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Feasibility Study of Using Short Wave Infrared Cavity Ringdown Spectroscopy (SWIR-CRDS) for Biological Agent Detection

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Prepared for the U.S. Department of Energy
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Final Report for Project # 49284

Feasibility Study of Using Short Wave Infrared Cavity Ring Down Spectroscopy (SWIR-CRDS) for Biological Agent Detection

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1. Executive Summary

This project focused on determining the feasibility of using short wave infrared (SWIR) cavity ring down spectroscopy (CRDS) as a means for real-time detection of biological aerosols.

The first part of the project involved identifying biological agent signatures that could be detected with SWIR CRDS. After an exhaustive search of the open literature it was determined that whole biological spores and/or cells would not be good candidates for direct SWIR CRDS probing because they have no unique SWIR signatures. It was postulated that while whole cells or spores are not good candidates for SWIR CRDS detection, their pyrolysis break-down products might be. A literature search was then conducted to find biological pyrolysis products with low molecular weights and high symmetry since these species most likely would have overtone and combination vibrational bands that can be detected in the SWIR. It was determined that pyrrole, pyridine and picolinamide were good candidates for evaluation. These molecules are formed when proteins and porphyrins, proteins and dipicolinic acid, and dipicolinic acid are pyrolyzed, respectively.

The second part of the project involved measuring quantitative SWIR spectra of pyrrole, pyridine and picolinamide in PNNL's FTIR Spectroscopy Laboratory. Spectral information about these molecules, in the vapor phase is sparse – there were only a few prior studies that measured line positions and no information on absorption cross sections. Absorption cross sections are needed in order to estimate the SWIR CRDS detection sensitivity, and line position determines what type of laser will be needed for the sensor.

The results of the spectroscopy studies allowed us to estimate the SWIR CRDS detection sensitivity for pyrrole to be 3×10^{12} molec cm^{-3} or 0.1 ppmv, and for pyridine it was 1.5×10^{15} molec cm^{-3} or 0.6 ppmv. These detection sensitivity limits are close what we have measured for ammonia. Given these detection limits we then estimated the amount of biological material that would have to be collected for analysis in a sensor that combined pyrolysis with SWIR CRDS. Using conservative estimates of pyrolysis yields and precursor species concentration we determined that it would be necessary to collect and pyrolyze biological aerosol samples in the 10's of mg. This is a large amount and is far larger than required for current sensors. It is therefore concluded that while possible, the large amounts of material required preclude using SWIR CRDS for detecting biological agents at this time.

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3. Introduction

The real threat of terrorists or hostile nations using biological agents, such as anthrax or smallpox, to attack US interests has emphasized the need for developing new biological sensors and biodetection technologies. This has provided the scientific community with a daunting challenge. Because biological agents can be effective in low doses, biological sensors and detection systems must have high detection sensitivity. The large array of environmental backgrounds against which detection must take place also means that the sensors or systems need to be highly selective (i.e. very low false alarm rate). And to best mitigate the extent of a biological attack, the sensor or system should be capable of real-time detection, or at least have a sampling/analysis time that is on the order of minutes, as opposed to hours or days. Also taken into consideration is the amount of consumables and power required by the sensor and its cost – with the goal being to minimize these parameters.

Considerable progress has been made in the last few years in developing new biosensors and technologies[1, 2] and first generation biological aerosol sensing systems have been field tested.[3] There is still room for improvement however. The requirements of high sensitivity, high selectivity and short analysis time are orthogonal to each other, at least with respect to practical sensor design. As a result it has been decided that the best approach is to combine two or more vastly differing sensing technologies on one platform. The choice of the sensing technologies used depends upon the attack scenario (i.e. will the attack be on a particular food or drinking water supply chain, or will biological aerosols be dispersed over a local area), the method of detection (i.e. point or remote monitoring capability), and also upon the information that is needed from the sensor (i.e. detect-to-warn versus detect-to-treat).

This project is concerned with point detection of biological agent aerosols and more specifically determining if short wave infrared (SWIR) cavity ring-down spectroscopy (CRDS) has the potential to be used in a detect-to-warn strategy. In this case, the sensor platform must be capable of screening an aerosol sample to a) determine if it contains biological material, and b) decide if the biological material represents a threat.

CRDS has previously been used to study systems that mimic, or systems that use, atmospheric aerosols.[4 – 11] These experiments have typically involved measuring particulate extinction (absorption plus scattering) coefficients at one or two laser wavelengths. Experiments have been done using light ranging from the visible into the SWIR, and extinction detection sensitivities in the 10^{-6} – 10^{-8} m^{-1} range reported[6-8] – which corresponds, roughly, to mass concentration changes in the 1 – 10 $\mu g\ m^{-3}$ range.[8]

The Paldus group has recently reported using CRDS to measure extinction and spectrally resolved fluorescence (in two 100 nm wide bands) of specially doped micro-spheres using 488 nm excitation light.[12] Their observations led them to suggest that different biological aerosols could be differentiated by monitoring the fluorescence spectra. However it is not clear that this CRDS detection approach is viable given the recent observations that the fluorescence spectra seen from a variety of natural biological aerosols and bioagent aerosols are quite similar and, in addition, are highly sensitive to the conditions that the biological species were in while they were growing.

The Schechter group has recently reported measuring broadband CRDS spectra of laser dye-doped aerosols.[11] This result suggests that it may be possible to use tuned laser CRDS to spectrally monitor absorption in biological aerosols and distinguish between biological agents and natural species. Given this, the first focus of this project was to determine if there were any specific SWIR spectroscopic signatures that could be used to distinguish bioagent aerosols from

natural biological aerosols (such as pollen or non-toxic bacterial spores) and ambient aerosol species (such as sulfate or soot aerosols).

4. Survey for Short Wave Infrared (SWIR) Signatures of Biological Agent Aerosols.

Fourier transform infrared spectroscopy (FTIR) in the long wave infrared region (typically 600 – 4000 cm-1) has been used to monitor and distinguish biological material. The work of the Naumann[13] and Goodacre[14] groups in particular has led to major advances understanding the spectroscopy of pathogenic microorganisms. PNNL has also done high quality quantitative spectroscopic studies on several different *Bacillus* strains in both vegetative and sporulated forms. PNNL determined that a straightforward analysis of the transmission spectra at 1070 and 1230 cm^{-1} could be used to differentiate vegetative bacteria from sporulated bacteria; and that different strains could be identified by comparing second-derivative spectra using a six component principal analysis.[15] PNNL has also demonstrated that second derivative photoacoustic FTIR spectra can also be used to speciate bacterial spores.[16] Several other groups have also reported using long wave FTIR for monitoring whole cells during bioprocessing[17], and for determining LWIR optical (absorption and/or extinction) cross sections and other optical properties.[18,19]

Given that whole bacterial species have characteristic LWIR signatures, and that SWIR spectroscopy is routinely used to monitor food and drug processing and production,[20] it is not unreasonable to speculate that whole cell signatures might also be present in the SWIR. However, after an exhaustive search of the open literature, it was determined that whole biological spores and/or cells would not be good candidates for direct SWIR CRDS probing because they have no unique SWIR signatures. SWIR spectroscopic studies on *Erwinia herbicola* bacteria and *Bacillus subtilis* spores show that absorption in the 1.0 – 2.5 μm region is flat and contains no unique identifying peaks.[21,22] A comparison of the second derivative spectra in the 2.2 -2.5 μm region of aqueous solutions of several strains of E. Coli and Listeria innocua show some differences.[22] However, this region corresponds to fundamental CH and methylene stretch modes that are present in every biological or organic system, so this region is going to be subject to considerable background interference. It is also worth noting that tunable lasers are not currently available for this spectral region.

5. Feasibility of Using SWIR CRDS to Detect Biological Agent Pyrolysis Products

While whole cells or spores are not good candidate species for detection using SWIR CRDS, their pyrolysis break down products might be. The genesis for this idea comes from the considerable amount of research that has gone into developing small form factor bioagent detectors - devices which combine pyrolysis with gas chromatography, ion mobility spectroscopy, or compact mass spectrometry.

Thermal pyrolysis has been found to be a viable method for the production of biological markers from whole microorganisms,[23] and several groups have demonstrated that unique biomarkers are associated with bacteria and bacterial spore pyrolysis.[24-40] Some of the products have extremely high molecular weight, but there are also a number of low molecular weight, high volatility species that are produced. As would be expected, the lower molecular weight species prevail when the pyrolysis temperature is high and the residence time is long.

While varied, the main chemical components of cells are macromolecules made up of four classes of monomers – sugars, fatty acids, nucleotides, and amino acids (monomeric constituents of proteins). Sugars (carbohydrates) combine into long polymers called polysaccharides. Cell walls are composed of polysaccharides. Phospholipids play a major structural role in the cytoplasmic membrane.[41]. Bacterial spores are composed of the exosporium (multilayered, found in *B. cereus, B. thuringiensis* and *B. anthracis*), the spore coat (inner coat and outer coat), the cortex, DNA, and ribosomes.[42-47] The exosporium which protects the spores from chemical and environmental attack consists of several amino acids, lipids, sugars, glucosamine, phosphate, RNA and degraded carbohydrates. The spore coat, which comprises about 50% of the spore's volume, is comprised of many layers with up to 25 cross-linked polypeptide species. Proteins, especially amino acids rich in tyrosine and cysteine are the main constituent of the spore coat, but small amounts of carbohydrates and lipids are also present. The cortex is composed of peptidoglycan which consists of long chains of glycan cross-linked with fragments of peptides.

Simmonds et al. have assigned pyrolysis products from *Bacillus subtilis* and *Micrococcus luteus* to biological classes.[23] They note that pyridine is a product of protein and nucleic acid pyrolysis, while pyrrole is a product of protein and porphyrin pyrolysis. Pyridine is also a thermal degradation product of dipicolinic acid (DPA) which is found in bacterial spores but not in vegetative cells. Pyridine is a small organic molecule that has significant use in the chemical and pharmaceutical industries however – therefore it is a common background interferent so its presence is not a definitive marker for spores. However observation of pyrrole together with pyridine will confirm that species undergoing pyrolysis are biological in character. Picolinamide is a primary product of DPA pyrolysis – it's presence along with pyridine and pyrrole will be a key indicator of gram-positive bacteria and/or spores.

Since pyridine, pyrrole and picolinamide have low molecular weight and a fair degree of symmetry we expect that they will have overtone and combination bands that lend themselves amenable to detection with SWIR CRDS. To determine the feasibility of this detection strategy, quantitative measurements of these species SWIR spectra need to be made, since this information is not available. It is to be noted that pyridine, pyrrole and picolinamide are not the only, nor perhaps not the best, bacterial pyrolysis biomarkers. They have been chosen primarily because they contribute appreciably to the pyrolysis yield (under certain conditions) and may have measurable N-H overtone spectra that can be used for species discrimination (note they may show C-H overtones but this is not unique since most other pyrolysis products will be comprised of CH moieties).

6. SWIR Spectroscopy Studies of Gas Phase Pyrrole, Pyridine and Picolinamide.

To determine the feasibility of using SWIR CRDS to detect pyrolysis biomarkers it is necessary that a) the spectra in the SWIR be known in order to determine the wavelength and spread of the laser needed for CRDS measurements, and b) the absorption cross sections (or alternatively transition line strengths) be known to determine if the SWIR CRDS technique is sensitive enough to detect the low concentrations of species that are expected to come from pyrolyzing a small amount of suspect biological material.

An extensive search of the literature revealed that limited information is available about the overtone vibrational transitions of pyrrole and pyridine and nothing for picolinamide. Kjaergaard et al. have measured room temperature pyridine CH stretching overtones in the visible region (500 – 900 nm) in both vapor and liquid phases using intracavity laser

photoacoustic and conventional absorption spectroscopies.[48] They assigned the observed peaks by comparing the experimental results with ab initio calculated bond lengths and they report theoretical and experimentally measured oscillator strengths. More recently McNesby et al. used SWIR FTIR spectroscopy to measure vapor phase pyridine broadband absorption between 1.62 – 1.70 μm (peak at ~ 1.67 μm), and then did a narrow band diode laser differential absorption measurement to obtain an absorption cross section of 3.6 x 10^{-22} cm^2/molecule at 1.63 μm.[49] They did not identify the transition they accessed however. Mellouki at al.[50], Held and Herman,[60] and Douketis and Reilley,[61] have reported measuring pyrrole N-H overtones in regions from the visible to the long wave infrared fundamental.[50] The first N-H overtone is centered around 1.44 μm. No information was given with regard to line strengths or absorption cross sections.

6.1 Experimental

Quantitative near-infrared cross-sections were determined using a slight variant of the method of Chu et al.[50] and Sharpe et al. [51] In this method, a series of Fourier transform IR measurements is made, each corresponding to a different mixing ratio of the pure analyte vapor in a stream of ultra-high purity (UHP) nitrogen gas. After the measurements, a composite absorption spectrum $A(\lambda)$ is generated from the individual spectrum; the individual (298.1 K) measurements cover a large range (~2 orders of magnitude) of analyte burdens, each burden pressurized with N_2 to one atmosphere. The fit is derived by generating a Beer's law plot at each wavelength channel to the individual burdens. However, in order to account for various nonlinearity effects in the A = f(P) fit, the y-axis intensity values in each spectrum are weighted proportional to T^2 (where $T = I/I_o$). All absorbance values $A > 1.6$ (that is $T < 0.025$) are simply weighted with zero. This multiple burden method greatly improves the S/N ratio by using the high burden measurements to enhance the signal for the weak bands that might not exceed the noise floor in any given measurement (while down-weighting the nonlinear strong peaks), but also using the low burden measurements to bring out a better fidelity for the strong peaks by accounting for Beer's law saturation and detector nonlinearity effects. Chu et al.[51] and Sharpe et al. [52] have demonstrated the advantages of this method compared to a simple linear absorbance data fit at each wavelength bin, since the raw data also have a weighting factor to account for nonlinearity mechanisms. In this method the residual fit vector is, moreover, carefully analyzed since any chemical impurities, including the uncommon ones, readily manifest themselves in the fit residual. The resulting error analysis (Sharpe[53]) shows that the maximal expected systematic errors in absorbance are 3% for the PNNL static-system measurements and 7% for flow-system measurements, including the near-IR values of this work. Random values are less than this, and these results have been vetted against NIST for a host of different molecules as described by Sharpe et al. [52,53] and Johnson et al. [54], which contain further details of the error analysis.

The three chemicals whose spectra are presented here were purchased as neat liquids from Aldrich Chemical with reported purities of 99.9%, 99.8% and 98% for chloroform, pyridine and pyrrole, respectively. Chloroform is not a potential biomarker species; we have used it in these studies to ascertain the validity of our spectroscopic measurements since its SWIR spectrum has been measured by other groups. The liquids were introduced into the PNNL flow system via a Hamilton 500 μℓ syringe whose plunger was depressed at a constant rate by a Harvard Apparatus lead-screw driven constant flow syringe pump as given previously by Johnson et al. [54] The spectrometer and White cell arrangement were also re-designed to accommodate a better

thermostatted measurement, as well as to redress known artifacts such as the "warm aperture" problem (Johns et al. [55], Birk et al. [56], and Johnson et al. [57]) and detector nonlinearity (Sharpe et al.[52], Birk et al. [56]). Due to the weaker cross sections in the near-infrared, the White cell path length was increased to 14.52 m (± 0.5%). Other general experimental description can be found in Johnson et al.[54], Masiello et al.[58] and Johnson, Sharpe et al.[59] (2005). The only adaptations to the experiment to convert for near-infrared vapor-phase measurements involved alternate optical components on the Bruker IFS 66v/S to improve the NIR signal/noise: A near-infrared quartz beamsplitter and W-lamp source were employed, as well as a photovoltaic InSb detector. The mirror scan speed was adjusted to 20 kHz, but all other collection parameters were as for the mid-infrared measurements (Sharpe et al.[52]). The NIR spectra covered the 2,700 to 11,000 cm^{-1} range, for a total range of 550-11,000 cm^{-1}. Data is only plotted to ~7,000 cm^{-1} due to the limited number of bands at higher frequencies.

Picolinamide was purchased as a solid from Aldrich Chemical with a reported purity > 98%. It was dissolved in research grade CS_2 and the resulting solution injected into the spectrometer as described above. Because the temperature was around 25 ^oC the picolinamide condensed on the spectrometer cell walls and windows. High quality spectra could therefore not be collected. It may be possible to get high quality data by increasing the spectrometer internal temperature to 60 ^oC. Budget limitations precluded us from doing these experiments on this project.

6.2 SWIR Spectroscopy Results

Figure 1 reports the quantitative near-IR spectra of chloroform, pyridine and pyrrole; the upper two spectra have been vertically offset for clarity by 5 x 10^{-6} and 10 x 10^{-6}, respectively. The data are quantitative as each represents a concentration-path burden of 1 ppm-meter. The y-axis has been multiplied by 10^5 such that, for example, the 7,127.98 cm^{-1} peak of chloroform has an absorbance of 1.34 x 10^{-6} for mixing ratio of 1 ppm through an optical path of 1 m. The raw data have been used to form a composite quantitative fit (Sharpe et al.[52] and Johnson et al.[54]). The only other manipulation was to subtract trace amounts of H_2O vapor from the spectrum and also subtract a small but broad feature due to condensed ice on the detector near 3100-3300 cm^{-1}. The spectra are all pressure broadened to 760 Torr (high purity N_2 ballast gas) yet still display several resolved ro-vibronic peaks as detailed below.

Several peaks are of note, but we focus primarily on those that suggest themselves for trace detection using SWIR laser spectroscopy. In general, the C-H stretching region (2,800 - 3,100 cm^{-1}) often has strong bands, but only the narrowest of peaks provide any specificity; broad peaks are susceptible to interferences since almost all organic species absorb at these wavelengths.

The bottom trace in Figure 1 is the near-IR spectrum of pyrrole. Pyrrole, C_4H_5N is a planar aromatic heterocycle of C_{2v} symmetry. Its quantitative near-infrared spectrum is reported for the first time in Figure 1. The C-H peaks near 3100 cm^{-1} have been reported as a composite of three of the four C-H stretching fundamental bands, namely ν_{12}, ν_2 and ν_{13}:[60] These bands are resolved at low pressure and have been assigned,[60,61] but are not resolved in Figure 1 since the spectrum was taken at 760 Torr. The C-H peaks of the first overtone show up around 6150 cm-1 in Figure 2. These have also been previously assigned. In terms of SWIR CRDS monitoring the peak that offers the most promise is the first overtone ($2\nu_{24}$) of the N-H stretch made at 6924.29 cm^{-1}. It has A1 symmetry – i.e. there are a-type bands with a resolved Q-branch. These features

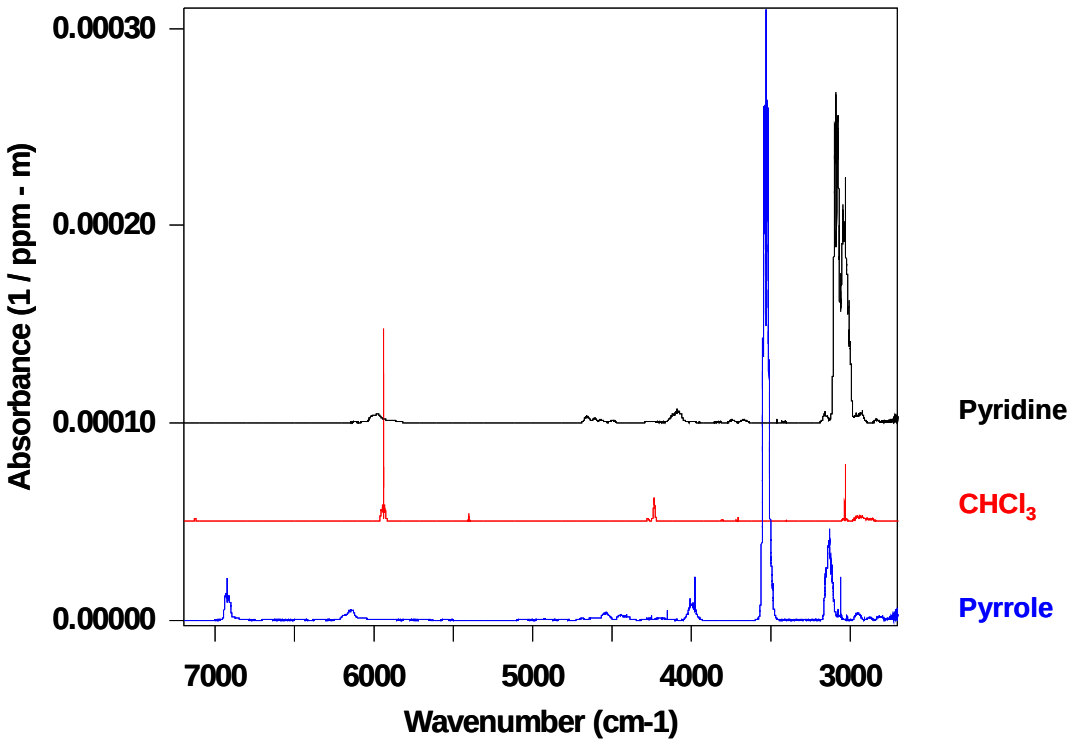

Figure 1. Measured FTIR spectra of vapor phase pyridine, chloroform and pyrrole. Spectra were taken at 760 Torr total pressure.

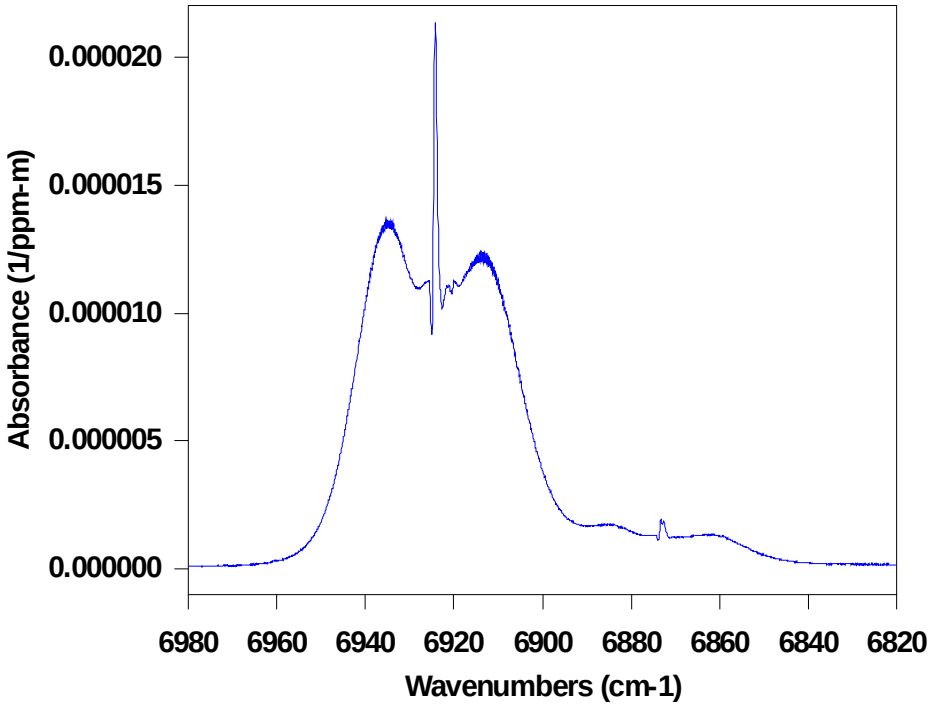

Figure 2. Expanded view of the $2\nu_{24}$ first N-H overtone band of pyrrole. Even though the spectrum was measured at 760 Torr with 1 cm^{-1} resolution, the fringe-like structure on the P and R branches indicate that many lines could be targeted in low pressure cavity ring down measurements.

are shown in detail in Figure 2. It is interesting to note that even though the spectrum was measured at 760 Torr with 1 cm^{-1} resolution, the fringe-like structure on the P and R branches indicate that many lines could be targeted in low pressure cavity ring down measurements. From the spectrum it can be seen that the pyrrole $2\nu_{24}$ N-H overtone Q band peak transition absorbance is 2.2 x 10^{-5} $(ppm\ m)^{-1}$ and has a bandwidth ~ 17 cm-1 (FWHM). The absorbance corresponds to an absorption cross section of 2.1 x 10^{-20} cm^2 $molecule^{-1}$. This is, to our knowledge, the first quantitative measurement of the pyrrole N-H overtone absorption cross section.

The pyridine near-infrared gas-phase spectrum is also displayed in Figure 1. The pyridine spectrum is much sparser than that seen for pyrrole and the bands in the shortwave region (~4000 - 7000 cm^{-1} do not have quite the sharp structure as was seen with pyrrole N-H overtone. Two transitions can be seen, one at around 4600 cm^{-1} and the other around 6000 cm^{-1}. The first transition, which is blown up and shown in Figure 3, is a series of combination bands, corresponding to ν(CH) + Pyr(8a), ν(CH) + Pyr(8b), ν(CH) + Pyr(19a), and 2Pyr(8b)+ Pyr(1), as per the assignments given by Van Meervelt et al.[62] The second transition, which is blown up and shown in Figure 4, consists of 3 bands that include 1) a combination of aryl ring 2,6 and 3,5 C-H stretch fundamentals, and 2) the first overtones of the 2,6 and 3,5 aryl ring C-H stretches.

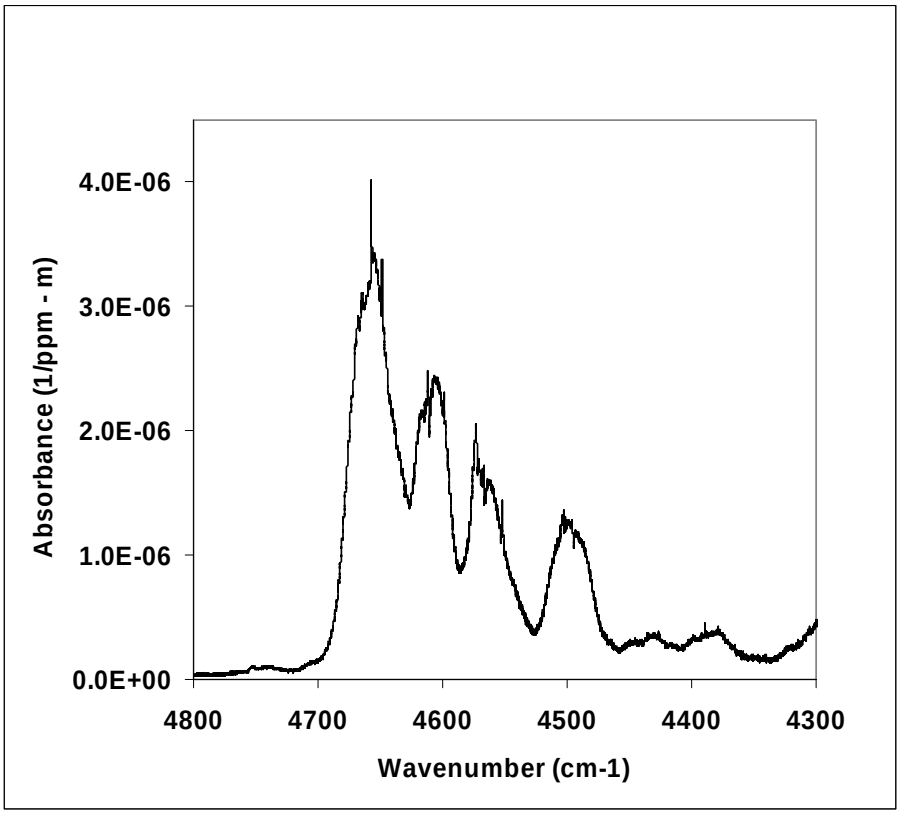

Figure 3. SWIR spectra of the combination bands associated with the pyridine ν(CH) + Pyr(8a), ν(CH) + Pyr(8b), ν(CH) + Pyr(19a), and 2Pyr(8b)+ Pyr(1) vibrations.

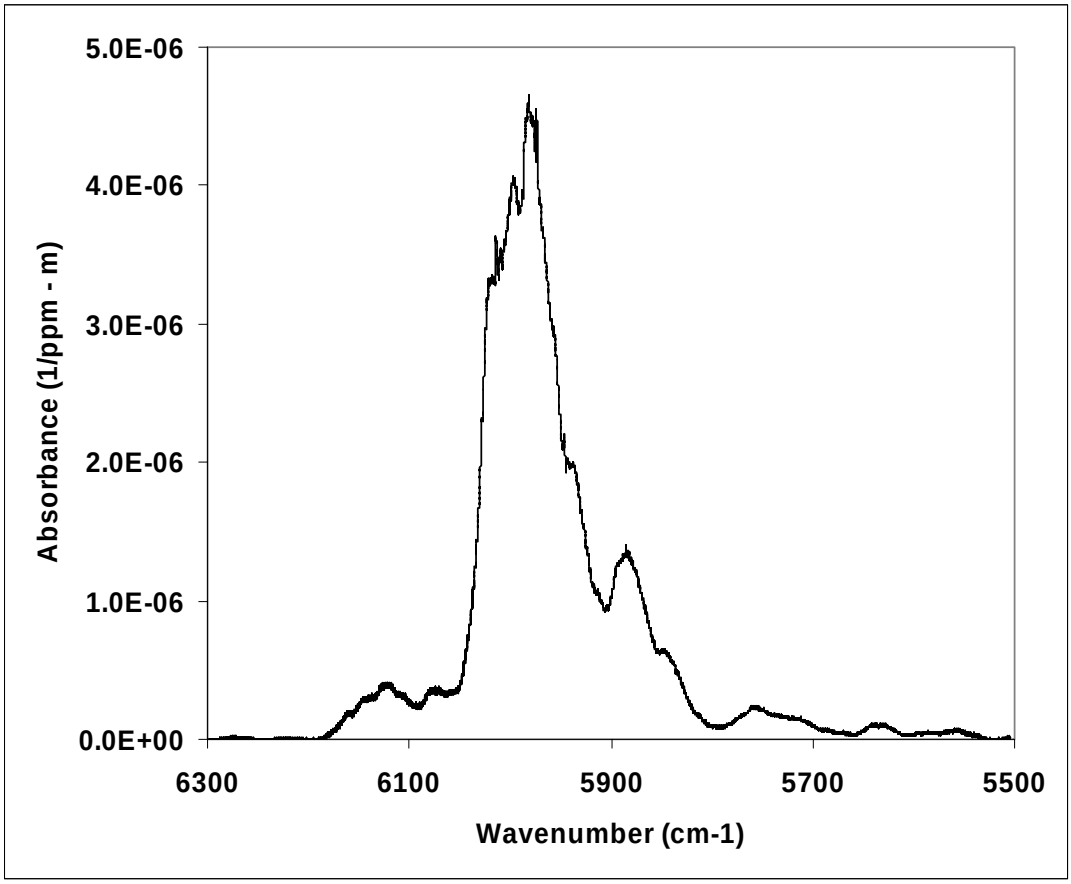

Figure 4. SWIR spectra of pyridine 2,6 and 3.5 CH stretch combination band, and 2,6 and 3,5 C-H stretch first overtones.

While most of the SWIR bands seen for pyridine are associated with C-H stretches, the spectra are unique enough that these bands could be used to positively confirm this molecule's presence, even in the presence of other hydrocarbon species. The peak absorbances observed in the 4600 and 6000 cm-1 regions are 4 x 10^{-6} and 4.6 x 10^{-6} ppm^{-1} m_{-1}, respectively. These correspond to absorption cross sections of 3.7 x 10^{-21} and 4.3 x 10^{-21} cm^2 $molec^{-1}$, also respectively.

6.3 Estimated SWIR CRDS Detection Sensitivity for Pyrrole and Pyridine Biomarkers

PNNL has built and fielded a SWIR CRDS system which is described in detail in Williams et al., 2002, 2003, 2004 and 2005.[63 – 66] The system detection sensitivity limits for ammonia have been determined to be 0.5 ppmv at 335 Torr operating pressure using the absorption line at 6528.76 cm^{-1}. This corresponds to a molecular detection sensitivity of 5.75 x 10^{12} molec cm^{-3}. The absorbance for this line at 1 atm pressure (so that comparison with spectroscopy results for pyridine and pyrrole is relevant) is 1.21 x 10^{-5} ppm^{-1} m^{-1} which corresponds to an absorption cross section of 1.12 x 10^{-20} cm^2 $molec^{-1}$. The pyrrole first N-H overtone absorption coefficient was determined to be 2.1 x 10^{-20} in this study. Since CRDS detection (as measured from the ring down time) scales linearly with absorption cross section and species concentration we can estimate the detection sensitivity for pyrrole to be;

$$[\text{pyrrole}]_{\text{min det}} = (\sigma_{NH3}/\sigma_{\text{pyrrole}}) \times [NH_3]_{\text{min det}}$$
$$= (1.12 \times 10^{-20}/2.1 \times 10^{-20}) \times 5.75 \times 10^{12} \text{ molec cm}^{-3}$$
$$= 3 \times 10^{12} \text{ molec cm}^{-3}.$$

For pyridine the best detection sensitivity would occur from monitoring the band in the 6000 cm^{-1} region, here the absorption cross section was determined to be 4.3×10^{-21} cm^2 molec-1. The SWIR CRDS detection sensitivity for pyridine should therefore be 1.5×10^{13} molec cm^3.

7.0 SWIR CRDS Feasibility for Detection Biological Agent Pyrolysis Products

PNNL's SWIR CRDS instrument is outfitted with a cavity that has a volume of 490 cm^3. If it is assumed that a sampling apparatus could be designed such that all gas phase products from biological agent pyrolysis would be swept into a prior evacuated CRDS cavity, then the amount of pyrrole and pyridine that needs to come from pyrolysis in order to be detected is 1.47×10^{15} and 7.35×10^{15} molecules, respectively.

There is very little quantitative information on pyrolysis yields of particular chemicals from particular biological species. Most of the published studies on the pyrolysis of bacterial spores have not chemically identified all of the pyrolysis products, reactions, and reactants that are involved in the production of biomarkers. Typically there have been only superficial investigations of particular target compounds and there is, to our knowledge, no data relating observed biomarker signals (i.e. GC, MS, IMS, etc.) to absolute concentrations. Schmidt et al.[35] have reported that their particular pyrolysis apparatus is capable of converting 0.28% of *Escherichia coli* bacterial mass into a gas chromatographic/flame ionization detector response. We will take this as a lower limit for conversion, since this figure does not account for instrument response to different chemicals and necessarily includes loss of gas phase pyrolysate to the GC capillary walls. Fractional pyrolysis yields of pyridine and pyrrole have also not been determined for whole bacterial species. Kuckuk et al. have reported that pyrrole accounts for 0.5% of the gas produced when humus is pyrolyzed.[67] Since humus is expected to contain far less protein (the major source of the pyrrole pyrolysis product) than bacterial spores, we will take this as a lower limit for the production of both pyrrole and pyridine. Another important source for pyridine is the pyrolysis of dipicolinic acid, which comprises 5 = 15% of dry spore weight, but we will ignore this here since we are doing a conservative estimate.

For every microgram of biological spore sample put into a pyrolysis chamber, and assuming that all the gas phase effluent is flown into the SWIR CRDS cavity, the amount of pyrrole or pyridine produced will be;

1 μg biological sample x 0.0028 pyrolysis x 0.005 fraction gas phase products = 1.4×10^{-11} g
= 1.4×10^{-11} g/67.09 g/mol x 6.022×10^{23} molec/mol = 1.256×10^{11} molecules.

This number is 4 orders magnitude below the estimated SWIR CRDS pyrrole and pyridine detection sensitivities which are 1.47×10^{15} and 7.35×10^{15} molecules, respectively. To generate a detectable amount of pyridine or pyrrole it would therefore be necessary to collect and pyrolysis a 12 mg biological sample. This is far larger than is required for current sensors. It is to be noted that we have used very conservative estimates for the overall pyrolysis efficiency and product fractions, however it is difficult to imagine that these could be changed by a factor of 10,000 by optimizing the pyrolysis conditions. It is therefore concluded that while possible, the large amounts of material required preclude using SWIR CRDS for detecting biological agents at this time.

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