

Rapid Detection of Low Concentrations of Aqueous Species in the Presence of Spectral Overlap Using Planar Array Infrared Spectroscopy

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Index Headings: Planar array infrared spectroscopy; PA-IR spectroscopy; Aqueous solutions; Spectral subtraction; Focal plane array; FPA.

INTRODUCTION

It is well known that water is the Achilles' heel of infrared spectroscopy. The rotational-vibrational manifold of gas-phase water spans several hundred wavenumbers in the fingerprint region and necessitates that instruments be continuously purged with dry gas. This problem is especially vexing when studying aqueous systems, since the absorptivity of condensed-phase water is relatively high, necessitating a balance between path length and sensitivity when attempting to study dilute aqueous samples.

A common approach to the removal of spectral contributions from water is to perform a spectral subtraction, using pure water as a reference. This is only possible if the path length of the sample cell is small enough to prevent saturation of the absorption signals from water itself. Typically, path lengths of several micrometers are employed to keep the absorbance intensity of water within the linear range. However, this short path length requires that high concentrations of the species of interest be employed to maximize the signal-to-noise ratio of the final spectrum. Many species of physiological relevance exist at concentrations well below 1% w/w *in vitro*. Of particular interest is the analysis of proteins in the aqueous humor of the human eye, which has a typical total protein concentration of approximately 0.1% w/w.¹ Additionally, specific proteins of interest to the detection and diagnosis of eye diseases, such as vascular endothelial growth factor (VEGF), exist at much lower concentrations, on the order of 10^{-7} % w/w.² Thus, in order to study these species under

realistic conditions, care must be taken to remove any spectral interference.

The common approach to avoiding interference from H₂O is to use D₂O solutions, but it is well known that this can cause changes in the structure of proteins.³ Several instrumental and data processing approaches have been devised to minimize water interference, including attenuated total reflection,^{4,5} Fourier self deconvolution,⁶ spectral subtraction,⁷ and optimization of transmission cell thickness.⁸

Recently, we have developed planar array infrared (PA-IR) spectroscopy as a method to acquire infrared spectra rapidly, from both static and dynamic chemical systems.^{9–11} Here, we use PA-IR as a means of acquiring spectra of dilute aqueous solutions that are free from the interference of both gaseous and liquid water.

EXPERIMENTAL

All experiments were carried out using a planar array infrared (PA-IR) spectrograph described in detail in previous publications.^{9,10} Data were acquired using 50 and 150 grooves/mm gratings and a 256 × 256-pixel mercury cadmium telluride focal plane array detector (Santa Barbara Focal Plane). All spectra were the result of 10 co-additions of 100 frames each, which required approximately 10 seconds to acquire. Aqueous solutions of poly(acrylic acid) (PAA) and poly(N-isopropyl acrylamide) (PNIPAM) were prepared using deionized water. The solutions were analyzed in transmission mode by placing a portion of the solution in a liquid cell (BioTools, Inc) that was equipped with CaF₂ windows and provided a sample thickness of approximately 3 μm. Reference spectra were acquired with the same sample cell filled with deionized water. The spectral processing, including absorbance calculation, subtraction, and slit curvature correction, was carried out using custom-written Matlab routines, which have also been described previously.¹¹

RESULTS AND DISCUSSION

Several approaches were taken to acquire spectra that were free from interference from water. It was found that the subtraction of liquid water was easily accomplished, but the subtraction of water vapor was the limiting factor in the ultimate sensitivity achievable using PA-IR. An example that demonstrates the difficulty in achieving this goal can be found in Fig. 1, which shows the absorbance spectra of neat water and a 1% w/w PAA solution. The PAA carbonyl stretching band at 1729 cm⁻¹ is barely visible as a shoulder on the high frequency side of the H–O–H bending mode of H₂O at 1640 cm⁻¹ and is on the same order of magnitude as the residual water vapor bands. The result of spectral subtraction for a series of PAA solutions is shown in Fig. 2. This shows that the liquid water can be successfully subtracted, while the residual water vapor absorption bands are still the major contributors to the “noise” seen in the baseline. These results were obtained with an instrument designed for studying systems at higher concentra-

Received 27 September 2007; accepted 8 January 2008

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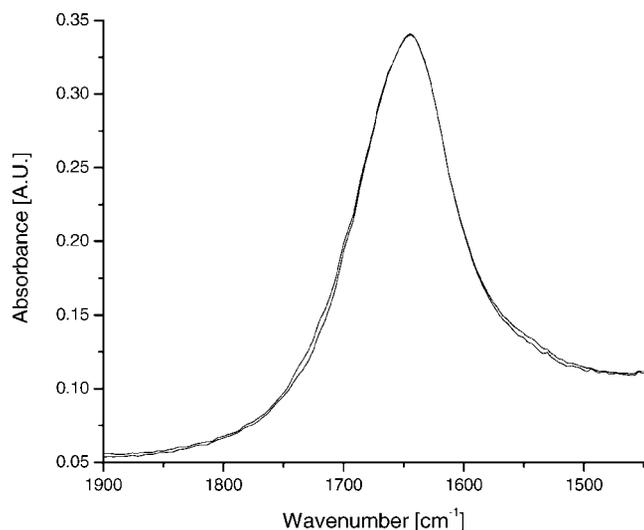


FIG. 1. Spectra of water and a 1% w/w aqueous poly(acrylic acid) solution.

tions, where water vapor interference is easily handled by simply purging the instrument with dry air.

Through a series of systematic trials, it was found that the key to achieving a successful subtraction of both liquid- and vapor-phase water was twofold: minimizing stray light and achieving a constant, low concentration of water vapor in the optical path. It was found that even very small amounts of stray light led to baseline artifacts after subtraction. To avoid these complications, baffles constructed from corrugated cardboard were placed throughout the optical setup to prevent any stray light from reaching the detector. It was found that a constant purge environment was more critical than a low absolute humidity, and the results shown in the figures above were acquired with a relative humidity in the vicinity of 25–30%.

Due to small differences in the single beam spectra between the background and sample, the small changes that are normally not noticed in the collection of spectra from samples of higher concentrations become significant. Likewise, it becomes difficult to perform these experiments using the

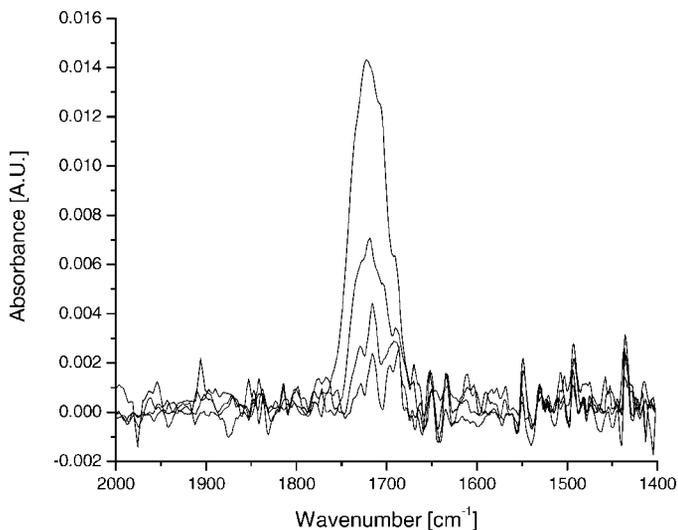


FIG. 2. Results of subtracting a pure water spectrum from aqueous poly(acrylic acid) spectra at concentrations of 2.0, 1.0, 0.1, and 0.05 % w/w.

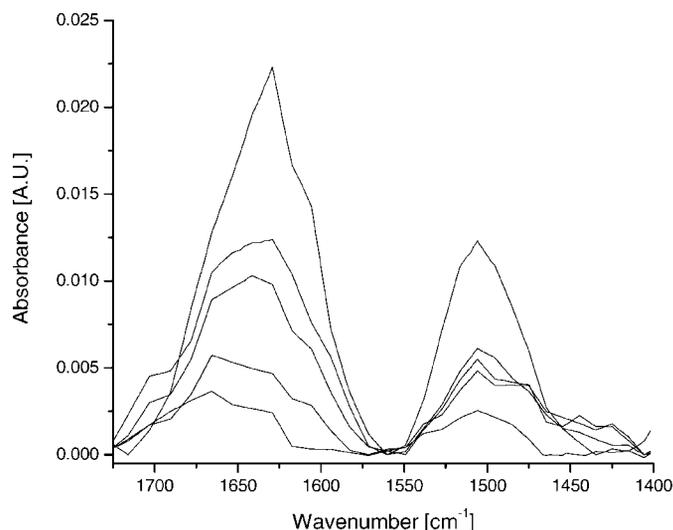


FIG. 3. Results of subtracting a pure water spectrum from aqueous poly(N-isopropyl acrylamide) spectra at concentrations of 2.0, 1.0, 0.5, 0.05, and 0.005 % w/w.

previously described dual-beam setup.¹¹ It is seen that, even for small changes in the amount of water vapor present in the spectra, the water vapor cannot be completely subtracted out due to spectral artifacts arising from the curvature correction routine. It is therefore necessary to perform the experiment in a serial fashion. Due to the small amount of time required for data collection, this does not pose a major limitation, since the fluctuations in the water vapor concentrations are small on this time scale. Additionally, the signal-to-noise ratio can be improved in this approach by row binning, as described in previous PA-IR studies.^{9–11}

The best results were achieved by carefully purging the instrumental setup with liquid nitrogen blowoff that was passed through a purge gas generator. A nearly leak-tight enclosure was constructed that allowed for maintaining a controlled purge environment, and the dry nitrogen was fed uniformly into the enclosure through perforated tubing placed throughout the bottom of the enclosure. Using this approach, it was possible to keep the relative humidity to around 15%, even though the enclosure volume was approximately 1 m³. The results obtained from PNIPAM solutions acquired with this setup are shown in Fig. 3, where it is shown that solutions down to a concentration of 0.005% w/w can be successfully studied. These results are quite remarkable, given the acquisition time of only 10 seconds, the use of only a single row of data from the image, and the direct overlap of the Amide I band of PNIPAM and the H–O–H bending mode of H₂O. It should be noted that the curvature correction routine, which includes an interpolation step, was not employed in this case, and the data therefore appear to have a lower signal-to-noise ratio. These results demonstrate that PA-IR is capable of analyzing low concentrations of aqueous species, despite the presence of strong band overlap from vapor- and liquid-phase water. The developments described here allow for the routine study of low concentration solutions without resorting to the use of D₂O or more complicated spectral processing routines. We anticipate that this approach will be particularly relevant in the study of proteins at physiologically relevant concentrations, and these investigations are currently being explored in our laboratory.

ACKNOWLEDGMENTS

The authors thank the National Institutes of Health for funding this work under grant number 4R33EB03288-03.

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Indirect Determination of Molecular Chlorine by Fourier Transform Infrared Spectrometry

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Index Headings: **Fourier transform infrared spectroscopy; FT-IR spectroscopy; Gas analysis; Chlorine gas.**

INTRODUCTION

Nowadays, Fourier transform infrared (FT-IR) spectrometry (both extractive and open-path techniques) is widely used for the detection of air pollutants^{1–6} because the method is fast, reliable, and nondestructive. It is capable of detecting simultaneously all compounds except non-infrared active homonuclear diatomic molecules.

Molecular chlorine gas has many anthropogenic sources, including water treatment plants, chemical production facilities, ceramic brick industries, and paper production processes,^{7–9} and it can also be formed in marine environments by reactions with sea salt particles.^{10–13} Various sensors have been applied to detect chlorine, including chemical sensors based on semiconductor metal oxides.¹⁴ Mass spectrometry also can be used for the determination of halogen molecules at atmospheric

pressure.^{10,15} A mist chamber and ion chromatographic detection has been employed to detect molecular chlorine indirectly at the parts per trillion (ppt) level.¹⁶ Spectroscopic techniques (e.g., ultraviolet (UV), Raman) are also capable of measuring molecular chlorine directly. An artificial chlorine cloud was investigated by Edner et al.¹⁷ by the differential absorption LIDAR (light detection and ranging) method.

Fourier transform infrared spectrometry has already been used to detect a homonuclear diatomic molecule. Larsen and co-workers¹⁸ measured the concentration of oxygen in a gas cell by determining the concentration of ozone produced upon UV irradiation of the sample. The authors established a linear correlation between the concentration of oxygen in the original sample and the concentration of ozone produced by photolysis of the sample. The aim of the present work was to investigate whether it is possible to detect chlorine molecules by means of FT-IR spectrometry as predicted by Hanst.¹⁹ If successful, a new analytical method can perhaps be developed based on this investigation.

EXPERIMENTAL

A Bio-Rad FTS-185 series spectrometer and a multipass gas cell (Infrared Analysis Inc., Anaheim, CA) with a 25.7-m effective path length were used for the experiments. The special cell was equipped with a mercury UV-radiation source, whereby ozone and reactive radicals can be produced. The source of the radiation was a medium-pressure quartz mercury lamp. This type of lamp emits one or two percent of its radiation in the 185 nm mercury line and about 90% in the 254 nm line, with the remainder in lines that fall in the near-UV and visible ranges. The 185 nm photons dissociate oxygen molecules and the resultant oxygen atoms react mainly with molecular oxygen to form ozone. The ozone can react with other molecules, or it can absorb the 254 nm photons and be photo-dissociated, giving oxygen atoms in the energetic singlet-D state. These energetic atoms react with water to produce hydroxyl radicals. The ultraviolet photons can also directly photo-dissociate pollutant molecules.¹⁹

The concentration of molecular chlorine gas was measured with a wide-range vacuum gauge (Kurt Lesker Co, KJL902074) connected to a vacuum system. Following introduction of a known quantity of chlorine gas and normal air (according to the analysis of the infrared spectra, this air contained 6650 ppm water vapor, 333 ppm carbon dioxide, 1.77 ppm methane, 0.32 ppm nitrous oxide, and 0.30 ppm carbon monoxide) at atmospheric pressure into the evacuated gas cell, the UV lamp was switched on after recording the first single beam spectrum. Next, a kinetic study was carried out during the photolysis: a spectrum was recorded every 15 s.

RESULTS AND DISCUSSION

Since there are many possible reactions (e.g., Eqs. 1–5) beyond ozone and reactive radical formation under the influence of the UV lamp, it is not practical to try to explain everything that could occur in the gas cell. In the course of the reactions, chlorine will be produced in atomic form and serve as a catalyst for ozone depletion. Some possible reactions are given here:^{20,21}



Received 19 June 2007; accepted 7 January 2008.

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