 22 % of its weight [302]) than the chiller, this degree of difference is beyond the optical detection limit of the device, where the absorption cross-sections due to water vapour are incredibly small.

In addition, it is also important to investigate the longevity of the molecular sieve, i.e. is there a point at which it stops adsorbing acetone? To study this, an experimental arrangement mimicking that involving the optical cavity was set up: the molecular sieve was attached to an empty cell, which had a connection to the pump and a second tap from which the mass spectrometer could sample the vessel's contents. This ensures that the sample is drawn through the molecular sieve in exactly the same way as it is with the optical device. On filling the vessel, it can then be moved (in contrast to the optical device) to the mass spectrometer to analyse the sample that has passed through the molecular sieve. The acetone (and isoprene) levels recorded can then be compared to those seen in the portion of the sample still within the breath bag. It was found that it was not until the 50th sample of breath that ~ 200 ppb of acetone was recorded on the other side of the molecular sieve. The change was rather sudden, with the first indication of any acetone passing through coming with the 47th sample. Given the sample passing through the molecular sieve will be the I_0 sample, at this point (200 ppb) the absorption readings will become unreliable and it will be necessary to replace the molecular sieve. It can be regenerated from inducing a pressure change, heating or sometimes purging with gas that will remove the acetone (and water) adsorbed to the molecular sieve. However, in this instance the most straightforward solution is to replace the molecular sieve; and this may represent the solution for the end product. Interestingly, isoprene diffuses through rather

more rapidly: the first indication of isoprene (10 ppb) was from the 10th sample, whilst approximately all of it was passing through by the 36th sample; this, and the influence of isoprene in general, will be discussed in more detail later in the chapter.

With a mechanism in place for dealing with both water vapour and methane, the device was now ready to be tested on real breath samples.

5.3.3 Breath

The mass spectrometer used (AIRSENSE Compact, V&F) was calibrated against a 1 ppm acetone, 1 ppm isoprene and 1 ppm ethanol in N_2 mix and a 5 ppm of acetone in N_2 mix (both BOC Special Gases). Breath samples were collected in an identical manner to that described in Chapter 3: the subject was asked to inhale normally and to exhale the last part of the breath into a collection bag (Fischer Analysen Instrumente GmbH, F201-VP-05c, 1.5 litres).

To investigate how much the acetone (and isoprene) levels degrade over time in the breath bags, a number were prepared with breath samples of varying levels of acetone and isoprene and the contents were evaluated using the mass spectrometer over the course of 2 weeks. The results are illustrated in Figure 5.14, and they demonstrate that within the first few days the level of acetone does not drop considerably (the change is greater for isoprene), but once this stretches to weeks the levels are reduced quite significantly. Although one could still record measurements from samples taken a couple of days previously, to ensure the greatest accuracy possible, all samples were analysed on the day on which they were taken.

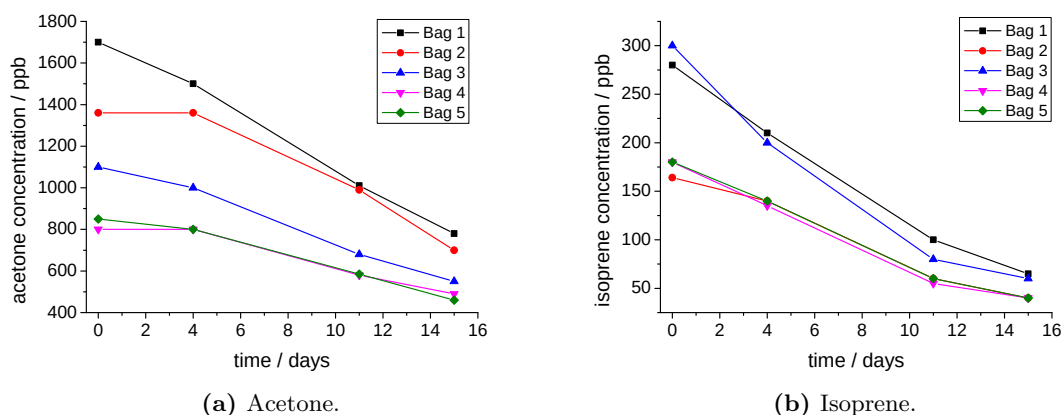


Figure 5.14: Figure (a) shows the drop in acetone concentration measured from the breath bags over the course of two weeks, whilst Figure (b) illustrates the loss in isoprene concentration over the same time period.

Prior to measurement with the optical device, a portion of the collected breath sample was fed into the mass spectrometer and the concentrations of acetone and isoprene noted. Next, a portion of the sample was passed through the molecular sieve and into the cavity so that an I_0 measurement could be recorded. The same breath bag was then attached to the chiller, which was set to -30°C , and an I measurement was obtained. Finally, the breath bag was reattached to the molecular sieve and a second I_0 measurement was taken. Whilst this was happening, the chiller was switched off and allowed to come up to room temperature so that once all three readings had been taken, the whole instrument (optical cavity, molecular sieve and chiller) was flushed with N_2 for a few seconds before the next sample was analysed. For calibration purposes, a breath bag full of the 5 ppm acetone in air mix was also analysed by the optical device and had its levels verified against the mass spectrometer. Using the calibrated mix, a mirror reflectivity of $\sim 99.996\%$ was consistently returned.

The average value of the two I_0 measurements was then used as the background measurement for the I recorded to produce an absorption level. Strictly speaking, this absorption is due to both acetone and isoprene, as the latter also absorbs in this region. Isoprene tends to be found at much lower levels in breath than acetone, but its absorption cross-section is approximately 1.5 times larger. Therefore, it does have a significant effect on the level of absorption seen: indeed Figure 5.15 illustrates a plot of acetone level indicated by the optical device against that recorded by the mass spectrometer, and this yields an offset of 329 ppb.

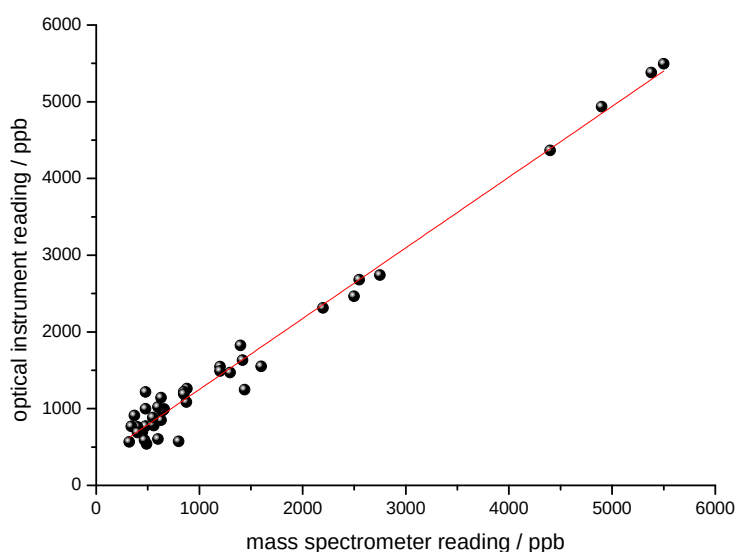


Figure 5.15: A plot illustrating the absorption measured by the optical device against that recorded by a mass spectrometer; the optical device data points have not been corrected for the isoprene contributions, yielding straight line fit parameters of $y = 0.922x + 329$.

When the same figure is plotted with the isoprene concentration for each sample taken into account (using the values from the mass spectrometer), this offset effectively disappears (31 ppb), as illustrated in Figure 5.16. A secondary issue, briefly mentioned previously, is that isoprene tends to pass through the molecular sieve more readily: this means that over the course of some measurements, the degree of isoprene included in the I_0 measurement will increase over time, or more significantly, it will vary. Consequently, the degree to which the level of isoprene is negated (in a similar way to methane) will also vary and may perhaps explain some of the variation seen even when isoprene is corrected for.

A potential solution to the problem with isoprene could be to just take many more samples so that a statistically significant mean isoprene level can be determined, and thus taken into account automatically when the device is used. Alternatively, a spectral region in which isoprene has a much reduced absorption cross-section could be probed; this is investigated further in the following chapter.

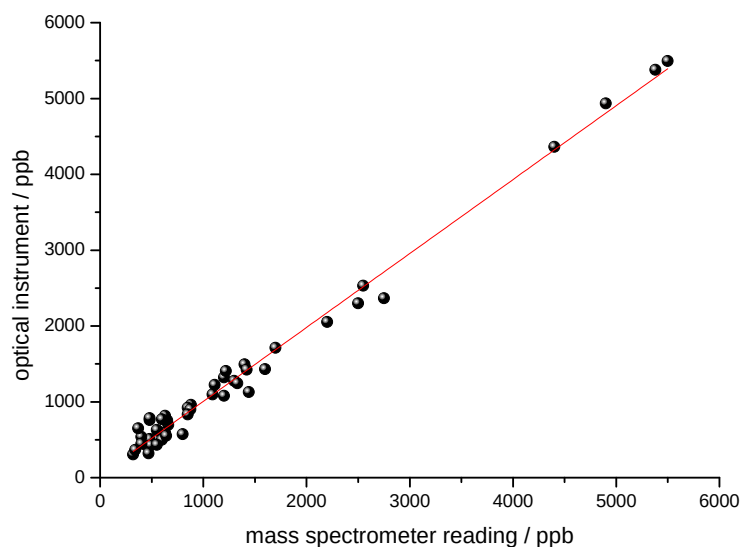


Figure 5.16: A plot illustrating the absorption measured by the optical device against that recorded by a mass spectrometer; the optical device data points have been corrected for the isoprene contributions using the values recorded by the mass spectrometer, yielding straight line fit parameters of $y = 0.975x + 31$.

What is promising is that the uncertainty in both sets of measurements (whether corrected for isoprene or not) is ~ 200 ppb. This is derived from the standard deviation of the respective linear fits to the data points measured (excluding calibration data points) and it represents a very reasonable uncertainty in the device's performance and in conjunction with the sensitivity demonstrated by the instrument, suggests that it could conceivably be used to detect acetone at the levels found in the breath of healthy individuals.

5.4 Conclusions

This chapter has provided a clear demonstration of the viability of laser-based CEAS for the detection of broadband absorbers, where care must be taken to account for all possible sources of absorption level change. This has been found particularly important in breath where there is a large quantity of water, which provides a continuum absorption over a very broad spectral region. A unique solution has been found here by removing water and also using breath samples to generate their own background measurement, which has allowed breath acetone levels to be measured within an uncertainty of 200 ppb. One way to circumvent, or at least reduce, the problems associated with interfering species is to move to a spectral region where their absorption is less significant and the acetone absorption cross-sections are larger, a possibility which is explored in the following chapter.

Chapter 6

Detection of Acetone in the Mid-Infrared

6.1 Introduction

The work carried out previously in this thesis has focused solely on the use of near-infrared radiation sources, primarily because these are readily available, relatively cheap (the exception being the SC source), robust and reliable thanks to their use in the telecommunications industry. However, in terms of molecular spectroscopy, the mid-infrared (MIR) is a far more desirable region to probe as moving from the weak overtone and combination bands of the near-infrared (NIR) to the fundamental transitions in the MIR allows access to transitions whose absorption cross-sections are often at least fifty times larger, providing the potential for greater detection sensitivities. This enhancement in $\sigma(\nu)$ on moving from the NIR to the MIR is illustrated in Figure 6.1 for acetone. Traditionally, the MIR spectral region has been probed using Pb-salt diode lasers, in addition to CO and CO₂ gas lasers. More recently, non-linear optics-based techniques have been used to generate MIR radiation from NIR sources; a brief overview of these MIR sources is presented in Appendix A.

The advent of the quantum cascade laser (QCL), a relatively high power and widely tunable source in the MIR with inherent low bandwidth, offers the exciting prospect of achieving greater levels of sensitivity at very high resolution for trace gas detection. QCLs are potentially available over a broad wavelength range (3 - 250 μm , although they are not commercial available for all wavelengths within this range), with the particular QCL used in the present studies operating near 8 μm over a quoted region of 1215 - 1223 cm^{-1} . As will be discussed at length later, this region is an ideal one in which to probe the spectroscopy of acetone. Initial studies will revolve around direct absorption measurements, utilising the high resolution of the laser to probe the rotational structure of acetone, before

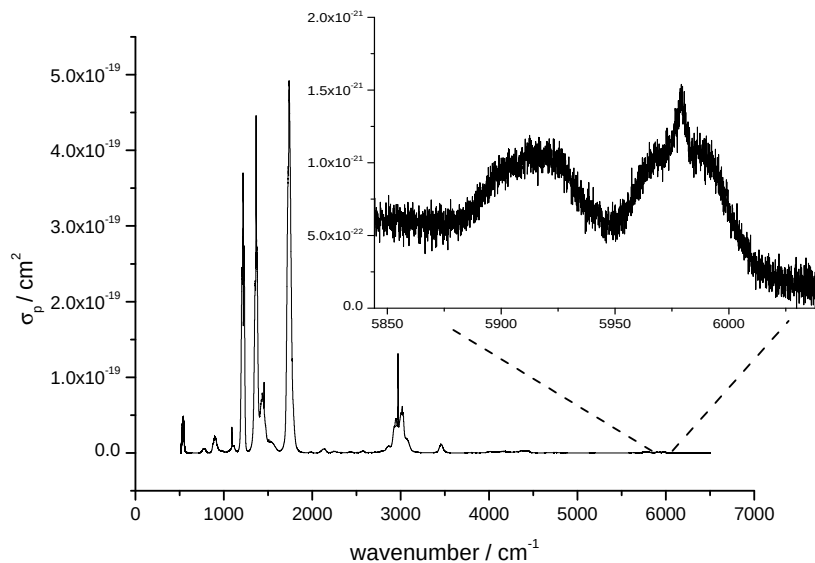


Figure 6.1: Survey spectrum from the PNNL database [246] showing the marked increase in absorption cross-sections on moving from the NIR to the MIR for acetone; inset, the feature studied in the NIR work presented earlier in this thesis.

taking advantage of the large cross-sections present in the MIR to apply the system, via pathlength enhancement techniques, to the detection of acetone in breath. Before this the device will be characterised and its spectroscopic performance on application to a variety of techniques evaluated using the ro-vibrationally resolved transitions of methane found in the same region.

6.2 Characterising the QCL

The QCL (Alpes Lasers 8 μm cw DFB-QCL, $P_{\text{max}} \sim 35 \text{ mW}$) is mounted in a custom-built housing, illustrated in Figure 6.2. The laser and its submount are mounted on a copper bed which acts as a heat sink, thermally connecting the laser submount to the water cooling system behind. The QCL chip is located in the central section of the mount, and to prevent any possibility of water crystallising or condensing onto it as the laser is cooled, a dry pump (KNF, N 84.3 ANE) is utilised to keep the chamber under vacuum and water-free. A thermoelectric cooler (TEC), at the back of the copper heat sink, is used to control the QCL temperature, which in turn is monitored by a thermocouple on the copper block. Both the temperature controller and the laser current driver are homemade, with the latter based on the work of Libbrecht and Hall [303].

The advantage of using a homemade laser current driver over a commercial one is that it can be tailored specifically for this QCL set-up, with earlier studies in this laboratory [305]

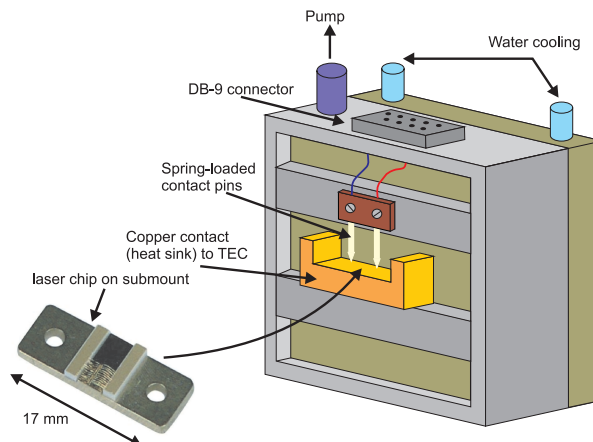


Figure 6.2: Depiction of the QCL mount; adapted from [304].

demonstrating that the homemade device results in a vast reduction in noise on the current output and far superior reproducibility in returning to the same frequency on repetitive scanning of the laser, thus reducing the spread of the laser output frequencies, or the effective laser linewidth.

As with diode lasers, both the laser temperature and injection current can be varied to select different wavelengths. In conjunction with an FTIR spectrometer (Perkin Elmer Spectrum 100), the spectral coverage of the QCL was probed by varying both of these parameters, and the results can be seen in Figure 6.3.

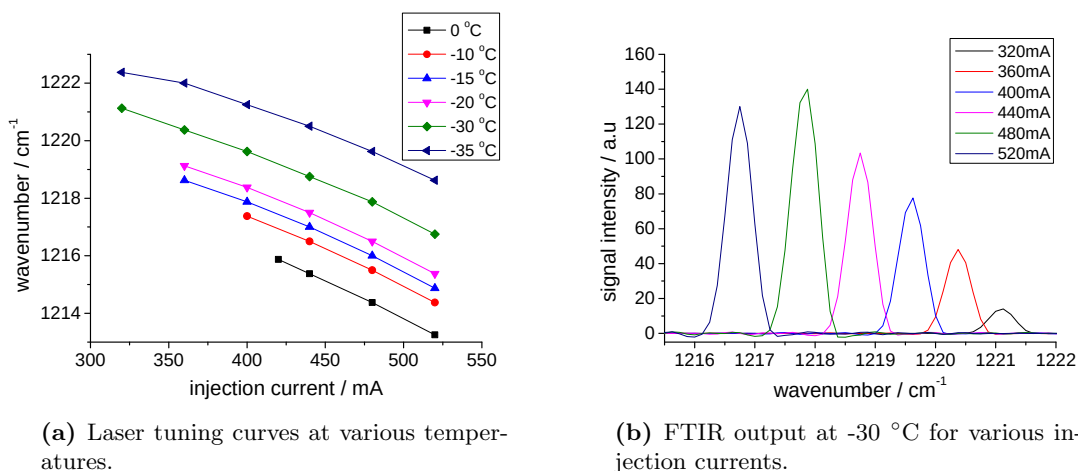


Figure 6.3: Graph (a) illustrates the laser tuning curves at various TEC temperatures for a range of injection currents, whilst Graph (b) features the FTIR output for a range of injection currents at $-30\text{ }^{\circ}\text{C}$. The FTIR spectrometer has a resolution of 0.5 cm^{-1} , so the outputs depicted do not reflect the linewidth of the QCL.

In addition, it is essential that the beam is collimated. The mid-infrared radiation diverges rapidly on emission from the QCL chip, so in order to minimise this divergence before the collimation optics, the chip is placed as close to the BaF_2 window mount as possible. A

4 mm focal length lens (Thorlabs, C036TME-F), placed in front of the laser facet on an x, y, z translational mount, was then used in conjunction with the MIR camera (Pyrocam, Spiricon) to ensure the beam was reasonably well collimated over ~ 6 m. Figure 6.4 shows a typical image obtained of the laser output with the MIR camera.

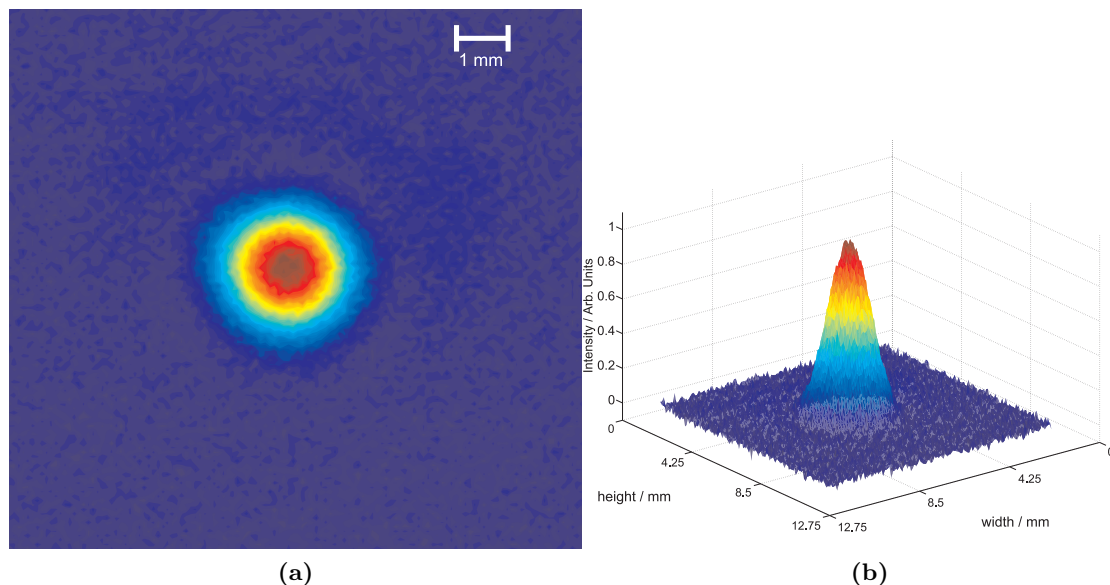


Figure 6.4: (a) is an image obtained of the collimated QCL beam at a distance of ~ 40 cm from the laser, whilst (b) depicts a 3D representation of the same image. A Gaussian lineshape fitted to the beam profile yields a beam waist of ~ 1 mm.

6.2.1 Methane

Although ultimately acetone is the target molecule, it is useful to first study methane. The reasons for this are two-fold: firstly, methane is a breath biomarker in its own right, as described in Chapter 3. As one in three people are methane producers, it is essential its spectroscopy is also considered, as it may interfere with the detection of acetone. Secondly, as a narrowband absorber with well defined transitions and absorption cross-sections, it allows the system to be easily characterised and a sensitivity deduced.

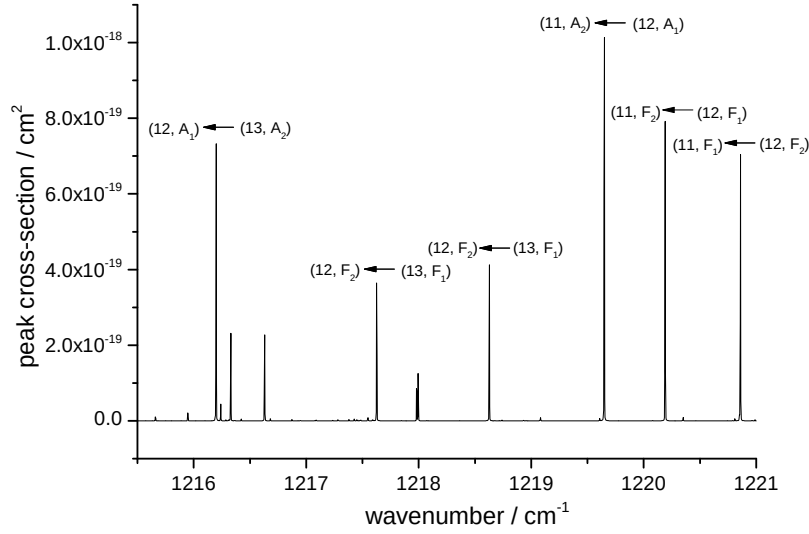
The methane transitions in the 1220 cm^{-1} region form part of the ν_4 band, due to the excitation of one vibrational quantum into the triply degenerate T_2 deformation mode. Details of the normal modes of CH_4 are given in Table 6.1.

In general each transition is defined firstly by the band (ν_4 throughout this work) and, as described in Chapter 3, then by its rotational quantum number, J , its ro-vibrational symmetry, C , and α , the counting integer for levels of the same J and C , incremented in order of increasing energy [170], such that $(J', C', \alpha') \leftarrow (J'', C'', \alpha'')$. Figure 6.5 summarises

Mode	Predominant motion	Symmetry	Frequency / cm^{-1}
ν_1	Symmetric stretch	A_1	2917
ν_2	Degenerate deform	E	1534
ν_3	Degenerate stretch	T_2	3019
ν_4	Degenerate deform	T_2	1306

Table 6.1: The fundamental vibrational modes of methane.

the methane transitions in this spectral region of interest using data from the HITRAN database [251].

**Figure 6.5:** Methane cross-sections in the spectral region covered by the QCL.

The precisely defined transitions of methane can be utilised to investigate the stability of the laser system over time. When taking spectroscopic measurements, a reference I_0 measurement, where no absorber is present, is taken before the sample itself is analysed. If the laser intensity varies on the time scale of the experiment, the subsequent absorbance measured will be incorrect. This is of particular importance with the detection of acetone, as its broad spectrum means that absorption occurs across the whole region scanned and this lack of baseline means that any laser intensity variation must be accounted for.

One way of potentially correcting for any variations is to introduce a second detector, as illustrated in Figure 6.6. The QCL radiation was initially diverted via a beam splitter onto a 2'' focal length off-axis paraboloid (OAP) which focuses it onto the first detector. The remaining light was then directed through a 10 cm sample cell, before being focused onto the second detector (both Vigo, PVMI-3TE-10.6) with another 2'' focal length OAP. Therefore, any variations seen in the first detector can be used to correct the signal from the second; what remains is then purely due to absorption and not laser drift.

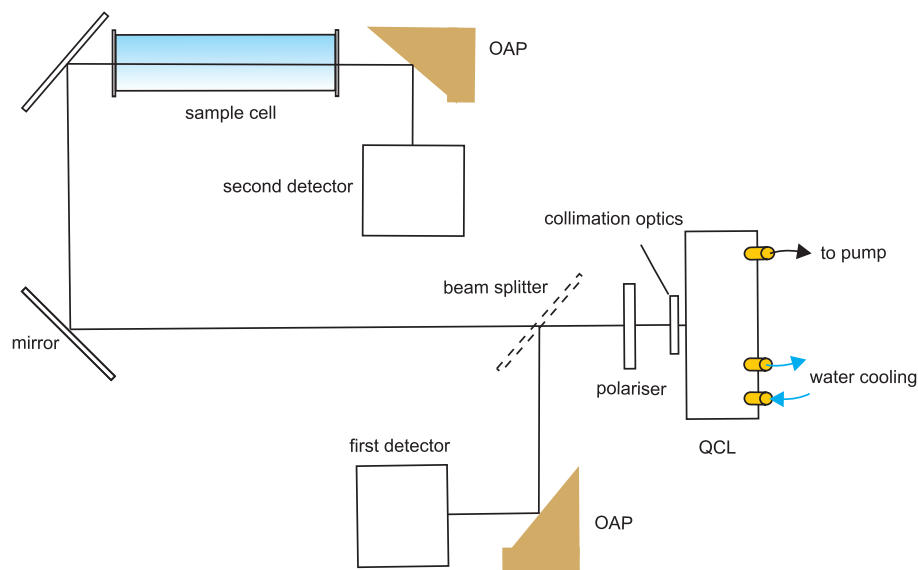


Figure 6.6: Two detector experimental set-up for monitoring the output of the laser before and after the sample cell.

Initially, the variation of the laser output was monitored when it was not being scanned: i.e. it was lasing at a fixed frequency. The laser output on both of the detectors was then recorded on the oscilloscope over 30 minutes, in one minute intervals. As one can see from Figure 6.7, the signals from the two detectors mirror each other, thus confirming that the entire beam was being focused onto the detector and that any variation seen was not due to the beam only partially being detected due to its large size and/or positioning instabilities. Although there is a pseudo-sinusoidal variation seen over time, between consecutive measurements this amounts to $<1\%$ variation. The overall variation is due to the stability of the laser temperature controller as it oscillates about the set temperature, thus varying the lasing frequency and consequently the detected signal slightly. However, of greater importance is the stability of the system as the laser is being scanned, as this is how it will be used ultimately.

For this, the set-up was identical to that in Figure 6.6, with the addition of a small pressure of methane in the sample cell. Once again, the detector output was taken every minute over a 30 minute period. The laser was scanned over the region $1219.75 - 1220.15 \text{ cm}^{-1}$, which includes the ν_4 methane $(11, F_2, 6) \leftarrow (12, F_1, 3)$ transition at $1220.1904 \text{ cm}^{-1}$. As the frequency of the methane absorption is known, it is used as a marker and its position in each scan used to determine any variation in the laser frequency. There was negligible variation in the signal intensity but as Figure 6.8 illustrates, the laser drifts in wavelength over time. This drift can be readily corrected by including a cell of methane in the beam path throughout measurements so that any shifts in the position of the methane transition can then be accounted for in the final analysis.

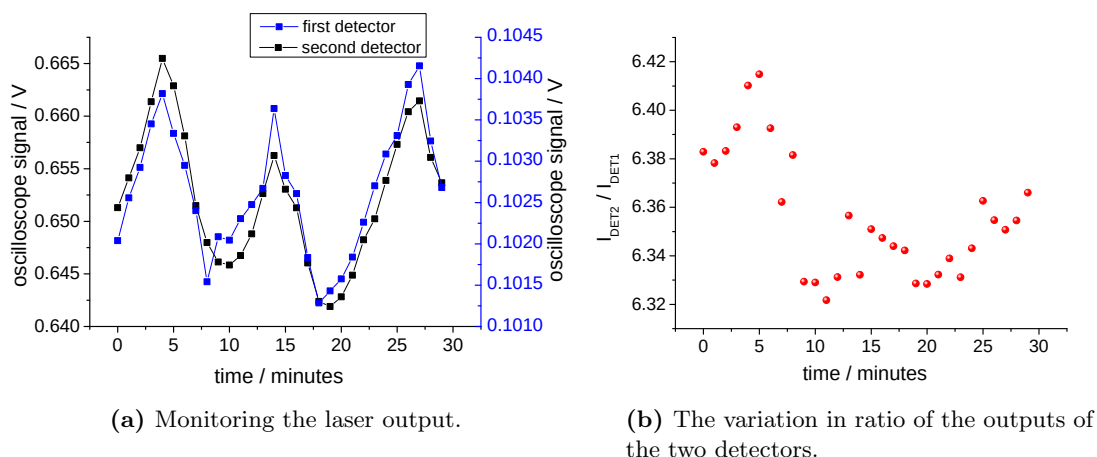


Figure 6.7: Graph (a): monitoring the QCL output at a fixed laser frequency using two PVMI-3TE-10.6 detectors placed pre- and post- the sample cell respectively. Graph (b) shows the variation in the ratio of the second detector and first detector output.

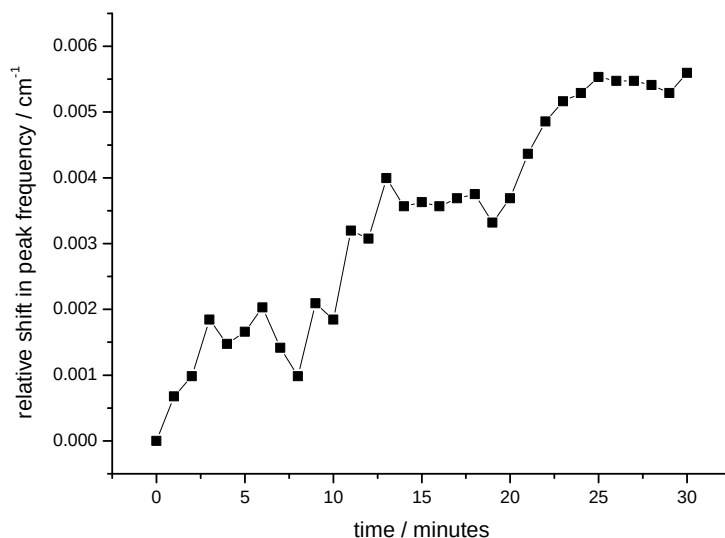
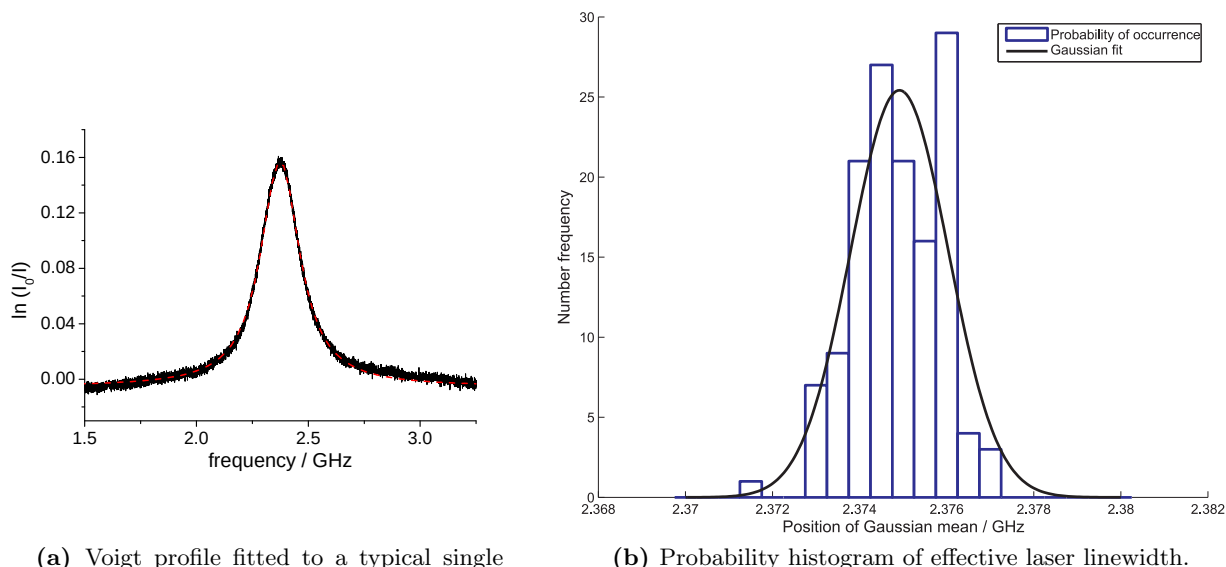


Figure 6.8: Shift in the position of the methane transition at $1220.1904 \text{ cm}^{-1}$ over a 30 minute period, corresponding to a total frequency shift of $\sim 180 \text{ MHz}$ which is $\sim 1.6\times$ that of the Doppler width of the transition.

To determine an approximate effective laser linewidth, and hence the resolution of the QCL when used with the homemade controller, the laser was scanned repeatedly over the methane $(12, F_2, 8) \leftarrow (13, F_1, 3)$ transition at 1218.63 cm^{-1} in a direct absorption measurement on $\sim 4.5 \text{ Torr}$ of CH_4 in the 10 cm sample cell. Each absorption feature was then fitted with a Voigt profile to determine the relative frequency position for each measurement, which were then collated and used to plot a probability histogram, as shown in Figure 6.9: each box represents the probability of the central frequency of the CH_4

transition being located at that spectral frequency. On fitting a Gaussian lineshape to the resultant histograms, the Full Width Half Maximum (FWHM) was determined and taken to be the effective laser linewidth. As the laser was scanned at a frequency of 15 kHz, and 150 scans were taken, it was assumed that any long term drift in the system will not affect the result. Under these conditions, an effective linewidth of 2.6 ± 0.1 MHz is produced.



(a) Voigt profile fitted to a typical single scan absorption spectrum.

(b) Probability histogram of effective laser linewidth.

Figure 6.9: Graph (a) shows a typical fit to an absorption profile obtained when determining the effective laser linewidth, whilst Graph (b) illustrates the determination of the effective laser linewidth from the FWHM of the Gaussian lineshape fitted to the frequency probability histograms.

6.2.2 Direct absorption

To investigate the spectroscopic performance of the QCL, pressure-broadening measurements on the ν_4 CH_4 ($12, F_1, 8$) $\leftarrow$ ($13, F_2, 2$) transition at $1217.6266 \text{ cm}^{-1}$ were carried out. Initially, low pressure samples of methane (BOC) were released into the cell and the resulting absorption recorded, before laboratory air was leaked into the sample cell and the broadened absorption feature measured. A relative frequency calibration was determined from the use of a Germanium etalon, with a free spectral range of 500 MHz. Sample spectra, with post-analysis data smoothing, are illustrated in Figure 6.10.

The spectra are fitted with a Voigt lineshape, with the Gaussian component fixed to the theoretical Doppler width for this absorption, 112.8 MHz. The subsequent HWHM Lorentzian widths determined from these fits were then plotted against the total pressure, as illustrated in the inset of Figure 6.10. The error bars, which are too small to be clearly visible in the plot, were determined from the error on each Voigt fit, and the gradient of

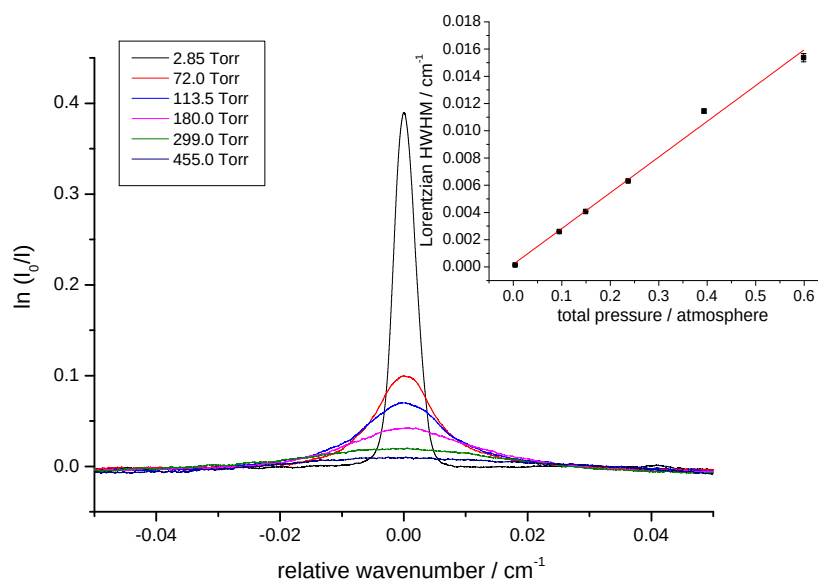


Figure 6.10: Absorption spectra of 2.85 Torr of methane, buffered with increasing amounts of air; inset, the HWHM Lorentzian widths derived from Voigt fits to the spectra, yielding the air-broadening coefficient for the CH_4 ν_4 transition $(12, F_1, 8) \leftarrow (13, F_2, 2)$. Please note that the central frequencies of the absorption profiles have been normalised to zero to compensate for the observed laser drift.

the graph gives the air-broadening co-efficient for the $1217.6266 \text{ cm}^{-1}$ CH_4 transition, γ_{air} , as $0.05251 \pm 0.00224 \text{ cm}^{-1}/\text{atm}$, which is within error of $0.0531 \text{ cm}^{-1}/\text{atm}$, the value given in HITRAN (with the 2σ uncertainty quoted derived from the error of the linear fit).

From these measurements it is possible to determine a sensitivity value for direct absorption, which can then be used as a benchmark to evaluate the merits of the subsequent sensitivity-enhancement techniques. Using the standard deviation of the linear fit to the baseline of the unsmoothed spectrum as a value for the noise, the signal to noise ratio for the absorption can be deduced and subsequently a minimum detectable absorption coefficient determined. In this case with direct absorption in a 10 cm cell, $\alpha_{\text{min}} = 2.34 \times 10^{-4} \text{ cm}^{-1}$ was derived.

Greater sensitivity can be achieved using modulation spectroscopy, and the next section will initially present a brief overview of the technique before describing an investigation into the application of wavelength modulation spectroscopy (WMS) with a cw QCL.

6.2.3 Wavelength Modulation Spectroscopy (WMS)

Modulation Spectroscopy

The detection sensitivity of an instrument can be increased by enhancing the magnitude of the absorption signal, or by decreasing the level of noise in the detected signal. Whereas CEAS and multipass cells increase the former with the enhanced pathlength they provide,

wavelength modulation spectroscopy (WMS) (developed first [306, 307]), and the related technique, frequency modulation spectroscopy (FMS) [308], improve the detection limit by reducing the noise in the absorption signal. Both techniques involve the application of a high frequency modulation on top of the relatively low frequency applied to the laser to scan its frequency over the absorption feature of interest. The detected signal then varies at the modulation frequency applied, which must then be demodulated at a harmonic of that modulation frequency to obtain the absorption feature. This demodulation is achieved using phase sensitive detection provided by a lock-in amplifier, which is capable of filtering out the components of the signal which are not at the same frequency and phase of the modulation originally applied to the laser.

As discussed in Chapter 2, there are three major noise sources: shot noise, white noise and external noise sources. The contributions from the latter are broadly categorised as $1/f$ noise and are signal-dependent. Therefore, by moving to a higher frequency regime, $1/f$ noise is necessarily reduced, though the degree to which the sensitivity is improved with respect to direct absorption is dependent on the relative contributions of the different noise sources.

Whether the modulation spectroscopy is classified as WMS or FMS depends entirely on the relative frequency of the modulation applied compared to the linewidth ($\Delta\nu$) of the spectral feature of interest. With WMS, the modulation frequency is much smaller than $\Delta\nu$ and tends to be in the kHz range, whilst the FMS modulation frequencies are much larger than the spectral linewidth, and occur in the MHz/GHz regime [309]. One might expect, therefore, that FMS will reduce the noise contribution further, but this is not always found to be the case as the higher frequency regime also requires detectors with faster response times and these higher bandwidth detectors often result in higher levels of white noise. Therefore, it is often preferable to take advantage of the less complicated electronics required for WMS, and this is the technique utilised here.

WMS Theory

The instantaneous frequency of laser light when a modulation is applied can be described with the following equation:

$$\omega(t) = \omega_0 + a_m \cos(\omega_m t) \quad (6.1)$$

where ω_0 is the unmodulated centre frequency of the laser light, a_m is the amplitude of the modulation and ω_m is the modulation frequency. From this, the time-varying intensity of the modulated light, $I(\omega_0, t)$, can be described as a cosine Fourier series, as it is a periodic, even function:

$$I(\omega_0, t) = \sum_{i=0}^{\infty} H_n(\omega_0) \cos(n\omega_m t) \quad (6.2)$$

where $H_n(\omega_0)$ is the n^{th} harmonic. The modulated intensity is necessarily composed of n harmonics of that frequency, each of which can be selected and detected by the lock-in amplifier. In the presence of an absorber the intensity, $I(\omega)$, is described by the Beer-Lambert law and $H_n(\omega_0)$ becomes:

$$H_n(\omega_0) = \frac{2}{\pi} \int_0^{\pi} I(\omega) \cos(n\omega_m t) d(\omega_m t) \quad (6.3)$$

$$= \frac{2}{\pi} \int_0^{\pi} I_0(\omega) \exp(-\sigma(\omega)CL) \cos(n\omega_m t) d(\omega_m t) \quad (6.4)$$

If one assumes firstly that the laser intensity is independent of wavelength tuning and secondly that the absorption is weak, such that $\sigma(\omega)CL \ll 1$, the Beer-Lambert Law can be approximated to $I(\omega) = I_0[1 - \sigma(\omega)CL]$, leading to:

$$H_n(\omega_0) = \frac{2I_0CL}{\pi} \int_0^{\pi} -\sigma(\omega) \cos(n\omega_m t) d(\omega_m t) \quad (6.5)$$

This illustrates how the demodulated harmonic of the signal is proportional to the species concentration, C - in other words, the peak-to-peak amplitude of the retrieved signal is directly proportional to the number density of the absorber.

In the situation whereby the amplitude modulation is much smaller than the linewidth of the absorption feature, $a_m \ll \Delta\nu$, $H_n(\omega_0)$ becomes proportional to the n^{th} derivative of the spectral lineshape, $\sigma(\omega)$, from a Taylor expansion of $\sigma(\omega)$:

$$H_n(\omega_0) \simeq \frac{I_0CL2^{(1-n)}}{n!} a_m^n \left(\frac{d^n \sigma(\omega)}{d\omega^n} \right)_{\omega=\omega_0} \quad (6.6)$$

This is known as the derivative limit and means that the WMS signal is the derivative of the absorption feature. This situation is depicted in Figure 6.11, where the sinusoidal forms at the top of the absorption lineshape represent the modulated laser frequency as it encounters the absorption feature. On sweeping across the feature, the intensity of the frequency component, $I(\omega)$, at ω_m will be attenuated by differing amounts, with a greater change, $\Delta I(\omega)$, observed where the slope is steeper and the WMS signal equaling zero at the top of the absorption line.

However, this regime is rarely used for sensitive trace gas detection as it does not produce the largest WMS signals. Instead, the modulation index (or depth), a dimensionless parameter given by $b = 2a_m/\Delta\nu$, is optimised to give the largest WMS signal by varying the

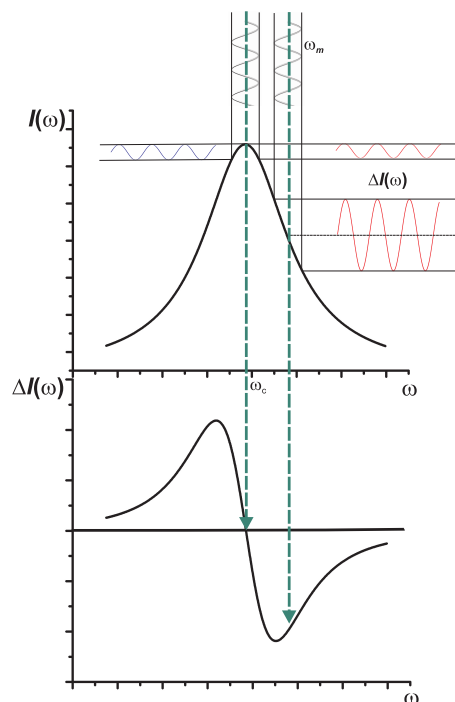


Figure 6.11: **Top:** illustration of the modulated laser frequency sweeping over the absorption feature; **bottom:** on demodulation, the resultant $1f$ signal.

modulation amplitude, a_m . However, increasing a_m also causes modulation broadening, as illustrated in Figure 6.12, which affects the spectral linewidth determined and so must be corrected for in the final analysis.

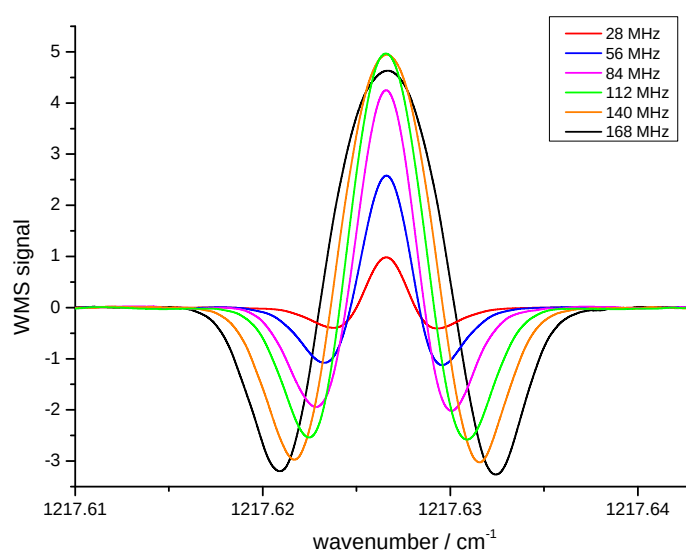


Figure 6.12: Illustration of modulation broadening on the ν_4 $(12, F_1, 8) \leftarrow (13, F_22)$ methane transition as the modulation amplitude is varied.

A second consideration is the harmonic at which to demodulate. Figure 6.13 demonstrates the demodulation of the first five harmonics on the ν_4 methane transition at $(12, F_1, 8) \leftarrow (13, F_2, 2)$. As can be seen, the lower the harmonic, the larger the signal. This would suggest $1f$ might be the optimum harmonic to use, but often $2f$ is used instead as it is less susceptible to residual amplitude modulation (RAM). RAM is caused by power variations that result from the modulation being applied to the laser, which is then converted into amplitude modulation at the modulation frequency of the laser. This causes background noise in the WMS signal, but it can be reduced considerably by instead demodulating at $n = 2$ (if the power variation is linear), as WMS signals resemble the n^{th} derivative. In other words, demodulating at $2f$ will often produce a larger signal to noise ratio (SNR) than demodulating at $1f$.

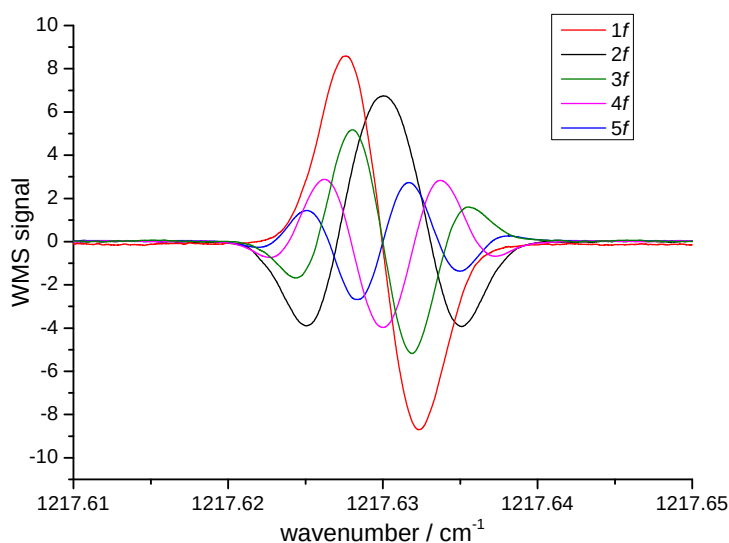


Figure 6.13: Demodulating at various harmonics at the ν_4 $(12, F_1, 8) \leftarrow (13, F_2, 2)$ methane transition; the amplitude modulation has been optimised for each harmonic and the modulating frequency is 20 kHz.

After the frequency at which to demodulate at (i.e. which harmonic) has been selected, it is necessary to optimise the bandwidth of detection within this frequency domain. The bandwidth (BW) of the detection system is inversely proportional to the time constant, τ . If too large a time constant is chosen, this will result in features in the signal being lost, or averaged out, as only a small portion of the frequency domain is considered, whilst the opposite is true for too small a value, where more noise is picked up as a result. As a consequence, it is necessary to optimise the time constant for each experiment undertaken.

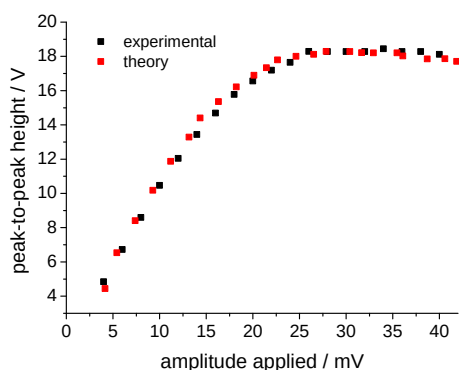
Finally, one of the major disadvantages of WMS is that the technique is not self-calibrating and consequently requires direct absorption measurements to be taken in tandem on a

strong absorption line to provide an absolute calibration for the technique. Alternative, self-calibrating, methods have been proposed and demonstrated, most notably by Henningsen and Simonsen [310] and Duffin, McGettick *et al.* [311–313], but both impose restrictions on the flexibility of the technique: the former developed algorithms to determine the molecular species density but the method requires the frequency tuning of the laser to be accurately known and can only be applied to the second harmonic detection of isolated absorption lines; whilst the latter, which utilises the RAM signal from a $1f$ trace for self-calibration, dictates the use of a large absorption in the derivative limit. Within a laboratory setting, such self-calibration methods are unnecessary and therefore are not employed in this work.

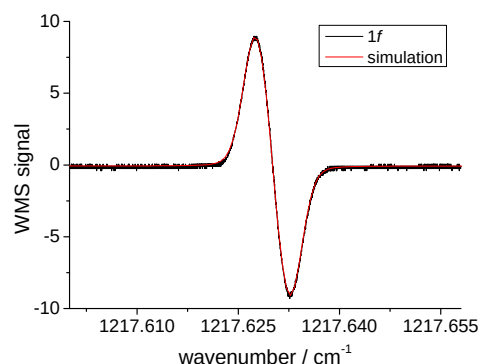
Experimental

The experimental set-up for WMS was identical to that utilised with direct absorption, the only change was the introduction of a lock-in amplifier to provide the modulation applied to the laser. A sample of 1.6 Torr of CH_4 was fed into the glass cell and to find the optimum value of the modulation amplitude (a_m) for the ν_4 $(12, F_1, 8) \leftarrow (13, F_2, 2)$ methane transition, the modulation voltage was incrementally increased and the resulting magnitude of the $1f$ and $2f$ peak-to-peak signals noted. Plotting the WMS signal against the applied voltage, as in Figure 6.14, clearly shows the plateau at which point modulation broadening dominates and increasing the voltage no longer causes an increase in the peak-to-peak signal. For $1f$, this optimum applied voltage was found to be 26 mV, whilst for $2f$ it was 44 mV. These optimum WMS signals are then utilised to determine the linewidth of the absorption feature under these conditions. As a low pressure of gas was used in this analysis, we can assume that negligible pressure broadening takes place and thus a Gaussian lineshape is expected. Work by Silver [314] has shown that the optimum modulation index, b , for a Gaussian lineshape is 2.1 for the second harmonic and 1.6 for the first. Using this, and the Doppler linewidth of the methane transition as determined from Figure 6.14b and 6.14d, the optimum modulation amplitudes were determined to be 100.4 MHz and 123.4 MHz for $1f$ and $2f$ respectively. In addition, signal to noise ratios were determined for both the optimum $1f$ and $2f$ WMS measurements, and it was found that the $2f$ harmonic produced a slightly higher SNR, 225 versus 200, and was thus used exclusively in the following experiments.

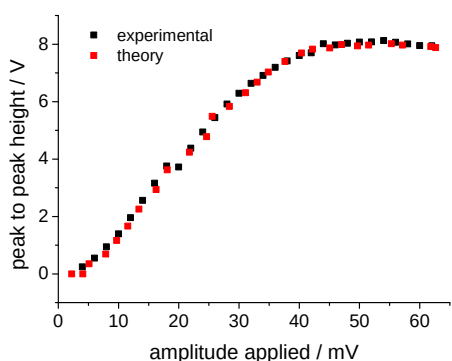
As previously mentioned, it is necessary to take a direct absorption measurement alongside a WMS measurement, in order to calibrate the technique. Therefore, initially a pressure of 0.27 Torr of methane was released into the cell and the $(12, F_1, 8) \leftarrow (13, F_2, 2)$ transition at $1217.6266 \text{ cm}^{-1}$ was first probed with no high frequency modulation applied to the laser. This allowed the exact methane concentration to be determined from the integrated area of the recorded transition, before the WMS measurement was taken and the resultant peak-to-peak amplitude attributed to the known pressure of methane in the cell. Sequential amounts of methane were then removed from the cell and WMS measurements taken until



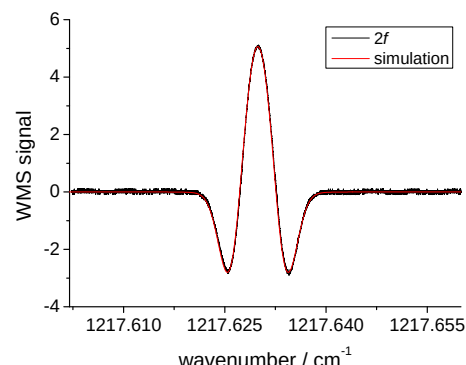
(a) 1f: Determination of optimum modulation amplitude.



(b) 1f Voigt fit to 1.6 Torr CH₄ absorption at 1217.622 cm⁻¹; FWHM = 125.5 MHz.



(c) 2f: Determination of optimum modulation amplitude.



(d) 2f Voigt fit to 1.6 Torr CH₄ absorption at 1217.622 cm⁻¹; FWHM = 117.5 MHz.

Figure 6.14: Determination of the optimum modulation amplitude and subsequent line-shape fit and FWHM determination under those optimum conditions. The difference in the returned FWHM for the methane absorption when measured by 1f or 2f principally reflects the uncertainty in determining the optimum modulation amplitude (as one can only apply 2 mV at a time).

the cell was completely evacuated. From these measurements, illustrated in Figure 6.15, it was possible to determine a minimum detectable absorption coefficient, $\alpha_{\min} = 1.2 \times 10^{-5} \text{ cm}^{-1}$ in the 10 cm cell. Thus it has been demonstrated that WMS can be applied to the QCL for spectroscopic applications, attaining a $\sim 20\times$ increase in sensitivity on direct absorption.

6.3 Acetone absorption cross-section determination

Having characterised the system using methane, it is now appropriate to apply the laser to the study of acetone, initially using direct absorption methods before utilising multipass cells and an optical cavity. The absorption cross-sections for acetone in the MIR are over

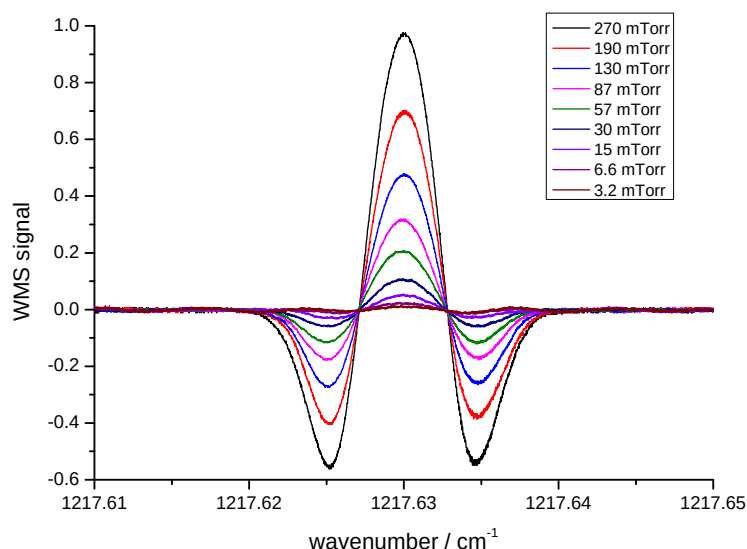


Figure 6.15: WMS applied to various low pressures of CH_4 .

100 times larger than those found in the NIR, as can be observed in Figure 6.1. This opens up the possibility of reaching the sub-1 ppm detection levels of acetone required for breath analysis without the need for a huge pathlength enhancement, and consequently a less complex optical arrangement.

Acetone belongs to the C_{2v} point group and its relatively large, bulky size results in rather more complex vibrational modes than those seen with methane, which manifest themselves as broad, congested absorption features. The infrared absorption spectrum of the equivalent of 1 ppm of acetone in an atmosphere of N_2 is given by the PNNL database [246] and is illustrated in Figure 6.16. It is a composite spectrum of several scans of different pressures of acetone, over a 1 m pathlength and with a resolution 0.112 cm^{-1} .

The band centred at 1216 cm^{-1} , pictured inset of Figure 6.16, is associated with the excitation of the ν_{17} vibrational mode, which has B_1 symmetry and corresponds to a C-C stretch. A full list of the vibrational modes can be found Appendix C [315]. This band is particularly well-suited for studying breath acetone because it represents a window in the absorption due to water vapour and other interfering species (such as CO_2 and CH_4), and the acetone cross-sections are relatively large.

However, given the broad, congested spectra associated with acetone, it is impossible to scan over a single, well defined absorption feature unless a broadband source, or an external cavity QCL, is used. Given this restriction when utilising a relatively narrowband source, it is essential to either a) find a region where nothing else absorbs and/or remove the species that do, as described in Chapter 5; or b) find part of the absorption feature that is

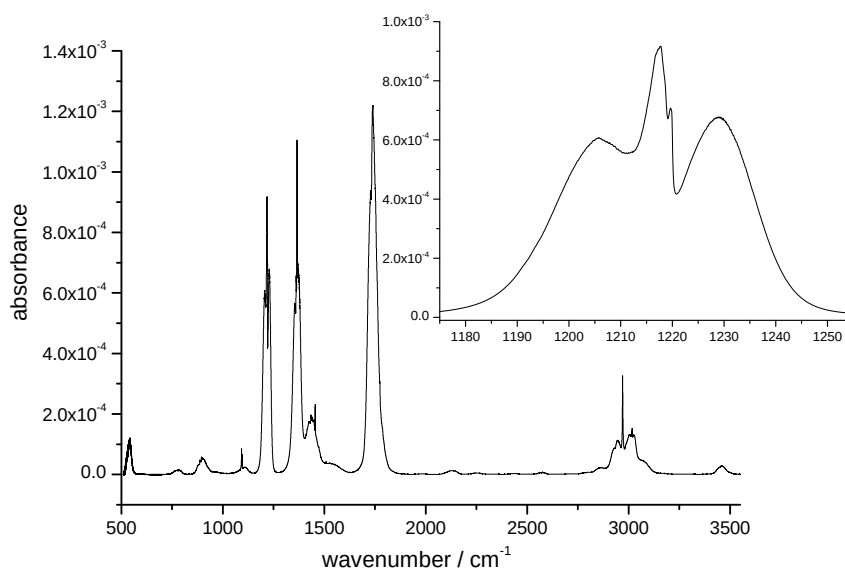


Figure 6.16: Acetone absorption spectra in the mid-infrared from the PNNL database [246]; inset, the ν_{17} band in further detail.

distinctive and thus easily identifiable. From the broadened acetone measurements in the PNNL database, it is clear that the regions located from $1218.6 - 1219.1 \text{ cm}^{-1}$ and $1219.7 - 1220.2 \text{ cm}^{-1}$ show a marked change in absorption cross-section over the length of a typical scan of the QCL ($\sim 0.5 \text{ cm}^{-1}$), as illustrated in Figure 6.17.

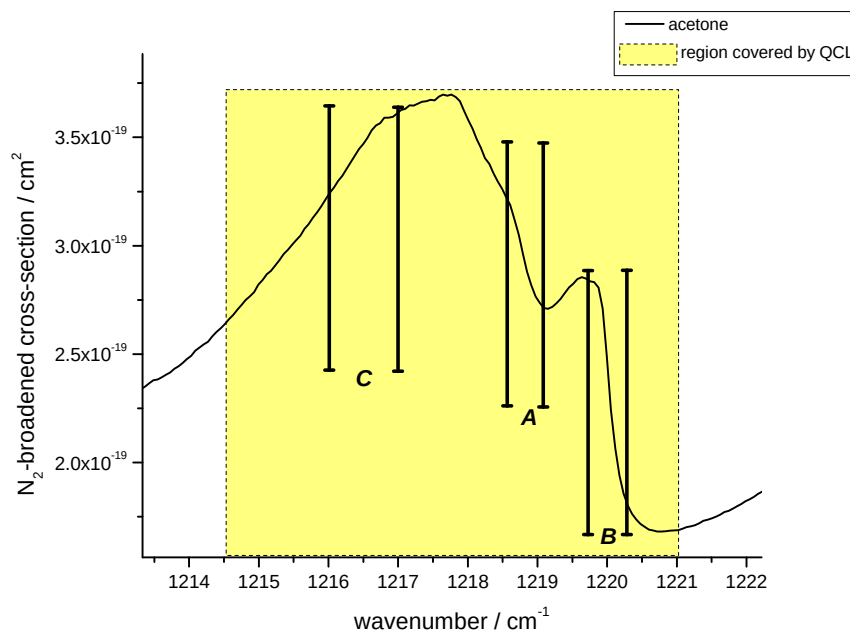


Figure 6.17: Spectral region of acetone absorption covered by the QCL. Highlighted potential regions of study: **A** = $1218.6 - 1219.1 \text{ cm}^{-1}$, **B** = $1219.7 - 1220.2 \text{ cm}^{-1}$ and **C** = $1216 - 1217 \text{ cm}^{-1}$.

Figure 6.18 illustrates the spectroscopy of typical breath constituents, methane, carbon dioxide and water vapour, in the spectral region covered by the QCL (using data taken from the HITRAN database [251]). The presence of a large water vapour transition at ~ 1218.5 cm^{-1} (in the ν_2 bending mode, corresponding to the transition $(6, 1, 5) \leftarrow (7, 4, 4)$, written in the form $(J', K'_a, K'_c) \leftarrow (J'', K''_a, K''_c)$ where J is the total angular momentum quantum number, whilst K_a and K_c represent the angular momentum quantum numbers along the a and c axes respectively) necessarily rules out using **A**, the first region described, despite the larger cross-sections found in that spectral region. The second region, **B** is flanked by two methane transitions, which could be utilised to provide an inherent absolute frequency scale and in addition, **B** shows a greater rate of change in absorption cross-section than **A**, providing greater selectivity. In addition, Figure 6.18 also highlights another potential region, $\sim 1216 - 1217$ cm^{-1} (**C** in Figure 6.17), which is relatively free of water absorption and has higher acetone absorption cross-sections than either **A** or **B**, although the region is less distinctive.

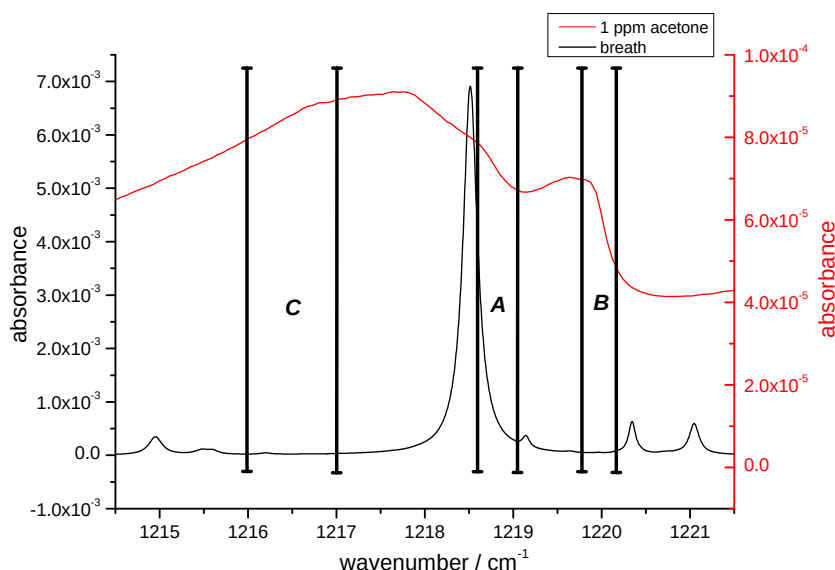


Figure 6.18: Simulation illustrating the absorbance seen in 10 cm of a sample of typical breath (5% H_2O , 4% CO_2 , 1.8 ppm CH_4 , < 91% O_2 and N_2), overlaid with the equivalent absorption seen with 1 ppm acetone (note the different scales) and with spectral regions **A**, **B** and **C** indicated.

Recent studies by Harrison *et al.* [316] have investigated the structure of the acetone absorption features in the MIR at various temperatures and pressures of air-broadened acetone samples using an FTIR of resolution 0.015 cm^{-1} , and normalised the results to the PNNL database. This relatively high resolution, coupled with low temperatures (197-198 K), has allowed ro-vibrational structure on the acetone absorption feature to be resolved, particularly in the $1367 - 1380$ cm^{-1} region. The group also identified distinctive detailed structure within the spectral range of the QCL in the 1219.95 cm^{-1} region (falling within

B of Figure 6.17) which is not explicitly highlighted in their paper, but can be observed in the raw data [317]. A selection of the Harrison measurements, at various temperatures and pressures, are illustrated in Figure 6.19. It clearly shows the aforementioned ro-vibrational structure at 1219.95 cm^{-1} , but also the evolution of the absorption feature with increasing temperature and pressure.

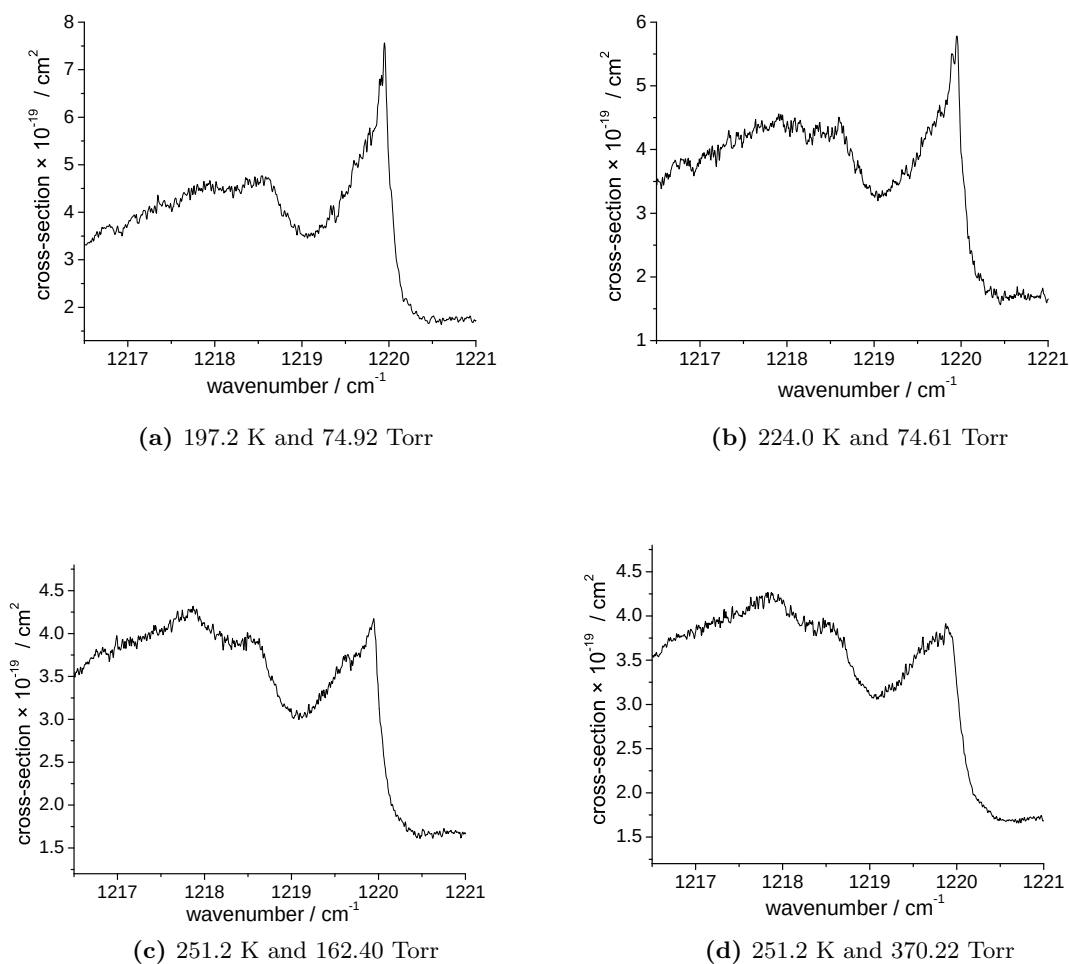


Figure 6.19: A selection of acetone spectra from the Harrison measurements [317]: Graph (a) shows a low temperature and low pressure spectrum, whilst (b) illustrates a spectrum at the same low pressure, but at a higher temperature. Similarly, the spectra in (c) and (d) are taken at the same higher temperature, with the spectrum depicted in (c) at a lower pressure than that depicted in (d).

Graphs (a) and (b) show lower pressure (~ 75 Torr) acetone spectra at 197.2 K and 224.0 K respectively. It is clear that the increase in temperature on going from (a) to (b) not only causes a broadening of the ro-vibrational structure at 1219.95 cm^{-1} , but also a significant decrease in the absorption cross-section. Graphs (c) and (d) show spectra at the same temperature (251.2 K), but at two different pressures: predictably, the higher pressure spectrum (d) shows greater broadening effects. In addition, it is also clear from all four

spectra that as the pressure, and especially the temperature, increase, the feature at $\sim 1218\text{ cm}^{-1}$ is at least as large as that at $\sim 1219.95\text{ cm}^{-1}$.

This would suggest that for low pressure measurements at least, **B** would be a desirable region to probe, given the ro-vibrational structure at 1219.95 cm^{-1} observed in the Harrison measurements. Thanks to the high resolution ($\sim 10^{-4}\text{ cm}^{-1}$) of the laser at our disposal, low pressure measurements on samples of pure acetone are not only possible to undertake, but they also represent an exciting prospect, as in addition to facilitating the determination of high resolution absorption cross-sections of acetone at room temperature, it may allow further ro-vibrational acetone structure to be probed. Consequently, this forms the starting point of this section, before the focus switches to detecting acetone at the levels found in human breath.

Experimental set-up

For the direct absorption measurements of acetone, a similar set-up to that described in section Section 6.2 was employed. A 10 cm cell containing several Torr of methane was positioned immediately after the QCL to allow an absolute frequency scale to be applied. From the previous studies with acetone in the near-infrared, it is known that acetone is difficult to handle, given its propensity to condense to surfaces, thus introducing uncertainties in accurate determination of the pressure of acetone samples. These uncertainties were reduced by connecting the sample directly to the vacuum line in which the samples were prepared. For low pressure measurements, a 10 Torr gauge (Leybold Piezovac) was used, whilst an atmospheric pressure gauge was used for the higher pressure measurements. The experimental set-up is illustrated in Figure 6.20.

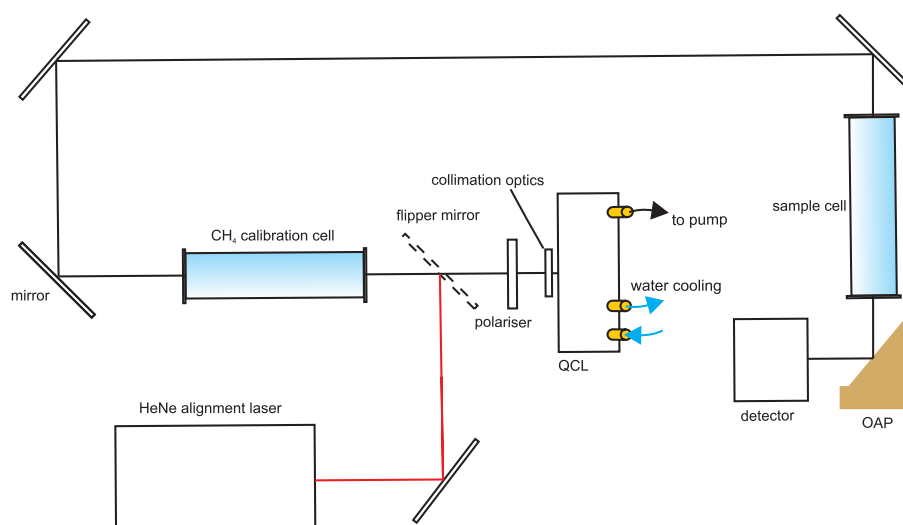


Figure 6.20: Experimental set-up for determining the absolute absorption cross-sections of acetone using direct absorption spectroscopy.

To confirm the suitability of the experimental and gas handling technique, experiments were initially carried out on determining the acetone cross-section at a fixed laser frequency,

$\sim 1217.99 \text{ cm}^{-1}$. This corresponds to the position of a methane doublet, thus allowing the lasing frequency to be confidently known. I_0 was determined from the empty cell measurement, before small, known quantities of pure acetone vapour (Fischer Chemical, HPLC grade) were leaked into the cell and the DC detector signal recorded at each pressure, to give a series of I measurements. As before, the acetone samples were prepared by freezing the liquid acetone and pumping on it for a few minutes to remove any impurities. From a plot of $\ln(I_0/I)$ against acetone pressure, shown in Figure 6.21, the cross-section at 1217.99 cm^{-1} was determined to be $\sigma = 3.62 \pm 0.1 \times 10^{-19} \text{ cm}^2$. This error does not include the uncertainty involved in the handling of substances like acetone, though it is anticipated that this is much lower than that quoted in the near-infrared measurements, thanks to the handling technique employed here. The uncertainties attributed to the data points were derived from the variability seen in repetitive scans and the error quoted for the cross-section from the 2σ uncertainty in the linear fit to these data points. Harrison, in measurements taken of 6.9 Torr of pure acetone at 295 K with an FTIR of resolution 0.00205 cm^{-1} [317], obtained a cross-section at this wavelength of $\sigma = 3.66 \times 10^{-19} \text{ cm}^2$, which is well within the error on our value.

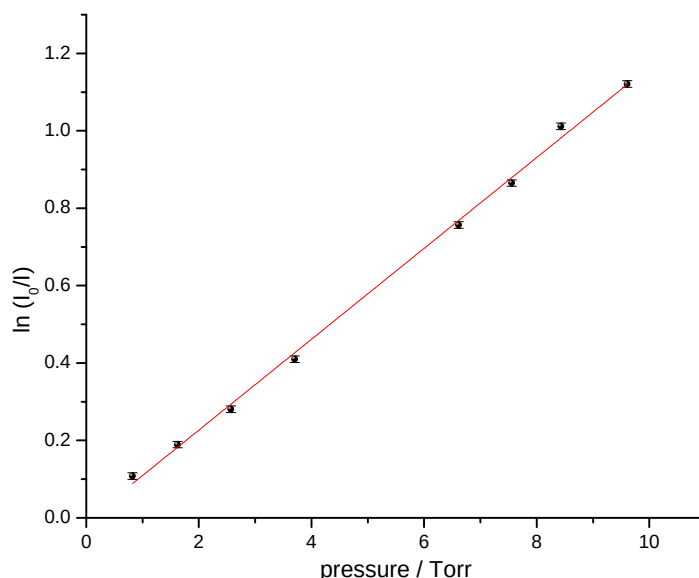


Figure 6.21: Acetone absorption at 1217.99 cm^{-1} as a function of pressure.

The laser current and temperature settings were selected so that the region $1219.7 - 1220.2 \text{ cm}^{-1}$ was scanned when the ramp was applied. The position of the methane $(11, F_2, 6) \leftarrow (12, F_1, 3)$ transition at $1220.1904 \text{ cm}^{-1}$ was used to convert the relative frequency scale derived from the Germanium etalon into an absolute one. Plotting the absorption cross-sections (determined from $\ln(I_0/I)$) against wavenumber yielded the spectra illustrated in

Figure 6.22a.

These initial measurements, taken at 2 Torr, 4 Torr, 6 Torr and 8 Torr, illustrate the presence of detailed structure, not visible in the broadened measurements from the PNNL database, or the room temperature pressure-broadened measurements of Harrison *et al* [316]. Low temperature studies from the same work highlighted the presence of rotational structure in acetone spectra under those conditions, but these results demonstrate that the structure can be resolved at room temperature with pure acetone samples. Most satisfying is the fact that further experiments conducted latterly by Harrison and co-workers at room temperature on low pressure (6.9 Torr) pure acetone samples produced cross-sections [317] that match very well with the results from this study, as illustrated in Figure 6.22b.

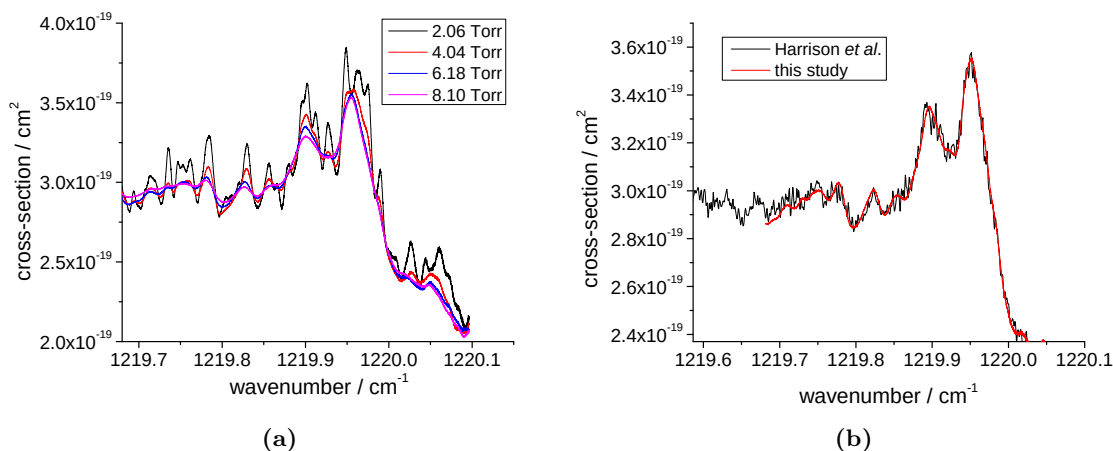


Figure 6.22: (a) shows the measured absorption cross-sections of acetone at low pressures, whilst (b) demonstrates the excellent agreement observed in the cross-sections measured in this study with those of Harrison [317] for ~6-7 Torr of acetone at 296 K.

The lowest pressure data from Figure 6.22a suggests that further ro-vibrational structure, in addition to the two features observed at ~ 1219.96 cm⁻¹ and ~ 1219.90 cm⁻¹, may be discernible at lower pressures. A series of low pressure measurements were recorded and the results are illustrated in Figure 6.23, clearly showing the presence of rotational structure. At these low pressures, self-broadening is reduced and in conjunction with the high resolution QCL, detailed rotational structure can be observed. With increasing pressure, the rotational structure starts to broaden out until the regime is reached where only the two familiar features at ~ 1219.95 cm⁻¹ are seen.

Following this, a series of measurements were undertaken at various different laser currents, so that a region spanning from 1218.6 - 1220.4 cm⁻¹ was probed. Each sub-region was selected in such a way that there was sufficient overlap with the adjacent sections and that a methane transition of known frequency was within each sub-region, so that a continuous

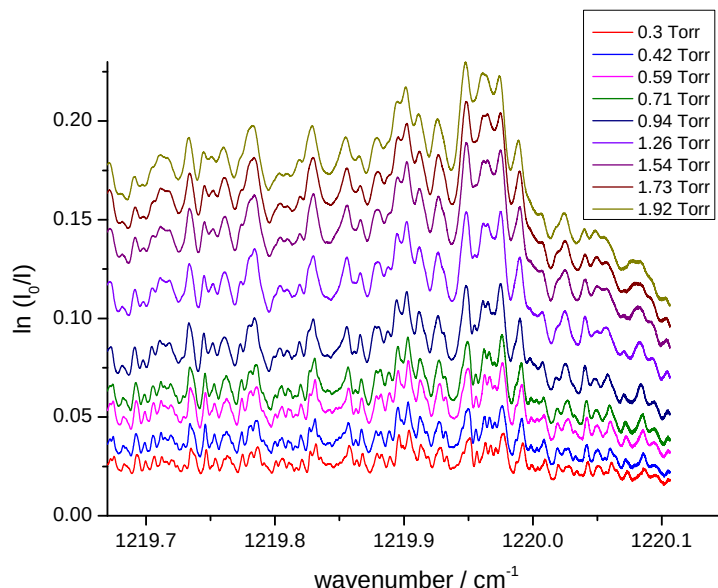


Figure 6.23: Direct absorption spectra of low pressures of pure acetone.

absolute frequency calibration could be applied and the separate scans stitched together to form the overall spectrum.

At every set laser current, an empty cell background was recorded for I_0 , before a pure acetone sample of ~ 6 Torr was added to the cell and the spectrum recorded. This process was then repeated 3 times before the laser current was changed for the next sub-region, where the same procedure was followed, and so on. This experiment was repeated several times with good reproducibility, and remarkable agreement is observed on comparison with the low pressure, room temperature measurements of Harrison *et al.* [317], as illustrated in Figure 6.24.

As acetone is a broadband absorber, there is no baseline from which to determine the level of noise in a spectrum when calculating a minimum detectable sensitivity value. Therefore, consecutive background measurements were taken (i.e. with no absorber present), and one was treated as the I_0 , and the other as I (in much the same way as described in Chapter 4). The resulting ‘absorption’ on applying the Beer-Lambert law represents the baseline, and the standard deviation of the linear fit to it provides the noise level for the system. This, in conjunction with the signal obtained from the acetone transition at 1219.95 cm^{-1} , results in a sensitivity of $\alpha_{\min} = 2.04 \times 10^{-4} \text{ cm}^{-1}$, which matches well with that achieved when studying methane. However, a second consideration is required when determining sensitivities for broadband absorbers: the intensity variation in the detected signal in the observed spectra. With narrowband absorbers, such as methane, it is not always necessary to record an I_0 spectrum before taking the sample spectrum itself, as there is normally a significant amount of baseline available either side of the transition from which to determine

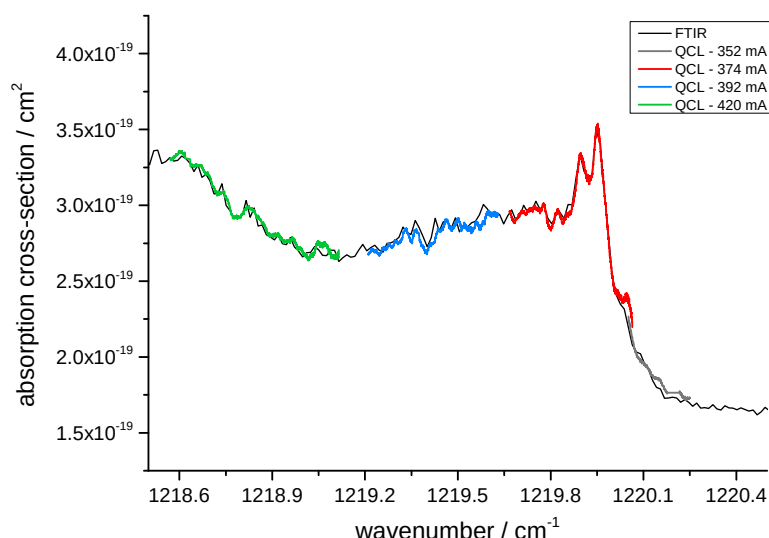


Figure 6.24: Extended acetone spectrum compiled from individual scans (gaps indicate removal of calibration methane transitions) compared to Harrison’s FTIR measurements [317] for ~ 6 -7 Torr of acetone at 296 K.

an I_0 level. With broadband absorbers there is no such baseline, as the entire scan is part of the acetone absorption and it is always necessary to record an I_0 measurement in addition to that of the sample. Any variation in the magnitude of the detected intensity during these measurements, independent of the absorption, represents a significant restriction on the level of sensitivity attainable. For example, if a linear fit is applied to the aforementioned ‘absorption’ of two background measurements and set to a slope of 0, an absorption level of -0.00204 is recorded. As there is no absorber present, this suggests that there is an uncertainty equivalent to $\pm 0.2\%$ absorption due to fluctuations in the experimental system. Therefore, although the sensitivities quoted can give an idea about the levels of acetone it is possible to detect, it is worth bearing in mind that the limiting factor as to whether those sensitivities can be achieved is the variability in the detected intensity over time.

It would be desirable to simulate a theoretical ro-vibrational spectrum for acetone to confirm that the structure observed experimentally is due to the rotational transitions of acetone. However, acetone is problematic to simulate due to the presence of internal rotation. A basic simulation was generated using ground state rotational constants found in the literature [318], but this failed to reproduce the ro-vibrational structure observed experimentally: studies by Groner *et al.* [318] have suggested that the internal rotation effects are significant and that these additional terms cannot be neglected. Previous simulations undertaken by Western *et al.* [319] have been able to simulate some molecules with internal rotors by adding a few terms to the effective Hamiltonian, but for acetone it is not straightforward to determine the required extra terms. Therefore, the PGOPHER simulation programme

[319] is unfortunately currently unable to simulate the rotational spectrum of acetone. To further investigate the spectroscopy of acetone at 8 μm it would be instructive to carry out further direct absorption measurements on low pressure samples of pure acetone over an extended spectral region so that the entire ro-vibrational spectrum for acetone in the region can be determined.

Acetone in breath is not going to display this detailed structure as it will be broadened by the other species present in breath. As water vapour in breath is a major problem to contend with, these early experiments will be conducted on acetone buffered with air, rather than breath, to determine the sensitivities of the techniques employed. To this end, glass bulbs of buffered samples were prepared on the vacuum line. First, a sample of pure acetone was allowed to fill the vacuum line, including the bulb. Then, the system was let up to an atmosphere of air and left overnight to mix. Samples were then analysed in exactly the same way as before in ~ 100 mbar (75 Torr) increments. Typically, samples of $\sim 1\%$ acetone were prepared and it is interesting to note that even with these buffered concentrations, some structure can still be seen at the lower pressures. Typical spectra, ranging from 100 mbar (75 Torr) to 600 mbar (450 Torr) in the region $1219.6 - 1220.2\text{ cm}^{-1}$, are shown in Figure 6.25. It is clear that as the total pressure increases, the cross-sections plateau at $\sim 2.9 \times 10^{-19}\text{ cm}^2$ in the range $1219.85 - 1219.95\text{ cm}^{-1}$ as the broadening reaches its maximum level under these conditions.

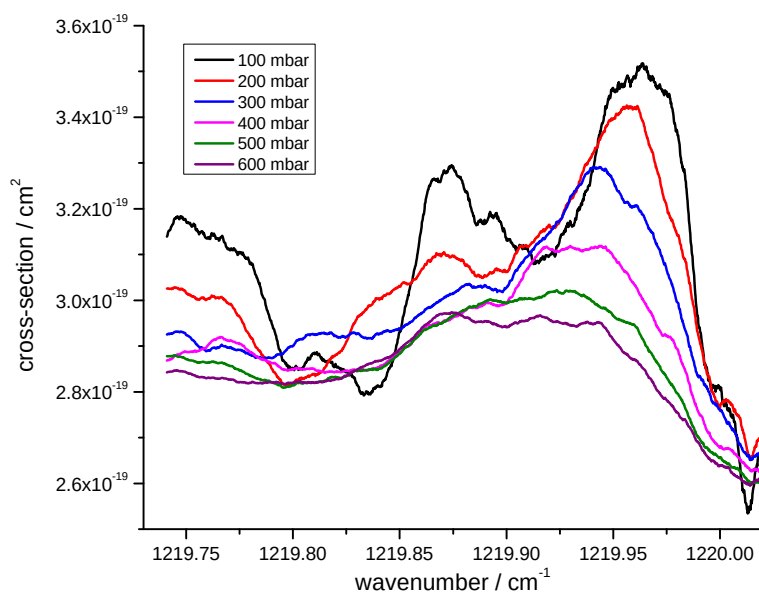


Figure 6.25: Absorption spectra of various pressures of 1.05% acetone buffered in air.

A sensitivity value for these broadened measurements can be determined in the same way as described in the previous section: although α_{peak} is necessarily smaller under these

conditions, a significant signal to noise ratio is still attainable, resulting in $\alpha_{\min} = 1.95 \times 10^{-4} \text{ cm}^{-1}$, which is marginally better than that determined for low pressures of pure acetone, and equivalent to ~ 26 ppm of acetone. This value is limited by the temporal variation in the detected signal intensity over the measurement of I_0 and I . This accounts for a variation in the zero baseline which is the absorption equivalent of $\pm 0.2 \%$, so that realistically the minimum detectable change in absorption is at the 1% level. With this taken into consideration, a minimum pathlength of approximately 15 m is required to detect a 1% change in absorption due to 1 ppm of acetone, though clearly sub-1 ppm detection is highly desirable and consequently, so are greater pathlengths. In order to achieve these levels of pathlength enhancement, multipass cells can be employed.

6.4 Extending the pathlength

White cell

As discussed in Chapter 3, the White cell [320] (Specac, Tornado; 2 m pathlength steps; 4.7 l volume) is a multipass cell, allowing a maximum pathlength of 20 m. It consists of three spherical, concave mirrors with the same radii of curvature, that are arranged in such a way to avoid optical interference on each pass. The radiation enters and leaves the instrument at the same end of the cell and the number of passes within the cell can be increased many times, limited only by the size of the mirrors (interference effects will occur if the beam spots overlap). The experimental set-up is identical to that depicted in Figure 6.20, with the White cell taking the place of the sample cell. In order to aid alignment, the HeNe laser was co-aligned with the QCL, and the beam paths directed around the edge of the laser table, with the laser collimation checked with the camera at various points. Following this, the HeNe was aligned into the White cell and focused onto the detector with the 2" off-axis paraboloid (OAP), before the lasers were switched via the aid of a flipper mirror and the alignment was optimised for the QCL.

In order to determine the pathlength enhancement due to the alignment of the White cell, a known concentration of methane was allowed to fill the cell and the absorption measured. The fitted absorption feature is pictured in Figure 6.26, and gives an integrated area which indicates a pathlength of 18 m. The value represents a pathlength slightly less than the maximum achievable with the White cell: this is attributed to the necessity to slightly alter the alignment into the White cell to reduce the effects of a 2 m pathlength etalon present within the instrument itself. From the signal to noise ratio of this absorption, a sensitivity of $\alpha_{\min} = 1.39 \times 10^{-6} \text{ cm}^{-1}$ is determined.

The design of the White cell is not ideally suited to frequent gas handling: with inlets positioned at the top of the vertical cell, the mirrors within it are susceptible to movement

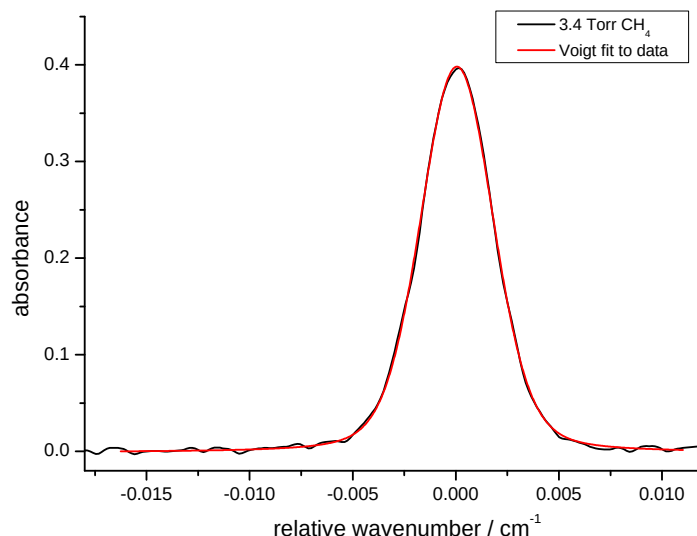


Figure 6.26: Voigt fit to the methane $(11, F_2, 16) \leftarrow (12, F_1, 8)$ hot band $(2 \leftarrow 1)$ transition at $1218.7398 \text{ cm}^{-1}$.

on addition or removal of gaseous samples, and as a result there can be some variability in measurements of identical samples. Repeated measurements with nitrogen demonstrated that careful manipulation of the gas inlet taps could significantly reduce the variability seen due to the release of gas into the system ($<1\%$ in successive identical samples), and that it was the evacuation of the cell that was the main culprit responsible for the change in alignment. For this reason, when measurements were taken on acetone, an initial empty cell was used as the background measurement (I_0), before consecutive samples of acetone were leaked into the cell. Given the increased pathlength available with the White cell, a calibrated 25 ppm acetone in air mix (BOC Special Gases) was used and samples ranging from 100 mbar to 900 mbar were analysed. However, it was noted that the White cell etalon, which had been reduced by altering the entrance alignment, periodically came back into prominence when filling the cell and pumping down on it. As a result, data taken still exhibit etalon structure to a greater or lesser degree, so it was also necessary to apply a Fourier Transform filter on the spectra to remove the effect analytically, the results from which are illustrated in Figure 6.27.

From this, the absorption levels at 1219.925 cm^{-1} for each data set were determined and plotted against total pressure of the acetone mix, resulting in Figure 6.28. The error bars are derived from the variation observed in the absorption of samples of the same concentration.

The gradient of the plot in Figure 6.28, $(\sigma L / kT)$, indicates a cross-section of acetone at this wavelength of $\sigma_{\text{peak}} = 2.78 \pm 0.03 \times 10^{-19} \text{ cm}^2$, which agrees well with that quoted in

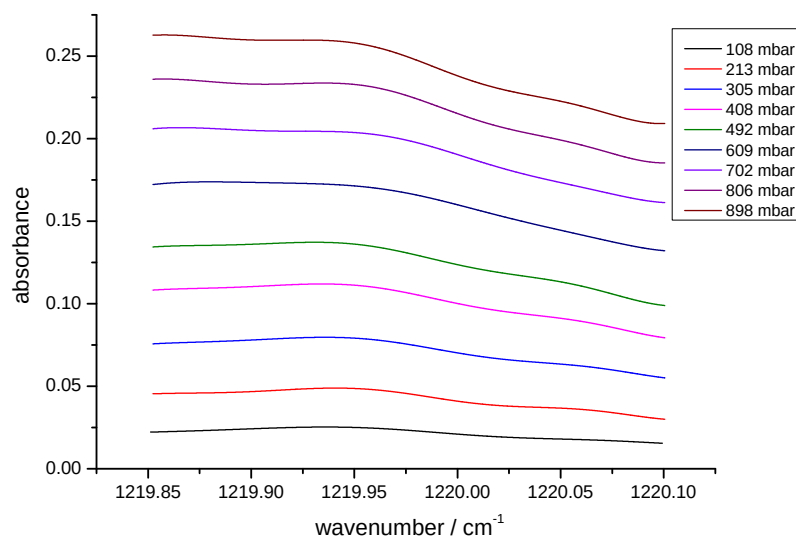


Figure 6.27: White cell absorption spectra of various pressures of a 25 pm acetone in air mix (FT low pass filter at 97 kHz applied).

PNNL [246] under the same conditions ($\sigma_{\text{peak}} = 2.73 \times 10^{-19} \text{ cm}^2$) and the $\sigma_{\text{peak}} = 2.79 \times 10^{-19} \text{ cm}^2$ determined by Harrison at 700 Torr [316].

Once again, the minimum absorption level is determined as described previously, and was found to be $\alpha_{\text{min}} = 2.3 \times 10^{-6} \text{ cm}^{-1}$, or approximately 350 ppb in terms of acetone levels. This is about the level of sensitivity required for detecting acetone in breath, though clearly a further increase in sensitivity is desirable, as this represents an absorption level equivalent to a signal to noise ratio of unity and the signal intensity variability amounts to an equivalent of 0.14 %. In addition, there is a rather large caveat to these conclusions: firstly, it is noted that this level of sensitivity is significantly worse than that obtained with methane. This is attributed to the fact that in order to determine a noise level, two measurements are necessary and this in turn requires a degree of gas handling (i.e. varying the pressure of nitrogen within the cell). This will increase the noise level in the signal. Secondly, of far greater consequence, is the effect of pumping down the cell. The sensitivity value and variability in detected intensity quoted have been determined without pumping the cell down in between measurements, but rather N_2 has been released into the cell (assuming that as a non-absorber it should always represent a zero level absorption, irrespective of the pressure it is at within the cell). If the White cell is pumped down in between zero absorber measurements, the variation in the signal intensity reaches an absorption equivalent of 5%. Clearly the White cell is impractical for this application.

In contrast, the Herriott cell not only has a more robust design (at least in terms of the fact that because it is home-built we have more control over its specifications), but it also

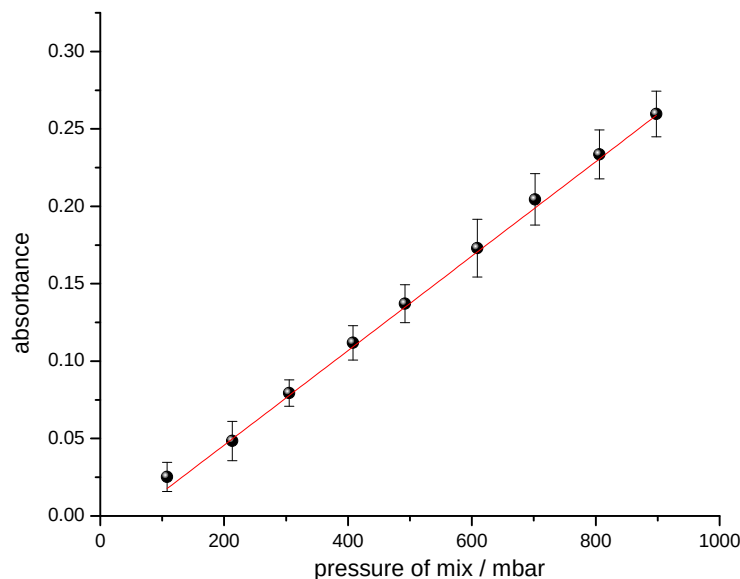


Figure 6.28: Absorbance at 1219.925 cm^{-1} due to a 25 μm acetone in air mix in the White cell, plotted as a function of total pressure.

promises a pathlength enhancement at least double that attainable with this White cell, providing a means to comfortably achieve the sensitivity levels required to detect acetone in breath. The following work was undertaken with both Dr Luca Ciaffoni and Simon Wood, a Part II student.

Herriott cell

A Herriott cell [135] is a type of multipass cell in which an off-axis ray is injected into the interferometer, which consists of two concave spherical mirrors. The series of reflections of the ray produces an elliptical pattern of spots on each mirror, the shape of which is dependent on the radius of curvature of the mirrors (R), the distance between the two mirrors, the position of the injected beam and the injected beam angle. The mirrors purchased for the construction of the Herriott cell had radii of curvature of 1.4 m, and the injection hole drilled into the near mirror was already pre-defined at (21 mm, -38.62 mm), measured from an origin at the centre of the mirror in Cartesian co-ordinates. Therefore, the variables to alter in order to maximise the number of passes whilst avoiding spatial and optical interference were the distance between the two mirrors and the angle at which the beam is injected into the cell.

In order for the input beam to return exactly to the same point on exiting the instrument after it has completed N passes within the cell, the re-entrant condition must be fulfilled:

$$N|(\theta)| = 2M\pi \quad (6.7)$$

θ represents the angle between successive reflections, as illustrated in Figure 6.29; therefore the angle between two successive spots on the same mirror is given by 2θ . θ can be positive or negative, depending on the direction in which the spots rotate around the mirror (positive θ for counterclockwise, negative for clockwise when looking along the z axis towards the far mirror). M represents the number of orbits the beam will make of the mirror before exiting the cell. In the original paper by Herriott *et al.* [135], M effectively was one; in other words, the spots are in consecutive order as one moves around the mirror. Given the number of spots on a mirror is the same for a given pair of mirrors, a greater number of orbits will result in a greater distance being traversed by the beam, and hence a longer pathlength.

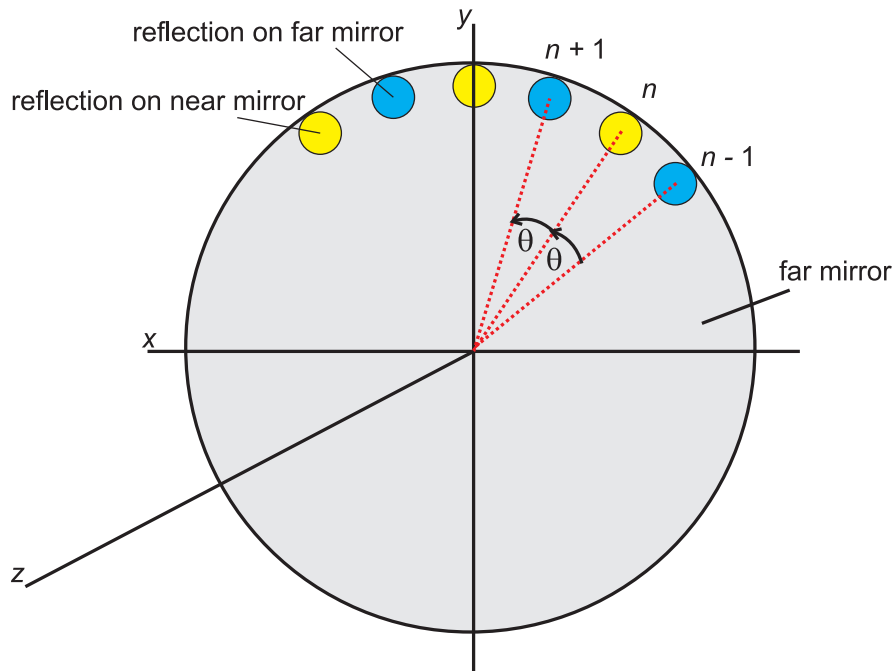


Figure 6.29: Looking at the far mirror of the Herriott cell along the z axis; the blue dots are the reflections observed on the far mirror whilst the yellow dots are a representation of the reflections that are found on the near mirror. The angle between successive reflections is given by θ , whilst the angle between successive reflections on the same mirror is given by 2θ .

Herriott *et al.* [135] showed that θ is linked to the radius of curvature of the mirrors, R , and the distance between them, d , via:

$$\cos(\theta) = 1 - \frac{d}{R} \quad (6.8)$$

For a given N and R , equations 6.7 and 6.8 can be solved simultaneously to give several solutions, which slightly differ in the pattern of spots produced on the mirrors. Tarsitano and Webster [321] introduced the variables k and p to describe these different patterns, where $k = \pm 2 \pm 4 \pm 6$ and p is a positive integer. p represents the number of spots on a mirror per orbit, such that the angular distance between two successive spots on the same mirror is given by $2\pi/p \approx 2\theta$. The magnitude of k dictates the number of spots between the n^{th} and $(n + 2p)^{\text{th}}$ spots via $|k|/2 - 1$, meaning for $k \pm 2$ the spots are adjacent to each other. The condition $k = \pm 2$ also means that the value of p for that solution signifies the number of intensity bands in the mirror pattern. The sign of k determines whether a full 2π orbit of the mirror is made between two successive spots on the same mirror (n and $n + 2p$), with a positive sign indicating an angle of $< 2\pi$ and a negative sign $> 2\pi$; consequently, the sign of k also determines the intensity distribution of the spot pattern. These definitions are illustrated in Figure 6.30.

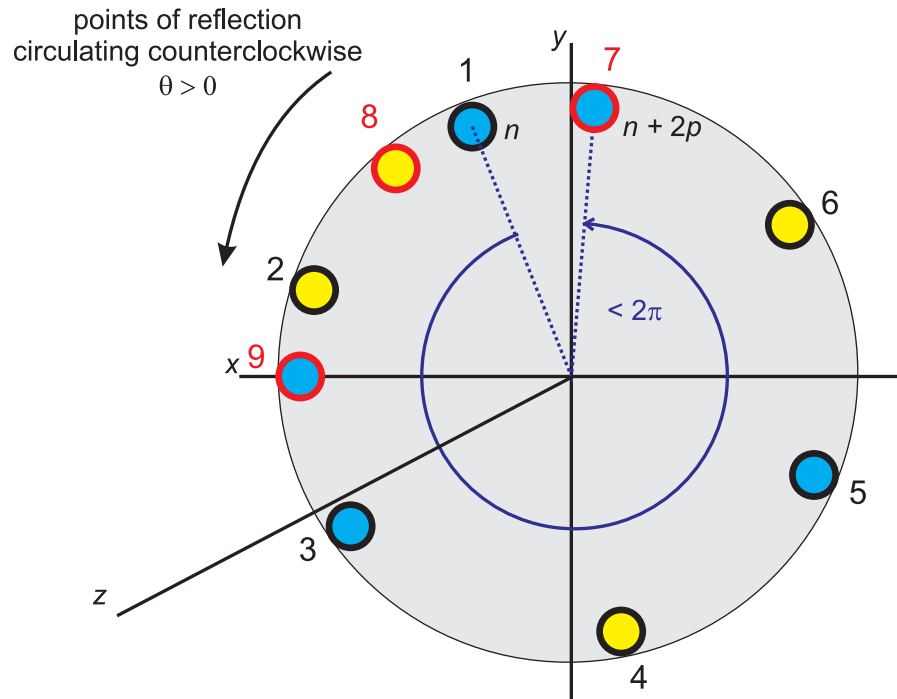


Figure 6.30: Looking at the far mirror along the z axis, the blue dots are reflections on the far mirror and the yellow dots are a representation of the reflections on the near mirror, with the numbers indicating the pass number. This pattern (which is incomplete for clarity) can be described by $\{20, 3, +2, 3\}$, with $N = 20$, $p = 3$, $k = +2$, $M = 3$. The black outlined dots refer to reflections on the first orbit, whilst the red outlined dots refer to the second (incomplete) orbit. The reflections sweep out in a counterclockwise manner, so θ is positive and the angle between the n^{th} and $(n + 2p)^{\text{th}}$ dots is less than 2π : k is positive.

The total number of passes in the Herriott cell is then given by [321]:

$$N = 2pM + k \quad (6.9)$$

The patterns are then grouped together according to the number of passes, N , which can then be further grouped according to their p for a given radius of curvature. Each solution is then written in the form $\{N, M, k, p\}$. Combining equations 6.7, 6.8 and 6.9 gives:

$$\left[1 - \cos\left(\frac{\pi}{p}\left(1 - \frac{k}{N}\right)\right)\right]R = d \quad (6.10)$$

Thus with a given radius of curvature and defining the number of passes desired, there are a range of solutions which will satisfy the equation and allow the distance required between the two mirrors to be determined. In order to decide the optimum values for p and k , it is necessary to consider the effects of interference fringes: spatial overlap between the exit point and neighbouring spots will result in some light exiting the cell before it has made its full complement of passes, resulting in the appearance of fringes in the Herriott cell output. These effects, essentially etalons, can be removed later, but it is desirable to reduce the effects as far as possible, and to select p and k in such a way that the fringes produced can be removed as easily as possible. A factor influencing the degree of spatial overlap is the size of the beam: on entering the cell it will be diverging, but on encountering a concave mirror it will be refocused, before starting to diverge again until it hits the second mirror and is refocused again. Consequently, the size of the beam will vary throughout its passage through the cell. Thus, p and k values which result in spots either side of the exit point which have traversed significantly different numbers of passes through the cell will have quite different intensities and spot sizes and as a result, produce two quite different interference patterns; therefore, it is desirable to have spots of similar pass number so that the resulting interference pattern is more easily removed. It is also necessary to ensure that the interference patterns do not have a free spectral range comparable to the linewidth of the absorption feature of interest (this is not really an issue for acetone, but needs to be considered with methane), as otherwise their removal will also subtract from the absorption spectrum.

These factors were optimised using a LabView programme (written by Dr Luca Ciaffoni), a screenshot of which is depicted in Figure 6.31, and it was found that for mirrors with a radius of curvature of 1.4 m and for 70 passes, $k = +4$ and $p = 3$. Thus the beam makes 11 orbits of the mirrors before exiting the cell, and there is one point of reflection in between the n^{th} and $(n + 2p)^{th}$ spots. From these values, a mirror separation of 628.74 mm was determined to be required, which should yield a theoretical pathlength of 44 m. It is desirable for a spherical spot pattern to be produced, as this will maximise the distance between the spots, reducing any spatial overlap. However, one of the mirrors arrived with a chip towards the edge of the mirror, which dictated that an elliptical geometry must be employed. This was simply achieved by altering the angle at which the beam entered the Herriott cell, and optimising it so that the reflection point patterns fill as much of the 10

cm diameter mirrors as possible. Figure 6.32 illustrates the simulated spot and intensity pattern for the parameters used to align the Herriott cell with a photograph of the spots observed on the Herriott cell near mirror with the HeNe laser.

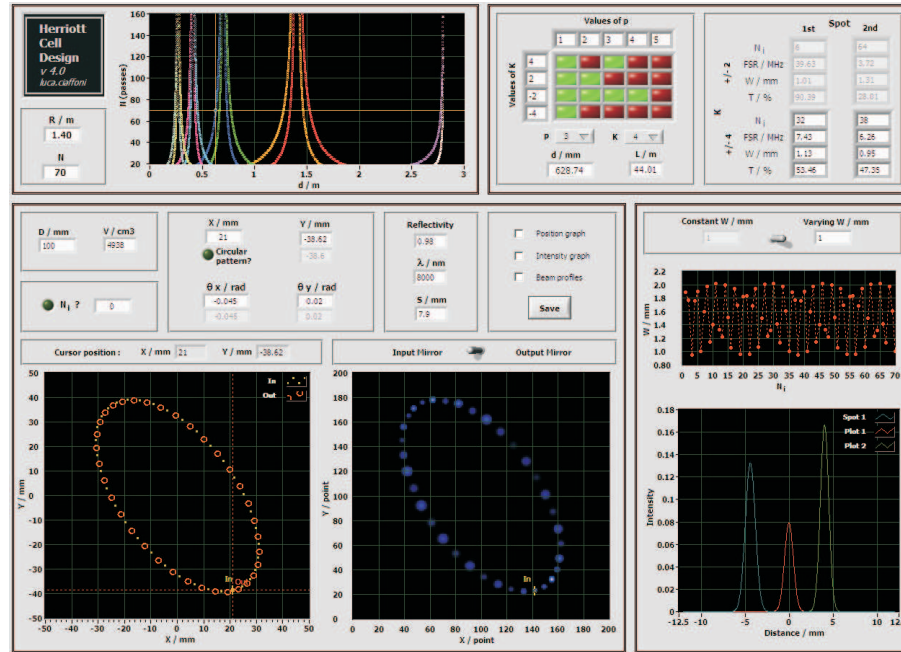


Figure 6.31: A screen-shot of the LabView programme used to optimise the conditions for the Herriott cell alignment. The top left panel illustrates the potential solutions for 70 passes and a pair of mirrors with radii of curvature of 1.4 m, whilst the top right panel highlights the allowed p and k values for those solutions. The right hand panel simulates the beam size variation as it passes between the two mirrors and the resultant spatial interference between the spots either side of the exit hole for the particular solution selected. Finally, the bottom panel illustrates the spot and intensity pattern expected for the chosen solution.

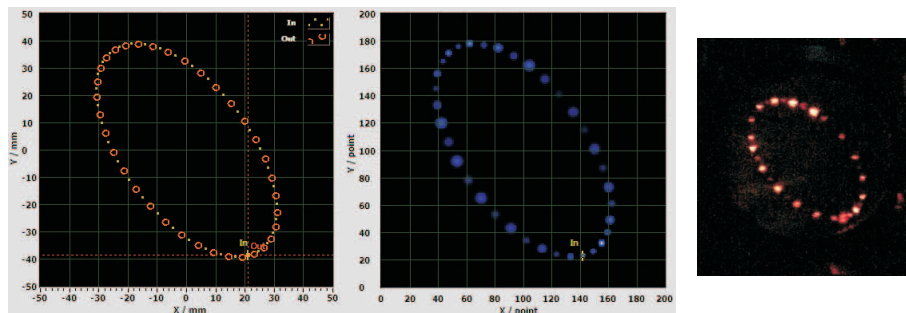


Figure 6.32: **Left:** the simulated spot pattern for the Herriott cell parameters; **centre:** the corresponding spot intensity pattern; **right:** a photograph of the HeNe spots on the near mirror of the Herriott cell.

The Herriott cell was constructed in a vacuum vessel with an approximate volume of $90.9 \times 24.9 \times 18 \text{ cm}^3$, from mirrors mounted on translational stages, with additional horizontal and vertical degrees of freedom on the mirrors themselves. It was initially aligned using the HeNe laser before the co-aligned QCL was injected into the device via a flipper mirror. The experimental set-up was identical to that used previously, with the exception that the

Herriott cell took the position of the White cell. Using a MIR camera (WinCamD-FIR2-16, DataRay), the beam was imaged both before entering the Herriott cell and on exiting, the images of which can be seen in Figure 6.33. Although the FWHM of the beam has increased from ~ 1 mm to ~ 2 mm on traversing the 44 m, reassuringly the beam shape is still nearly Gaussian and the size is still small enough to be comfortably focused into the detector (which has a 1×1 mm active area).

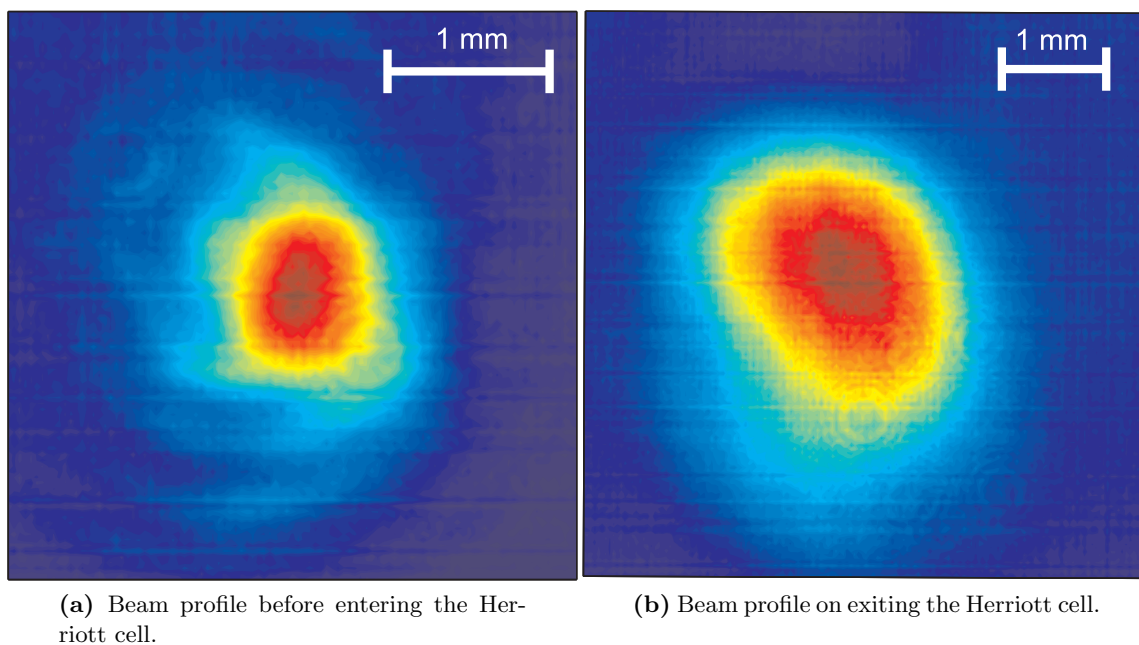


Figure 6.33: (a) is an image of the beam profile as it enters the Herriott cell; a Gaussian fit to the profile suggests a beam waist of ~ 1 mm. Similarly, (b) shows the beam as it exits the Herriott cell; the Gaussian profile fitted to it suggests a beam waist of ~ 2 mm.

To determine the pathlength of the Herriott cell, a calibrated mix of 100 ppm of methane in air was utilised; 152 mbar of the mix was released into the cell and the absorption $(11, A_2, 1) \leftarrow (12, A_1, 2)$ at $1219.6487 \text{ cm}^{-1}$ recorded, as depicted in Figure 6.34a. From the area determined from the Voigt profile fitted to the feature, the pathlength was calculated to be 44 m, thus agreeing with that expected theoretically. In addition, CH_4 was clearly detected in a 132 mbar sample of laboratory air, as illustrated in Figure 6.34b for the transition $(12, A_1, 3) \leftarrow (13, A_2, 1)$ at 1216.199 cm^{-1} . Assuming a pathlength of 44 m, the area of the absorption suggests a CH_4 concentration of ~ 2 ppm in the sample, which matches what would be expected for the typical methane levels in the atmosphere. This latter measurement demonstrates the sensitivity of the instrument, which can be further quantified to $\alpha_{\min} \sim 1.2 \times 10^{-7} \text{ cm}^{-1}$, which signifies a 19 fold increase in sensitivity in comparison to the White cell, and represents a minimum detectable acetone level of ~ 20 ppb. To determine the stability of the cell, it was repeatedly filled with a sample of nitrogen

before being pumped down: the baseline signal was found to show $\sim 0.9\%$ variation over several measurements, a vast improvement on the White cell.

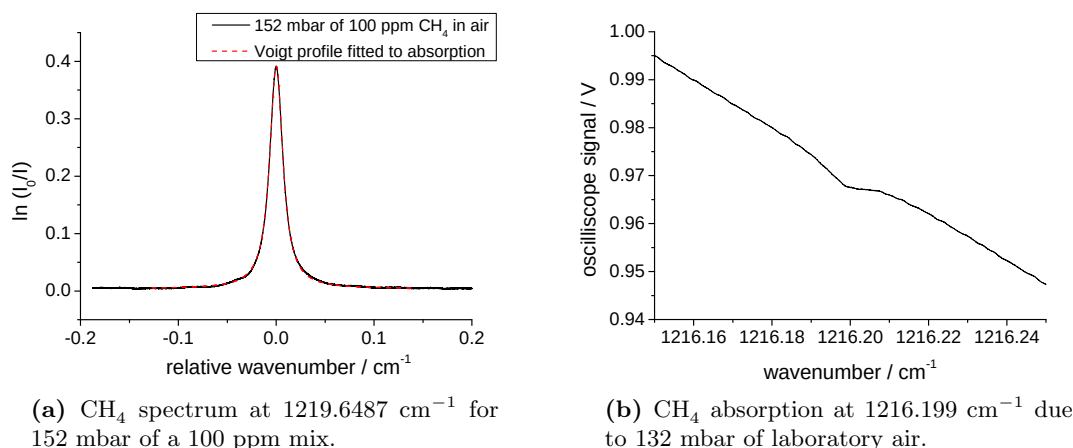


Figure 6.34: Graph (a) depicts the absorption spectrum obtained for 152 mbar of a 100 ppm CH_4 in air mix at 1219.6487 cm^{-1} ; the area of the fitted Voigt profile suggests a Herriott cell pathlength of 44 m. Graph (b) shows the absorption seen at 1216.199 cm^{-1} due to methane in the atmosphere for a pressure of 132 mbar.

Following this, the Herriott cell was used for the detection of acetone. An I_0 measurement was obtained for the cell transmission when evacuated, before samples of a 5 ppm acetone in air mix were allowed to fill the cell and the subsequent reduction in intensity recorded. The experiment was repeated several times and Figure 6.35 illustrates some sample spectra obtained. The 0.02 level of absorption observed at 1219.925 cm^{-1} for 150 Torr of 5 ppm of acetone, in conjunction with the previously calculated minimum detectable absorption, suggests that breath levels of acetone are within the sensitivity range of the Herriott cell.

Assuming a pathlength of 44 m, a simulation of the absorption due to breath can be determined, and the equivalent absorption due to 1 ppm of acetone overlaid. This is illustrated in Figure 6.36 and it demonstrates the problem caused by water vapour in breath. Clearly there are two regions where the acetone absorption at 1 ppm is greater than that observed due to the $\sim 5\%$ of water vapour in breath, the first found at the region studied previously (**B**), and the second from approximately 1216 to 1217 cm^{-1} (**C**), where a minimum absorption of 2% and 1% are attained respectively due to water. As discussed previously, this second region is the more desirable when considering the influence of water, as the absorption due to acetone is slightly higher than that found at $\sim 1219.9\text{ cm}^{-1}$, whilst that due to water is slightly lower.

However, as mentioned in Chapter 5, there is an additional absorption component in water, the water continuum. Although there is some debate over the physical mechanism behind it (the relative importance of far-wing absorption over dimer absorption), it is clearly present

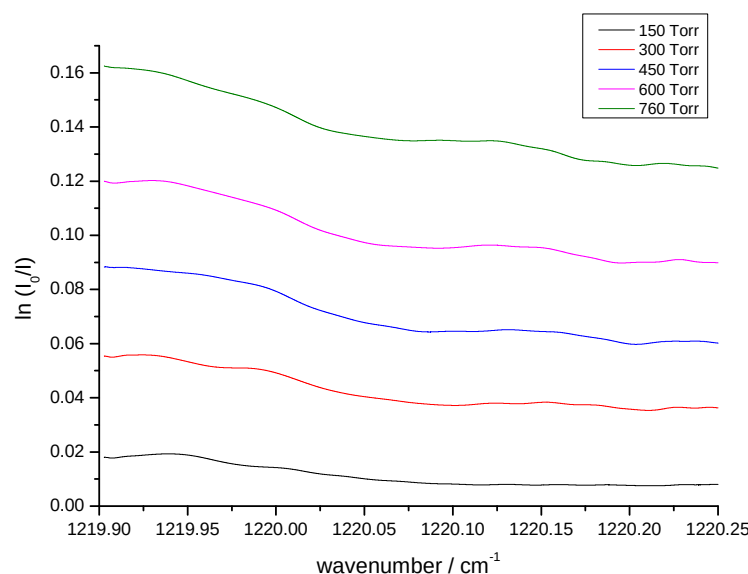


Figure 6.35: Sample spectra of various pressures of a 5 ppm acetone in air mixture. The level of absorption seen for 150 Torr of 5 ppm of acetone, 0.02 at 1219.925 cm^{-1} , suggests that the instrument has the required sensitivity to detect breath levels of acetone.

in spectra [267, 267, 287] and consequently must be taken into account. Using a cross-section of $\sim 7.53 \times 10^{-24}\text{ cm}^2\text{molec}^{-1}$ for the total (self- and foreign-) water continuum cross-section in the region $1215 - 1221\text{ cm}^{-1}$, determined from measurements in Paynter *et al.* [267], 5 % water vapour will give an absorption of ~ 0.041 . This smoother, broader absorption is in addition to the narrow absorption features seen as a result of the rovibrational transitions within the molecule. It follows that the combination of these two effects leads to a total absorption of $\sim 6\%$ and $\sim 5\%$ due to water vapour at 1219.87 cm^{-1} and 1216.5 cm^{-1} respectively. Therefore it is absolutely necessary for the water vapour from breath samples to be significantly reduced if one is to be able to detect breath acetone.

Whilst a device similar to that described in Chapter 5 could be used to remove the water vapour in breath, the sheer volume of the Herriott cell poses logistical problems as it would require several breaths to fill it to atmospheric pressure. Not only is this impractical for sampling, it also causes the very real problem of blocking the freezing device, given the quantities of water vapour that will pass through it. Although the Herriott cell has demonstrated that a great pathlength enhancement is not required for sub-1 ppm acetone detection, the decrease in volume provided by an optical cavity makes it far more practical for real breath analysis. As with the Herriott cell studies, the following investigation was also carried out with Dr Luca Ciaffoni and Simon Wood.

Optical cavity

A 30 cm optical cavity was constructed from two highly reflective mirrors (Lohnstar, radii

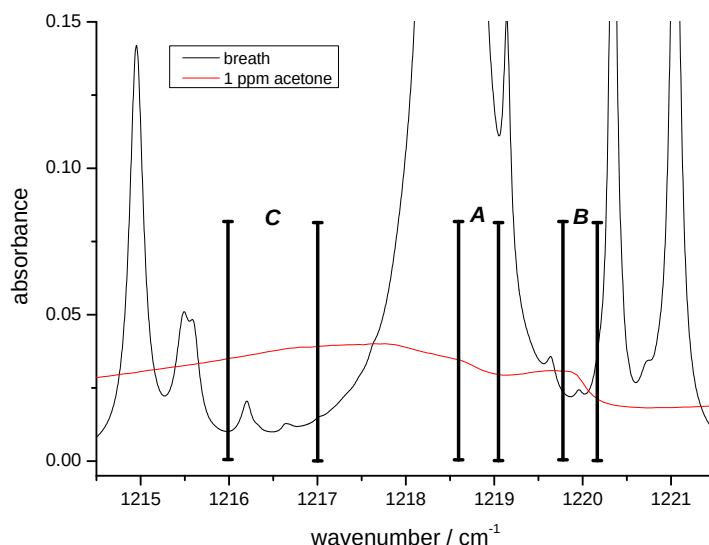


Figure 6.36: The absorbance due to an atmospheric sample of breath for a 44 m path-length, overlaid with the absorption due to 1 ppm of acetone under the same conditions; the spectral regions identified earlier are labelled accordingly.

of curvature 1 m and 5 m, $R \sim 99.9\%$), fixed within a vacuum cell box. The experimental set-up was identical to previous configurations, with the optical cavity taking the place of the multipass cell. As is frequently found with optical cavities in the MIR, feedback into the laser is an issue and it makes removing residual mode structure for off-axis CEAS very difficult, although precise alignment methods for dealing with it have been documented [29, 304]. However, it was found that scanning the laser rapidly (~ 8 kHz) and dithering the cavity mirrors helped to reduce the residual mode structure and, coupled with a filter (in-house built, 10 Hz low pass filter) to remove what remained, was sufficient for this study, where the species of interest exhibits a broad absorption profile which manifests itself as an absorption ‘offset’ over the spectral range covered by the laser.

The instrument was initially tested using samples from a 5 ppm acetone in air mix at region **C** for the detection of breath acetone, $1216.50 \pm 0.06 \text{ cm}^{-1}$. The laser was set to scan across a region of $\sim 0.1 \text{ cm}^{-1}$, within which the absorption cross-section of acetone does not vary greatly, allowing an average point to be taken as the absorption level. As before, increasing pressures of the mix were released into the cell and the absorption level recorded and compared to the level observed with the empty cavity. An example plot of sample pressure against absorption is illustrated in Figure 6.37, where the error bars are derived from the standard deviation (1σ) of repeated measurements. Assuming an absorption cross-section of $3.45 \times 10^{-19} \text{ cm}^2$ at 1216.50 cm^{-1} , a mirror reflectivity of 99.92 % is derived. In addition, the cavity was subjected to a series of measurements in which it was pumped down and refilled with nitrogen to determine the reproducibility of the signal.

The variability of the signal was found to be $<1\%$, falling within the drift of the laser and confirming that the system is reasonably stable.

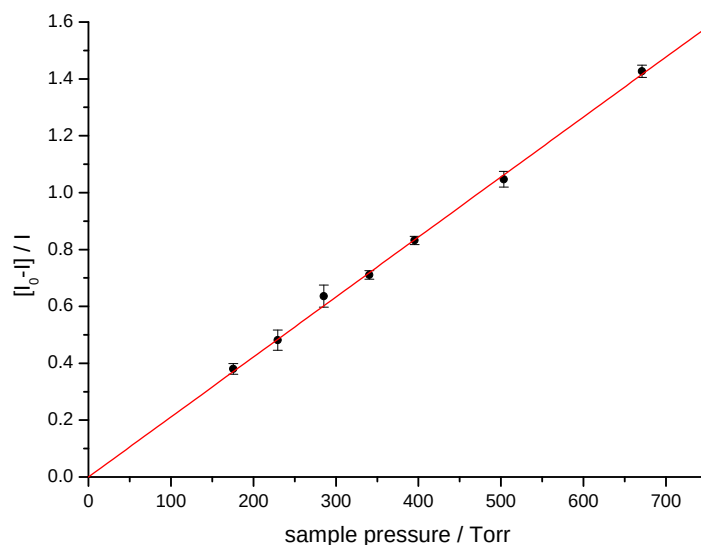


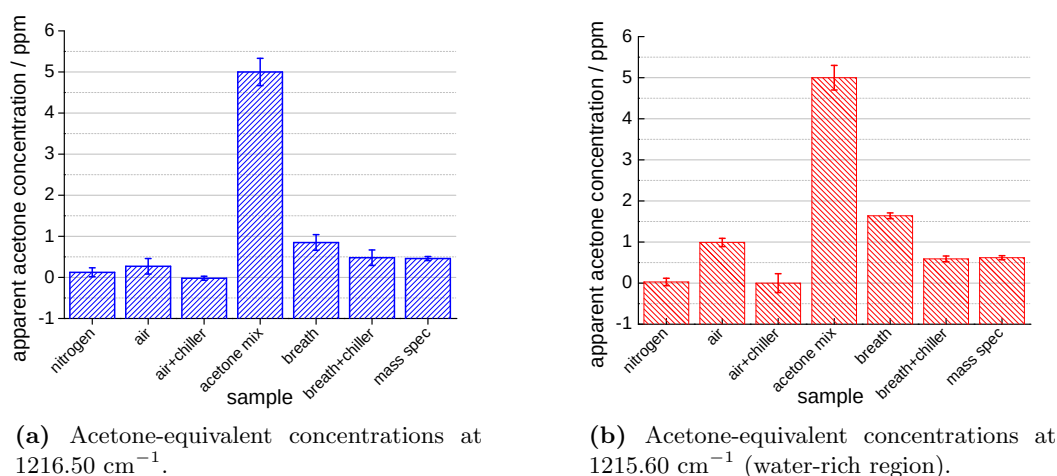
Figure 6.37: The absorbance observed due to various pressures of 5 ppm of acetone in air, at 1216.50 cm^{-1} .

A molecular sieve device, which removes both water and acetone from gaseous samples, was constructed and laboratory air samples fed through it. The absorption levels observed were then compared to those derived from samples of N_2 . Negligible difference ($<1\%$, within the drift of the laser) was found between the two, confirming that absorption due to methane (which can pass through the molecular sieve) is unlikely to pose a problem in this spectral region.

As previously, the cavity output of the empty cell was used as the I_0 measurement before a 200 Torr sample of N_2 was fed into the vessel, and the cavity output recorded over a 0.2 s acquisition time. Following this, samples of laboratory air, 5 ppm of acetone in air, and breath were also released sequentially into the cavity and the resultant absorption noted. The level of absorption of the 5 ppm acetone in air mix provided a calibration reference for the rest of the samples so that the levels of absorption could be described in terms of acetone concentration. A water freezing device, very similar to the one used in Chapter 5 but based on a commercial peltier system (DA-24-02-00-00, Laird Technologies), was loaned from OMD and set to $-8\text{ }^\circ\text{C}$. A laboratory air sample was then passed through the device and the resultant absorption recorded. Similarly, a sample of breath (from the same breath bag used in the previous measurement) was fed through the device and the absorption level also measured. It is immediately obvious on comparing the breath and laboratory air samples that have passed through the device and those that haven't from Figure 6.38 that the water freezing unit is removing water vapour from the samples. Previous work in Chapter 5 has

demonstrated that the levels of acetone are more-or-less unaffected on passing through the chiller at these temperatures, so the reduction in absorption can be attributed to the removal of water. Therefore, the absorbance that is recorded from the breath sample that has passed through the water freezing device is very likely to be solely due to the acetone present in that breath sample, and was measured at 480 ppb. The residual breath in the bag from which the breath samples were taken was then cross-checked against the reading from the mass spectrometer used in Chapter 5 and reassuringly found to contain acetone levels (460 ppb) which were well within the uncertainty of the measurement carried out using the optical device, as can be seen in Figure 6.38. The error bars depicted on the plot are derived from the standard deviation (1σ) of repeat measurements taken of each condition (in the case of breath, the samples are taken from the same breath bag), and suggest a precision of 170 ppb in the sub-second response time. Similar measurements at 1215.60 cm^{-1} where the interference from H_2O absorption is greater also return consistent values of the acetone concentration after the breath has been pre-treated in the water-removal device before being released into the cell, with 590 ppb recorded by the optical device and 620 ppb by the mass spectrometer, as depicted in Figure 6.38b. The reduction in absorption due to water being removed from the sample is approximately the same in both the laboratory air and breath samples: this is believed to be due to the fact that the breath sample within the bag is at a lower temperature than when it first leaves the mouth, this drop in temperature lowers the saturated water vapour pressure in the breath sample; in addition, the humidity of the laboratory air was very high on the day of the measurements which leads to a situation where the reduction in water vapour in the breath sample is equal to (or just greater than, with the uncertainty in the measurements taken into account) the reduction observed in the laboratory air sample.

Although these represent only very initial measurements on determining breath acetone with a MIR optical cavity, the system does seem to show promise and could be pursued and tested against further breath samples. In addition, the experimental set up could be refined to produce more accurate and sensitive results by dealing with the optical feedback better, such as aligning it using the methods described in [29, 304], using a more mechanically stable laser mount and placing the optical cavity in a bellow-type system (as used in Chapter 3) or a fixed mirror cell (as in Chapter 5).



(a) Acetone-equivalent concentrations at 1216.50 cm⁻¹.

(b) Acetone-equivalent concentrations at 1215.60 cm⁻¹ (water-rich region).

Figure 6.38: Both Graph (a) and Graph (b) illustrate the acetone-equivalent concentrations derived from absorption measurements in the optical cavity at two different wavelengths for a sample of nitrogen, laboratory air, breath, 5 ppm of acetone in air, a sample of laboratory air and breath having passed through a water chilling device, plus the level of acetone detected in the breath sample by a mass spectrometer; optical cavity absorption levels referenced to the calibrated 5 ppm of acetone in air and normalised to 200 Torr of sample.

6.5 Conclusions

This chapter has demonstrated the characterisation of an 8 μm QCL source using a narrowband absorber, methane, and its applicability to spectroscopic measurements. Following this, the intrinsically low effective laser linewidth was utilised to conduct high resolution studies on low pressure acetone samples, allowing the accurate determination of the molecule's absorption cross-sections in this spectral region and partially revealing its hitherto unresolved rotational structure. The second half of this chapter then dealt with applying the QCL to the detection of low concentration samples of acetone, with the aim of using the device for the detection of breath acetone. Initially a White cell was used, which achieved levels of sensitivity approaching what would be required for the detection of acetone in breath but it was found to be mechanically unstable for frequent gas handling operations. The Herriott cell demonstrated superior sensitivities, capable of detecting the levels of acetone found in breath. However, the large volume which the optical device occupied was impractical for breath sampling. This led to the construction of an optical cavity, which married the sensitivity required for breath acetone detection with a smaller volume more suited for breath analysis. Finally, combining the optical cavity with a water removing device demonstrated its potential as an instrument that could be used for breath analysis, with the acetone levels returned in good agreement with those determined by a mass spectrometer.

Although only a few samples have been tested with the device, these early results do look very promising and suggest that the MIR cavity constructed could be used for the detection of breath acetone. Spectral interference appears to be less of a problem in the 8 μm spectral region than in the NIR, making gas handling more straightforward, as there is no need for molecular sieve and $-8\text{ }^{\circ}\text{C}$ was found to be sufficient for removing water vapour to levels below the detection limit of the spectrometer. In addition, thanks to the larger cross-sections accessible with the QCL, a significant pathlength enhancement is not necessary to attain the levels of sensitivity required for detecting breath acetone; indeed, a Herriott cell with a smaller volume than that used here (perhaps using astigmatic mirrors) might represent a more suitable solution for detecting breath acetone given the relatively more straightforward optical arrangement. However, the bulky water-cooling system currently required for the laser renders the QCL-based system unsuitable at this stage for use outside of the laboratory. The following and final chapter will look at potential future developments in this field, including some initial results from a preliminary investigation into the possibility of a UV LED-based system for the detection of acetone, which could represent a way in which to combine the compactness of the NIR diode laser device with large cross-sections and the attractive possibility of very limited spectral interference from other species.

Chapter 7

Future Directions and Conclusions

This thesis has centred on the development of absorption spectroscopy-based technologies which have the potential to be applied to the diagnostics of breath analysis. The majority of this work has concentrated on the detection of acetone, a molecule strongly linked with the diabetic condition as a result of the utilisation of fat deposits as a primary energy source. However, acetone is a broadband absorber, which means that a broadband source is required to probe an entire absorption feature, providing the necessary selectivity to identify the molecule, as described in Chapter 4. The necessity for a bulky and expensive spectrometer within the detection system makes this difficult to be developed into a portable, bench-top device, although miniaturised, low resolution, spectrometers are available [322] and potentially offer a solution to this problem. If, instead, a narrowband diode laser is used to probe a broadband absorber such as acetone, all other interferents must be removed from the sample under analysis, or their influence negated. This was the technique employed in Chapter 5, where a NIR diode laser-based optical cavity device was developed. A spectral region where there were relatively few competing absorbing species was selected, and those that remained were either removed to a level below the detection limit of the spectrometer (water) or their presence was negated by using the breath sample of the individual to also provide the background measurement (methane). Similarly, the optical cavity constructed in Chapter 6 employed a water freezing device to remove the effects of water vapour from breath samples, and the larger cross-sections accessible in the MIR by the quantum cascade laser (QCL) ensured that a relatively low pathlength enhancement was sufficient to detect acetone at the levels found in breath. However, this arrangement required the use of a cw QCL: the MIR technology would have to markedly reduce in cost before it could realistically be used outside of the laboratory for breath analysing purposes on a large scale.

One potential solution could lie in the use of an ultra-violet light emitting diode (UV LED). Not only are these devices compact and relatively cheap in comparison to QCL

sources, but the ultra-violet regime also represents an attractive spectral region for the detection of acetone thanks to the sparsity of transitions from competing species, most notably isoprene [107, 315]. As discussed in Chapter 5, isoprene absorbs in the same spectral region as acetone in the NIR, and although it is found at much lower levels in breath than acetone, its NIR absorption cross-section is larger. Consequently, its spectral signature interferes with that of acetone and given the difficulty associated with separating it from acetone, moving to a spectral region where isoprene does not absorb removes this problem. Acetone has cross-sections reaching $\sim 5 \times 10^{-20} \text{ cm}^2$ at $\sim 278 \text{ nm}$, owing to the (symmetry forbidden) $X^1A_1 \rightarrow 1^1A_2$ ($n_y \rightarrow \pi^*$) electronic transition. This corresponds to the excitation of an electron from the non-bonding orbital localised near the oxygen atom to an anti-bonding orbital around the CO group, $\pi^* \leftarrow n$ [323]. The spatial overlap of the electronic and vibrational mode wavefunctions is poor, making the transition significantly weaker than similarly symmetry forbidden features; despite this, the absorption cross-sections are still over an order of magnitude larger than those studied in the NIR. Under atmospheric conditions and at room temperature, the absorption is broad and unstructured, as illustrated in Figure 7.1 from Koch *et al.* [323], and so, as in previous studies in this thesis, the absorption will appear as an ‘offset’ rather than with any well-resolved structure.

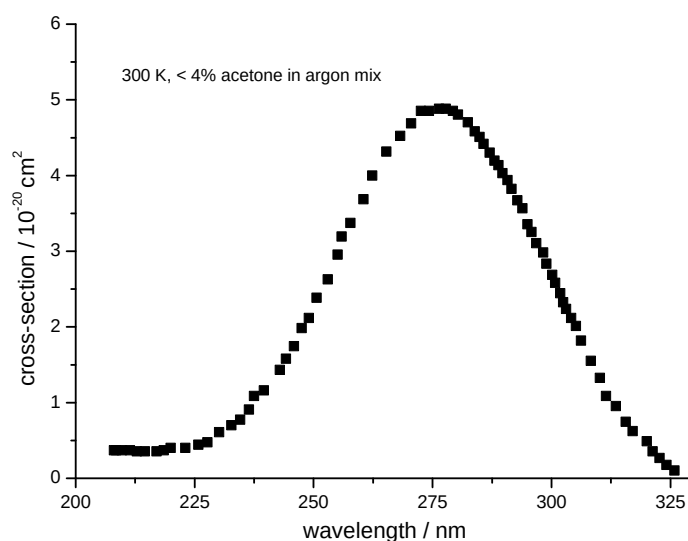


Figure 7.1: The absorption cross-sections for acetone in the UV, as determined by Koch *et al.* [323].

One major consideration when working in the UV is the effect of Rayleigh scattering, which is inversely proportional to the fourth power of the wavelength, resulting in a greater degree of scattering for UV radiation in comparison to the wavelengths used previously in this thesis. The total Rayleigh-scattering cross-section per molecule for air is given by [324]:

$$\sigma(\lambda) = \frac{24\pi^3(n_s^2 - 1)^2}{\lambda^4 N_s^2 (n_s^2 + 2)^2} \left(\frac{6 + 3\rho_n}{6 - 7\rho_n} \right) \quad (7.1)$$

where λ is the wavelength in cm, n_s is the refractive index of standard air (dry air with 0.03% CO₂, at a pressure of 760 mmHg) at λ , N_s is the molecular number density for standard air ($12.54743 \times 10^{-19} \text{ cm}^{-3}$) and ρ_n is the depolarisation factor. This takes into account the anisotropy of the ‘air molecule’, as Rayleigh scattering assumes that the scattering particles are isotropically spherical but the main components of air, N₂ and O₂, are diatomics and as such are slightly anisotropic. However, the polarisability of diatomic molecules can be reduced to perpendicular and parallel components and it is the ratio of the resultant scattering intensities in these two directions which yields the depolarisation factor, which also has a slight wavelength dependence [324]. Thus, at 280 nm the Rayleigh cross-section is $7.6 \times 10^{-26} \text{ cm}^2$ for air, which given the acetone cross-section at the same wavelength is $\sim 5 \times 10^{-20} \text{ cm}^2$, equates to a reduction in the light intensity at 280 nm with an atmosphere of air which is equivalent to the absorption due to ~ 1.5 ppm of acetone. Thus this is a very real problem that will need to be overcome if the detection of low levels of acetone is to be realised.

A preliminary investigation of the UV detection of acetone was undertaken using CEAS. A UV LED (Sensor Electronic Technology, Inc.) centred at 280 nm with a FWHM of 12 nm was mounted in a suitably modified Thorlabs Temperature Controlled Laser Diode Mount (TCLDM9), with current and temperature control provided by a Pro8000 unit (Thorlabs). This wavelength range was chosen due to its coincidence with the absorption maxima of acetone in this region. The LED has a hemispherical lens as its optical window, and as a result the output diverges rapidly. Therefore, a short focal length lens (3 cm) was placed directly after the LED and an iris placed at the subsequent focal point so that an effective point source was created. From this point source, a second lens was positioned to create a collimated beam, before a third lens was used to gently focus it into the centre of the cavity cell. Finally on exiting the cavity, the radiation was focused into the spectrometer (Shamrock SR-303i, Andor), an imaging spectrograph with a Czerny-Turner configuration and the resultant output is depicted in Figure 7.2a.

After recording the direct LED output, an optical cavity was constructed in a bellows-type cell from relatively low reflectivity mirrors (Layertec, radii of curvature 200 mm, $R \sim 99\%$), to ensure that the transmitted intensity was high enough to be detected (as discussed in Chapter 4). The signal detected on misaligning the cavity was then subsequently taken into account when the realigned cavity output was measured. The cavity output is depicted in Figure 7.2b, alongside the direct LED output, with the ripple structure clearly demonstrating variations in the mirror reflectivity across this spectral range. It is important to note, with regards to Figure 7.2, that whilst the spectrometer does not have an averaging

function, it does permit an increased number of accumulations. It is this which accounts for the relatively large signal intensity for the cavity transmission plot, where 1000 accumulations have been utilised to visualise the cavity output. From these measurements it is possible to determine a transmission curve, as illustrated in Figure 7.3. It is obvious from Figure 7.3 that the transmission is slightly higher than that quoted by the manufacturer.

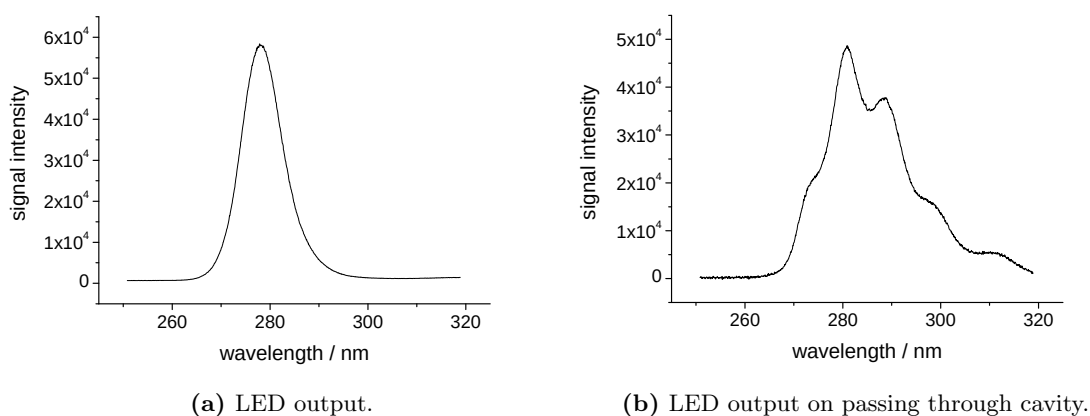


Figure 7.2: Graph (a) depicts the LED output for a 2×10^{-5} s exposure time with the injection current set to 8 mA; whilst Graph (b) shows the detected cavity output following 1000 accumulations, at an injection current of 20 mA.

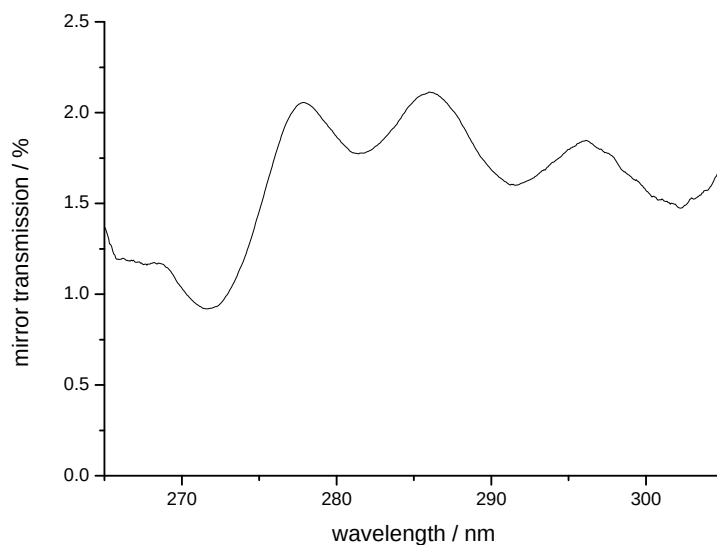


Figure 7.3: The experimentally determined transmission function for the cavity mirrors used in the experiment, determined from comparison of the direct LED output with that of the LED on passing through the cavity.

Given the low reflectivity of the mirrors (approximately 98 % at 280 nm), a 25 ppm acetone in air mix (BOC Special Gases) was used to test the instrument's response to acetone. It was found that the optimum number of accumulations for the spectrometer was 2000, which

was then used for all subsequent measurements. All samples were recorded at atmospheric pressure: the cell was filled with a 760 Torr sample of the 25 ppm mix and the cavity alignment optimised for that pressure. After recording the resultant absorption spectrum, nitrogen was slowly flushed through the cell until an atmosphere of nitrogen occupied the vessel and an I_0 measurement was taken. This ensured that the pressure within the cell did not vary drastically between measurements and minimised the effect of mechanical instabilities on the measurements. In addition, by using nitrogen as the I_0 measurement, with air (and thus nitrogen) as the majority component in I , the reduction in transmission due to the Rayleigh scattering of air is effectively negated. This was repeated several times and an example of the reflectivity-corrected absorption spectrum obtained is illustrated in Figure 7.4, alongside the theoretical absorption spectrum across the same spectral range, obtained from utilising the absorption cross-sections from the literature [323, 325–327] in conjunction with an assumed mirror reflectivity of 98 %. The absorption obtained experimentally has the same general shape as that predicted from the cross-sections, and is found to be almost at the same level. The apparent reduced absorption undoubtedly arises from the uncertainty in deriving the reflectivity curve; it has been assumed that $R = 1 - T$, where R is the reflectivity and T is the transmission. In reality, scattering losses from the mirrors become much more of an issue in the UV, meaning that there will be an extra term in the equation which will account for non-reflectivity-based losses on passing through the cell.

Despite being corrected for reflectivity fluctuations across the spectral range, the absorption (which has been divided through by the average mirror reflectivity to return values of αL for comparison purposes) still exhibits undulations. Whilst these could arise from the uncertainty in deriving the reflectivity curve, there is also a possibility that the features represent real structure in the acetone spectrum, as the cross-sections presented in the literature are at low resolution (2.6 nm in the Koch *et al.* [323] measurements illustrated in Figure 7.4) in contrast to the higher resolution of this study (0.07 nm). Indeed, a CRDS study by Wang *et al.* [106] on low pressures of acetone (1.5×10^{-3} Torr of $\sim 28\%$ acetone in argon) appeared to demonstrate a vibronically resolved spectrum of acetone in this region ($\sim 282 - 285$ nm) (however, the spectrum seemed to suggest that the cross-section dropped to zero in between each vibronic band, which is not consistent with other sources in the literature [323, 325, 327, 328]). More recently, a study by da Silva *et al.* [328] using synchrotron radiation to probe the UV spectrum from 115–330 nm on low pressure samples of acetone (0.023–0.938 Torr), also revealed vibronic structure. With a very similar resolution to the measurements taken in this study (0.075 nm), the structure satisfyingly seems to match quite well with the features observed here, as can be seen in Figure 7.4.

From considering two atmospheric measurements on N_2 and treating one as ' I_0 ' and the other as ' I ', it is possible to determine a noise level in the zero baseline of the instrument

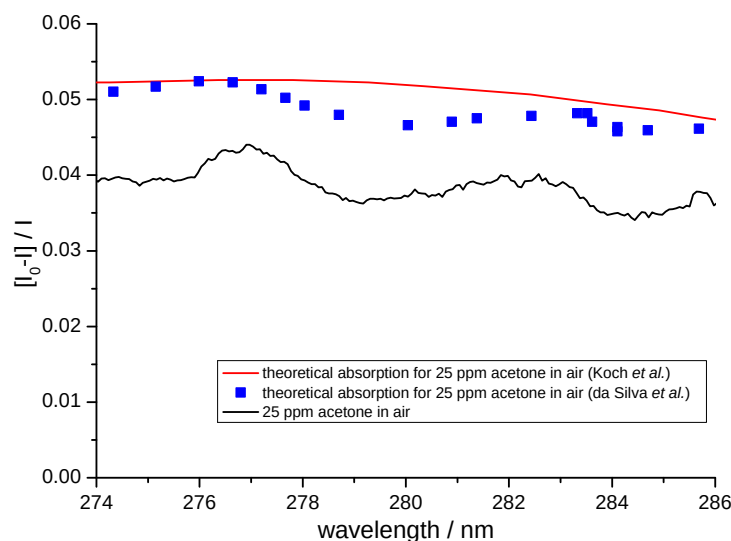


Figure 7.4: A CEA spectrum for 25 ppm of acetone over the FWHM of the UV LED, compared to that predicted theoretically assuming a mirror reflectivity of $\sim 98\%$.

from which the minimum detectable absorption co-efficient is calculated to be $1.6 \times 10^{-6} \text{ cm}^{-1}$ at 278 nm. Clearly, this level of sensitivity is too low for the detection of acetone in breath (for which $\alpha = 1.2 \times 10^{-6} \text{ cm}^{-1}$ for 1 ppm at 278 nm), but it could be improved dramatically by using mirrors of a higher reflectivity: even moving to 99.9% reflectivity could result in an approximate $20\times$ increase in sensitivity, which would be more than sufficient for detecting breath acetone levels. In addition, given the acetone absorption feature under atmospheric conditions is featureless, the spectrometer could be replaced with a miniaturised photomultiplier, such as those supplied by Hamamatsu [329]. This would allow a compact system to be developed, which could then be applied to breath acetone detection. Clearly a second problem with exploiting the UV spectral region is the aforementioned issues with Rayleigh scattering: this could potentially be overcome by employing a similar method to that used to deal with methane in Chapter 5, by initially passing a sample of the breath through some molecular sieve and using the subsequently acetone-depleted sample for the I_0 measurement. This would ensure that both the I_0 and I samples would be subjected to the same degree of Rayleigh scattering and thus the effect would be negated. In their 2008 paper on the development of a CRDS-based device for detecting acetone in breath, Wang *et al.* [107] identified CO_2 and O_2 as having a minor influence on the effective absorbance observed, which caused a certain degree of uncertainty in their measurements as they utilised laboratory air as their baseline measurement: clearly there is a discrepancy between the O_2 and CO_2 found in air and that found in breath. However, the molecular sieve method would reduce this uncertainty as the levels of these two molecules would be approximately the same in both the I_0 and I measurements, given

they originated from the same breath sample. Similarly, ozone is known to have a cross-section 70 times larger than that of acetone at 280 nm, and even taking into account its lower concentration (typically 50 ppb in air), it still poses a significant source of interference (with an absorption level ratio of 3.38:1 for 50 ppb ozone to 1 ppm acetone) [330]. However, it too can be taken into consideration by using the molecular sieve, ensuring the levels are approximately the same in both background and sample measurements, or, if necessary, it can be filtered out chemically [331, 332]. Thus, this represents a potential future direction in the application of absorption spectroscopy to breath analysis, and in particular to the detection of breath acetone.

Whilst the link between elevated levels of acetone in breath and diabetes is often quoted, it is more accurately merely an indication that the body is predominantly using fat deposits as a source of energy. Therefore, in addition to the potential application to diabetes diagnosis, there is the significant weight-loss market that could benefit from a device that is able to monitor breath acetone, particularly if it reduced to a hand-held size. Higher levels of acetone in breath are a direct result of fat-burning: thus, a subject could blow into the breath analysing device prior to exercise and then periodically breathe into it to monitor their fat-burning efforts.

The field of breath analysis itself is still very much in its infancy with regards to its widespread use in diagnostics, and the application of absorption spectroscopy to it even more so, but it is a rapidly expanding field, with increasing numbers of studies investigating the possibilities of the non-invasive diagnosis of diseases it provides. This thesis has sought to apply cavity enhanced absorption spectroscopy, in particular, to the application of breath analysis. Chapter 3 began with a simple CEAS system using a DFB diode laser to measure the levels of breath methane in a sample of 48 people. As a relatively straightforward molecule to detect given its narrow absorption features, and its significance as a breath biomarker in its own right, methane was an ideal molecule to start with. This was followed by the first demonstration of the application of a DS-DBR laser to cavity enhanced spectroscopy. This laser exhibits a wide tunability, making it an ideal source for monitoring multiple absorption transitions, which were demonstrated on another molecule found in breath, CO₂, illustrating its applicability in measuring CO₂ isotope ratios for the urea breath test. In Chapter 4 the focus shifted to detecting broadband absorbing molecules, butadiene, acetone and isoprene, in the near-infrared. This involved the development of a series of broadband cavity enhanced absorption (BB-CEAS) spectrometers, utilising both SLED and SC sources. Two SLED devices were coupled together to provide a 200 nm spectral range, which was used to demonstrate the possibility of monitoring multiple species using BB-CEAS detection. Hitherto undetermined absorption cross-sections were deduced for butadiene, acetone and isoprene over the 1.6 - 1.7 μm spectral range, before cavity enhanced techniques were utilised to achieve respective noise-equivalent sensitivities of 200

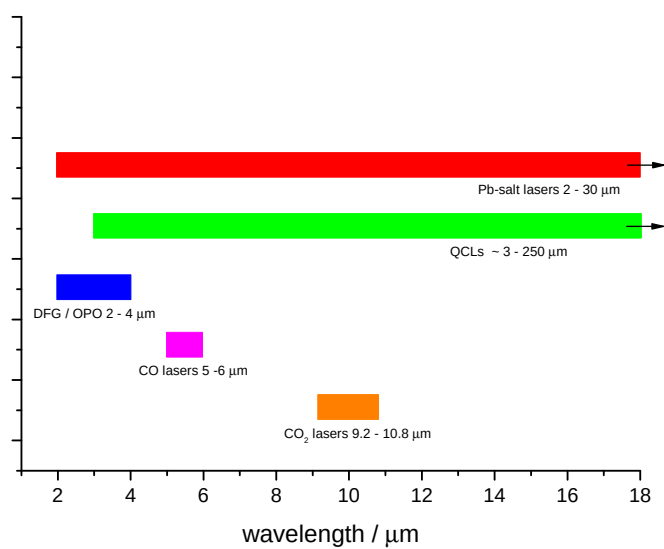
ppb, 400 ppb and 200 ppb for the three molecules respectively (with the SC source). This study helped to provide a basis for the development of a table-top breath acetone detecting device in work carried out at Oxford Medical Diagnostics Ltd. A combination of molecular sieve and a peltier-based chilling device were used to prepare the breath samples for analysis in the optical cavity. The levels of acetone determined by the optical cavity were found to agree with those measured by a mass spectrometer within an uncertainty of 200 ppb, adding confidence to the suitability of the device for measuring breath acetone. Chapter 6 saw the introduction of a cw QCL, which allowed the fundamental transitions of acetone at 8 μm to be probed. Utilising the narrow linewidth of the laser, high resolution measurements on low pressure, pure acetone samples were conducted and rotational structure discerned. Following this, the sensitivity of the system was incrementally increased using a White cell, Herriott cell and finally a cavity. Exploiting the small volume provided by the cavity, breath samples were analysed for acetone having first passed through another chilling device to remove the water vapour, and the levels deduced once again corroborated with those measured by the mass spectrometer on the same samples. Finally, an initial investigation into the detection of acetone in the UV has been presented in this chapter as a potential future direction for breath acetone detection.

Appendix A

Radiation Sources in the Mid-Infrared

MIR radiation can be accessed in a number of ways, and these are summarised in Figure [A.1](#). Radiation with $\lambda > 5 \mu\text{m}$ has traditionally been available via CO and CO₂ lasers, and the spectrally wide-ranging lead-salt solid state lasers. The gas lasers offer low laser linewidth, coupled with a high power, but are restricted in their spectral coverage and poor tunability. Limited by the position of their respective vibrational transitions, CO lasers cover the region 5 - 6 μm , whilst the CO₂ lasers emit from 9.2 - 10.8 μm . Pb-salt lasers, on the other hand, allow a much broader coverage, from 3 - 30 μm [\[333\]](#), and also offer narrow laser linewidth as they are essentially another type of diode laser, resulting in their use in numerous gas detection systems [\[76, 334, 335\]](#). However, they are limited by a low power output and their need to be cryogenically cooled, which restricts their use in any potential portable gas analyser. In the sub-5 μm regime, the use of non-linear optical processes to convert NIR radiation into MIR radiation come to the fore. Difference frequency generation (DFG) typically uses a non-linear crystal, such as periodically-poled lithium niobate (PPLN), to combine two initial frequencies, ω_1 and ω_2 , to produce a third, $\omega_1 - \omega_2 = \omega_3$. The radiation has to be phase-matched in order to preferentially select DFG over the other non-linear processes occurring within the crystal, and although this allows the generation of a narrow laser linewidth, it also results in a very low power output when used with cw sources (in the order of μW) as the conversion efficiency in the crystal is low. The power conversion can be improved if a waveguide architecture is used. In theory, as there is a degree of tunability with both of the input frequencies, this method allows a wide range of resultant frequencies to be accessible. However, in practice the necessity of fulfilling the phase-matching requirement restricts the tunability of the device, as the refractive indices of the crystal must also be tuned with the changing difference frequency, and this is achieved by varying the temperature of the crystal, which in turn

must be operated at a moderate temperature. DFG-based sources have been applied to many spectroscopic applications, including a device for monitoring multiple trace gas species [336, 337], measuring benzene concentrations [338] and notably in the field of breath analysis by Sigrist *et al.* [339], who applied a DFG system to the monitoring of carbon isotope ratios and to the detection of methylamines in breath. In addition, recent studies by Whittaker *et al.* [340] have demonstrated the use of a DS-DBR laser within a DFG system, which offers wide tunability in the MIR, coupled with narrow linewidth, for the high resolution detection of broadband absorbers. Optical Parametric Oscillators (OPO) also exploit non-linear optic effects to generate MIR. In this case, the non-linear crystal (also typically LiNbO_3 [341–343]) is placed within an optical cavity and the pump laser frequency ω_3 is used to generate radiation at $\omega_1 + \omega_2$, the signal and idler frequencies, respectively. The devices can either be singly resonant, where the signal frequency is resonant with the cavity; doubly resonant, where the idler frequency is also in resonance (though these are difficult to construct) and in special cases even triply resonant, where all three frequencies resonate with the cavity [333]. Thanks to the presence of the cavity, this process generates a lot more power (in the order of several Watts) than DFG-based systems, but the bandwidth of the radiation is typically greater than 3 GHz, making it unsuitable for high resolution studies (though it could be applied to broadband absorbers where resolution is less important). In addition, non-linear techniques are generally restricted to generating radiation in the 3–5 μm region due to the non-linear optics that are available commercially, although the generation of longer wavelength radiation using non-linear effects has been demonstrated using AgGaSe_2 ($\sim 7.2 \mu\text{m}$) [344], ZnGeP_2 (3.8 μm and 4.65 μm) [345] and GaSe (11.8 – 16.1 μm [346]; 8.8 – 15 μm [347]; and 8 – 19 μm , used for the detection of acetylene [348]) as the birefringent bulk crystal materials, whilst Lee *et al.* used AgGaS_2 in a dual-resonator scheme to access 9 – 11 μm [349]. 8 μm radiation, representing the region of study in Chapter 6, was generated via non-linear means by Levi *et al.*, who utilised orientation-patterned GaAs to produce the desired idler radiation on combining lasers at 1.3 and 1.55 μm [350].

**Figure A.1:** Radiation sources in the MIR.

Appendix B

Characterisation of the DS-DBR laser

The work described in this Appendix is based on the DS-DBR characterisation reported in [193], carried out primarily by Dr Luca Ciaffoni and Kim Whittaker.

In order to investigate fully the effects of changing the relative amplitudes of the current ramps applied to the front, rear and phase sections of the device, a 4 m multipass cell was filled with 85 Torr of CO₂ and the transmission spectra from both the cell and the spectrum analyser were recorded for each tuning setting. When just the rear current is scanned across its whole range, only a small fraction of the total spectral region is effectively accessed by the laser (4.65 cm⁻¹ out of 32 cm⁻¹), due to the occurrence of multiple mode hops. In contrast, when a current ramp is injected into the phase section only, mode hops occur far less frequently as it is effectively exploiting the maximum tunability of one of the longitudinal cavity modes. As illustrated in Figure B.1, this results in a larger accessible region of a narrower spectral window (3.3 cm⁻¹ out of 5 cm⁻¹). When ramps are applied to both the rear and the phase sections, intermediate responses are observed, also illustrated in Figure B.1. An example of the CO₂ spectra obtained under such conditions, where both the rear and phase sections have a current modulation applied, is shown in Figure B.2. It is immediately obvious from the spectrum analyser trace in this figure that the tuning rates during each mode hop-free section vary.

Figure B.3 further illustrates the tuning characteristics of a DS-DBR and depicts the boundaries between adjacent longitudinal modes (given by the dotted lines) for a typical supermode. This figure has been adapted from Ward *et al.* [189], with the tuning paths used to drive the laser in Figure B.1 depicted within the rear current (i_r) and phase current (i_p) plane. Each tuning path used stays within one supermode and is continuous in the

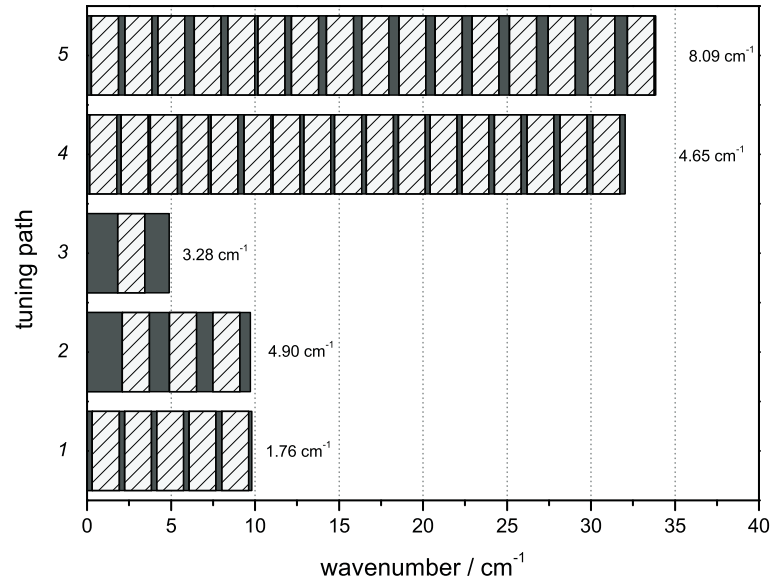


Figure B.1: Illustration of the tuning ranges observed from applying five different current ramping conditions; the mode hop regions are defined by the hatch pattern, whilst the spectral regions accessible by the laser for each tuning path are designated in black and the total wavenumber range this represents is quoted to the right.

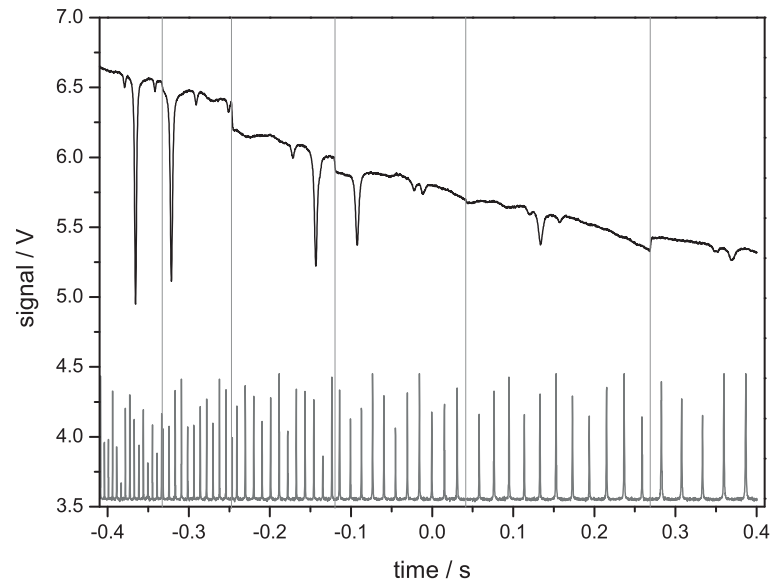


Figure B.2: An example of a transmission spectrum from the multipass cell ($L = 416$ cm) filled with 85 Torr of CO_2 ; below is the spectrum analyser trace recorded at the same time when both the rear and phase current are ramped simultaneously, whilst mode hops are represented as vertical lines.

current domain, but on crossing a dotted line a mode hop occurs, so it is discontinuous in the spectral domain [351].

Figures B.3 and B.1 indicate that the greatest accessible spectral coverage will thus be given by a combination of ramping the rear and the phase sections, as the rear covers a broader total spectral range, whilst the phase ensures a larger proportion of that range is actually

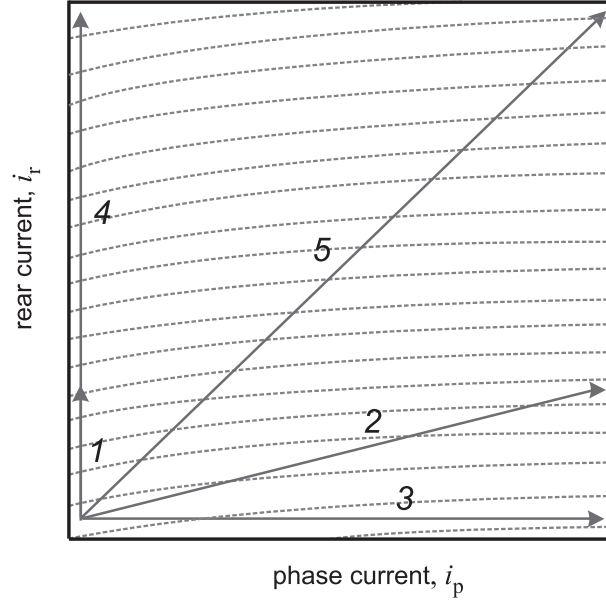


Figure B.3: A plot highlighting the boundaries between longitudinal modes for a generic supermode, adapted from Ward *et al.* [189]. The arrows indicate the different tuning paths in the i_p/i_r plane used in Figure B.1

accessed by the laser. In addition, it was found that ramping the front pair with the rear grating and/or the phase section had very little effect on the spectral response. Reid *et al.* [352] have attributed this to the wide front grating reflection which renders high accuracy in the front section unnecessary, as the overlapping supermode is much narrower and thus the selected wavelength is consequently defined only by the rear and phase sections. Figure B.1 also illustrates that the size of the mode hop jumps are the same within one supermode ($\sim 1.61 \text{ cm}^{-1}$ in this case). It should be noted, however, that the magnitude of the mode hop varies between supermodes: this is because selecting different supermodes involves changing the contact on the front grating (i.e. switching the front pair) into which the current is injected, which in turn effectively alters the length of the laser cavity [351].

Using these results, it is possible to combine the modulation settings of the front, phase and rear sections so that over 2 cm^{-1} can be scanned continuously without mode hops. Therefore, a sequence of these regions were identified for each front grating pair (i.e. each supermode) so that the whole 50 nm tuning range of the DS-DBR could be accessed without mode hops. The region was consequently split into ~ 80 segments, each of which contained at least one CO_2 transition and partially overlapped with the previous and following segment. Whilst the spectrum analyser traces recorded for each portion provided the relative frequency calibration, the presence of the CO_2 lines allowed an absolute frequency scale to be determined. Thus, the CO_2 absorption spectrum across the entire range of the DS-DBR, 1563-1613 nm ($6199.62 - 6397.95 \text{ cm}^{-1}$), was extracted from combining the spectra obtained from the individually recorded segments. This is illustrated in Figure 3.16a, along with a simulation based on the HITRAN database. Figure 3.16b shows the region at 1570 nm

(6369.42 cm^{-1}) expanded and the number of experimental points reduced for clarity, with the best fitting Voigt profiles superimposed. The Gaussian contribution to the Voigt profile was fixed to the theoretical Doppler width (0.0119 cm^{-1}) and the Lorentzian contributions subsequently deduced were found to be within 2 % of the values quoted in the HITRAN database [171].

Appendix C

The Fundamental Vibrational Modes of Acetone

Mode	Approximate type of mode	Symmetry	Frequency / cm^{-1}
ν_1	CH_3 degenerate stretch	A_1	3019
ν_2	CH_3 symmetric stretch	A_1	2937
ν_3	CO stretch	A_1	1731
ν_4	CH_3 degenerate deform	A_1	1435
ν_5	CH_3 symmetric deform	A_1	1364
ν_6	CH_3 rock	A_1	1066
ν_7	CC stretch	A_1	777
ν_8	CCC deform	A_1	385
ν_9	CH_3 degenerate stretch	A_2	2963
ν_{10}	CH_3 degenerate deform	A_2	1426
ν_{11}	CH_3 rock	A_2	877
ν_{12}	Torsion	A_2	105
ν_{13}	CH_3 degenerate stretch	B_1	3019
ν_{14}	CH_3 symmetric stretch	B_1	2937
ν_{15}	CH_3 degenerate deform	B_1	1410
ν_{16}	CH_3 symmetric deform	B_1	1364
ν_{17}	CC stretch	B_1	1216
ν_{18}	CH_3 rock	B_1	891
ν_{19}	CO ip-bend	B_1	530
ν_{20}	CH_3 degenerate stretch	B_2	2972
ν_{21}	CH_3 degenerate deform	B_2	1454
ν_{22}	CH_3 rock	B_2	1091
ν_{23}	CO op-bend	B_2	484
ν_{24}	Torsion	B_2	109

Table C.1: The fundamental vibrational modes of acetone, reproduced from the NIST website [315].

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