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Michael McClain April 20, 2011

Gated Proton Transfer Reactions at Low Temperatures in D2O

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Abstract

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Proton transfer reactions were studied using 2-nitrobenzaldehyde (NBA), a photoacid, as a proton donor and ammonium acetate as a proton acceptor in D2O. The reactions were performed at temperatures ranging from 20 K to 200 K and rapid scan FTIR was used to observe the reaction progression. At temperatures below 150K, a ketene intermediate in the photochemistry reaction of NBA was observed. Between 17K and 200K we were able to observe a proton transfer occur at a time scale over hald an hour. Rates were obtained for the protonation of ammonium acetate at these temperatures and were used to approximate the activation energy: 66 kJ/mol.

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1. Introduction

Proton transfer in water is one of the most fundamental reactions in numerous chemical and biological processes. For example, proton transport through proteins (proton pumping) is a crucial step in storing energy for prokaryotes and eukaryotes [1]. In many biological processes, protons are transported through narrow protein channels containing water wires [2]. Water wires are unidimensional networks of waters molecules where ionic transport occurs. Within these protein channels, higher diffusion rates than in bulk solution have been observed through both simulations and experiments [1,2]. This suggests that proton transport through water involves a process other than simple diffusion. The most accepted mechanism of proton transport is the Grotthus mechanism, where a proton is transported through water's extensive hydrogen-bond network through a series of rearrangements of water molecules [2]. Water's hydrogen bond network has been the subject of much research, and the effect of ionic species on its structure and ability to transport protons is an important problem with applications in numerous areas of scientific research [1-6]. Understanding what conformations the water molecules adopt during proton transport will greatly enhance current aims to engineer proton channels and our understanding of proton transport through proteins in general.

The study of proton transfer in water began with a paper published in 1806 by Freiherr von Grotthus, which is now refered to as the Grotthus paper [7]. The modern explanation of the original mechinsm is sequential transfer of protons between water through breaking and forming new bonds. However, it was not until

the early 20th century that the importance of this mechanism was recognized. During this time, there was much effort in predicting the mobility of ions in water and comparing them to empirical results. Diffusion was modeled as Brownian motion, or the random motion of a particle through a solution. While these predictions were sufficient for ions, the predictions for the mobility of protons were significantly less than what was observed. This anomaly was explained using the Grotthus mechanism and has been continually modernized to include structural rearrangements of water's hydrogen bond network, for example by rotation of water molecules into geometries more preferential for sequential proton transfer [7].

The proton's mobility is related to the structural rearrangement of the hydrogen bonds between water molecules to accomdate the proton. The rearrangements results in two solvated proton conformations: $H_5O_2^+$ and $H_9O_4^+$, the Zundel and Eigen cations (**Fig. 1)** [4,7]. Another possible contribution to the proton mobility is that rate of rotation of water molecules in the liquid state [7].

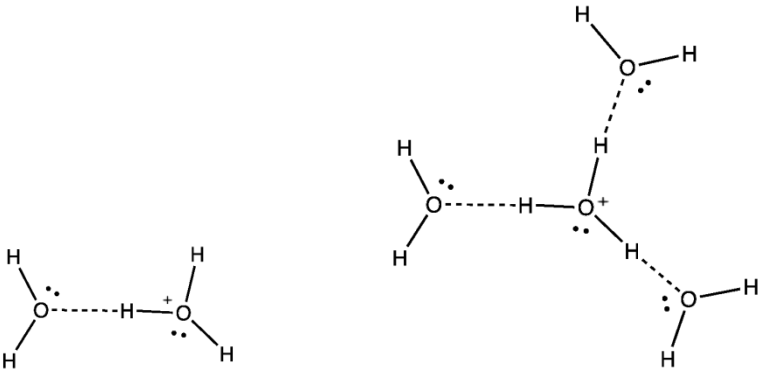

Figure 1. Diagram of the Zundel (left) and Eigen (right) cation species.

Between the process of structural rearrangement and the rotation of molecules, it is unclear which is the rate-limiting step. For almost a century after the revival of the

Grotthus mechanism, it was thought that the primary barrier to proton transfer is due to the rotations of the water molecules. Experiments done by Agmon suggesteded that at temperatures higher than 293K, water molecule rotation is the limiting step. As the temperature is lowered, the activation energy for rotation increases because water can form four hydrogen bonds; therefore, more bonds need to be broken before a molecule can rotate. At higher temperatures, the number of hydrogen bonds decrease, and this decreases the activation energy required for a water molecule to rotate. These results demonstrate that attempting to define proton transfer down to a single rate-limiting event is not possible, and the mechanism may vary depending on the environment [7].

Proton transfer is a vital process in many bioenergetic enzymes [1,2,7]. Many of these proteins have cavities of water molecules which are coordinated by residues to form virtually one-dimensional chains. It is hypothesized that the polar residues can facilitate proton transfer, and many important enzymes such as cytochrome c oxidases, and hydrogenases share this common characteristic. However, there are also examples of proton channels that lack hydrophilic residues and still promote efficient transfer of protons, such as gramicidin A [1]. Research using these model enzymes aims at elucidating how the proteins influence proton transfer rates: whether or not specific residues can help shuttle the protons and whether protons are shuttled via the Grotthus mechanism.

Another important system where proton transfer has been studied is ice. As it was mentioned before, as the temperature of water decreases it is more likely to form four hydrogen bonds. Once water has been frozen all water molecules form

four hydrogen bonds and although the water molecules may not be able to rotate, proton transfer still occurs at a high rate [13]. The mechanism in this case may be diffusion of a charge defect through reorganization of covalent and hydrogen bonds [7,13]. Despite the numerous constraints imposed by each of these systems: bulk solution, water wires, and ice, proton mobility remains high.

Infrared spectroscopy has been used extensively to study and characterize the dynamics of water structure and proton transfer. In infrared spectroscopy, molecules are subjected to light with wavelengths greater than 2500 nm that induce transitions to different energy states. Absorption of mid infrared (4000 cm^{-1} to 400 cm^{-1}) light induces transitions in the vibrational energy levels of the molecules, which will oscillate at the frequency of the light absorbed. Scanning the absorption of a sample over this region, one can obtain information about the bonds and structure of molecules present. To better understand how vibrations give insight into structure features of molecules, it is useful to use a model such as the harmonic oscillator, which oscillates at a frequency proportional to the square root of the spring force constant divided by the reduced mass. Each molecular bond has its own force constant which is proportional to the strength of the bond. In water, a large distribution of hydrogen bonds strengths are formed as a result of water's extensive hydrogen bond network. By forming a hydrogen bond, some of the electron density is shifted away from the H-O bonds, weakening it. As a result, water's IR bands are inhomogenously broadened. Furthermore, the presence of ions may also cause this increase in heterogeneity [8-11]. In an infrared spectrum this can make observation of certain absorptions difficult so a solution is to use

deuterium (D), a heavier isotope of hydrogen. This increases the reduced mass, which then lowers the frequency. H-D exchange is vital in infrared experiments where the required solution is water, because now the distribution of absorptions have shifted to different regions, opening new "windows".

Another area of research that has helped in elucidating the proton transfer mechanism is in theoretical predictions. Absorption frequencies for the Zundel and Eigen cations have been calculated from the case of a free ion to the species in bulk water [4,5]. These calculations have aided interpretation of spectroscopic data by giving researchers an idea of where to look for these transient species. For example, using ultrafast IR spectroscopy, a transient species has been observed on the timescale of picoseconds [5] and assigned as an Eigen cation on the basis of comparison with calculations.

We have used an alternative approach to capture proton transfer intermediates, by slowing down the reaction at cryogenic temperatures. We have investigated proton transfer at cryogenic temperatures (20K to 150K) using rapid scan Fourier transform infrared spectroscopy (FTIR). Rapid scan FTIR collects the spectrum by continuously moving one of the interferometer mirrors. The collected interferogram is then Fourier transformed to give a static spectrum of the sample in question. If we were to make an acidic solution, we would not observe proton transfer, only the time-averaged equilibrium state. The reaction must somehow be triggered in a coherent sense in order to follow the subsequent proton transfer dynamics. Photoacids are molecules that upon illumination are converted to an excited state or another molecule (via a photochemical reaction) with a significantly lower pK_a. In the previously mentioned ultrafast

experiment where the Eigen species was observed, the photoacid HPTS (8-hydroxy-1,3,6-trisulfonate-pyrene) was used [5]. However, this compound cannot be applied to our experiment because its excited electronic state has a lower pK_a and too short a lifetime to be observed with rapid scan FTIR. 2-Nitrobenzaldehyde forms a photoacid upon illumination of UV light in an irreversible process, making it ideal for our experiments. In this study we have used ammonium acetate as the proton acceptor and by monitoring the carboxyl stretching frequencies, we can determine whether or not it has been protonated.

By performing experiments at low temperature, the reaction can be slowed down enough to allow us to capture any intermediates in the reaction and the spectrum can be collected over a time scale longer than picoseconds. Collecting spectra over a temperature range gives significant insight into the kinetic barriers of the proton transfer reaction.

2. Materials and Methods

2.a. Reagents and Sample Prep

2-nitrobenzaldehyde (NBA) from Fluka >99.0% was dissolved in a solution of 100mM ammonium acetate from ACROS dissolved in D_2O atom D 99.9% from Sigma Aldrich. The supernatant was collected and filtered. A separate solution was prepared by dissolving NBA in D_2O.

2.b. Data Acquisition and Instrumentation

100mM solution of NBA and ammonium acetate in methanol and 100mM solution of NBA in methanol were prepared. Separate 100mM solutions of ammonium acetate were prepared in D_2O and H_2O and NBA was added to each. The

supernatants were extracted into two new vials. Solutions were prepared with minimum exposure to UV light and were stored with aluminum foil wrapped around the vial.

The samples for FTIR measurements were prepared by placing approximately 17μL of a solution between two CaF_2 windows with a 25μm thick two-compartment Teflon spacer. The spectra were recorded with an Excalibur series FT-IR using ResPro software (Varian). 5 measurements of 128 scans for each sample were obtained and referenced to a sample containing only solvent to confirm presence of NBA and ammonium acetate, or solvent and NBA to confirm presence of ammonium acetate. Acetate absorption spectra were obtained at room temperature and 20K using 100mM and 400mM solutions in D_2O respectively

A fresh sample was prepared with a cell containing only the NBA solution with a 25μm spacer. Then, a shutter was used to block the light as five more sets of 128 scans were taken and averaged. The resulting spectrum was ratioed [I know what this means, but I don't think it's a real word, and I'm not sure how to say it correctly without using a lot more words] to the average spectrum before illumination providing a light versus dark (LvD) difference spectrum. Another sample was prepared using the NBA and acetate solution and the LvD spectrum was obtained in a similar manner. For the low temperature experiments, a fresh sample was used at each temperature and for only one illumination. Once the cryostat had reached the desired temperature, the sample was given an additional two hours to ensure stability (baseline measurements taken over half an hour further ensured the sample was at equilibrium at a temperature). 128 scans were taken at 20 kHz

with a resolution of 4 cm^{-1}, illuminated for 15 seconds, and then 128 scans were taken sequentially over the next two hours. At the end the light spectra were ratioed to the dark spectrum at each temperature. LabView was used to record spectra and control illumination through an interface with a shutter.

3. Results and Discussion

The results and discussion are divided into three sections. The first section is regarding the LvD reaction of NBA to ammonium acetate in methanol. In the second section, the LvD reactions are performed in D_2O at room temperature and from 20K to 150K. Finally, the LvD spectra obtained between 170K to 200K are presented and discussed. In this temperature range we began to see the spectra evolve over time.

3.a. Proton Transfer Reactions in Methanol

3.a.i. Results

Since the overall goal of this experiment is to investigate the mechanism of proton transfer in a solution, the methods used should simplify the process as much as possible. For example, to study the rate of proton transfer, it would be much easier to arrive at the rate the proton travels over a distance if there are equal numbers of proton donors and acceptors present in solution because we can predict the average distance between the two species more easily. However, there is a problem if we have this goal in mind and are working with water as the solvent: o-Nitrobenzaldehyde is not very soluble in water. Methanol, on the other hand, can participate in hydrogen bonding, and also readily dissolves NBA, providing several practical advantages. The higher solubility of NBA means we can work with higher

concentrations of NBA to observe more generation of photoproduct with shorter illumination times. Greater yield of photoproduct with shorter illumination times produces larger IR signals while also minimizing the heating of the solution by the photolysis laser.

The LvD spectrum of NBA and AA in methanol is similar to that of the NBA LvD spectrum in D_2O. Both spectra contain the bleach at 1535 cm^{-1} and absorption at 1730 cm^{-1}. The LvD spectrum of NBA and AA in D_2O contains two bleaches at 1535 cm^{-1} and 1560 cm^{-1}, and two absorptions at 1710 cm^{-1} and 1600 cm^{-1}.

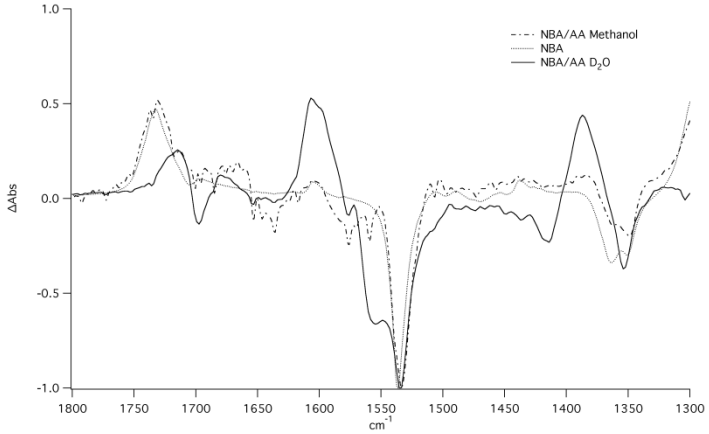

Figure 2. Light versus dark difference spectra of NBA and AA in methanol, NBA, and NBA and AA in D_2O recorded at 295K.

3.a.ii. Discussion

Unfortunately we encountered a problem with the use of methanol as a solvent for these experiments.

The LvD spectrum of NBA and AA in methanol is almost identical to that of NBA in D_2O. The peak at 1730 cm^{-1} is from the C=O bond of the carboxylic acid of the photoproduct, therefore protons are remaining on photoproduct and not

protonating the carboxyl group of AA. Protonation of ammonium acetate is signified by the bleach at 1560 cm^{-1} and absorption at 1710 cm^{-1}, features absent from the spectrum of the sample in methanol solvent. This is concerning because AA with a $pK_a \approx 8$, would be expected to be protonated upon formation of nitrosobenzoic acid, $pK_a \approx 3$. We confirmed there was AA in the solution by ratioing [same comment as before] the single beam spectrum to a sample containing NBA in methanol, so this eliminated a trivial conclusion. These results are understood in light of previous work by Bosch et al reproduced in Fig. 3 [16].

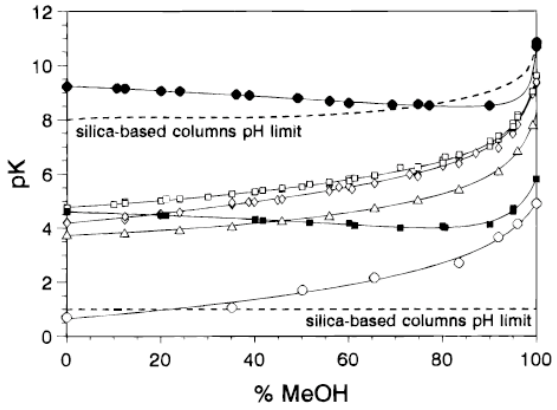

Figure 3. Variation of pK_a values of acids with varying methanol-water concentrations.: (◇) benzoic acid, (□) Acetic Acid. Figure taken from [16]

In **Fig. 3**, the pK_a of benzoic acid, which is similar in structure to our photoacid, is greater than acetic acid in solutions with less than 15% water. We assume that NBA acid is similar enough to benzoic acid for us to make a qualitative comparison. sentence fragment] Therefore, at the percent compositions of water in methanol we were using, we would not expect to see protonation of acetate. In our own experiment, we did not see significant protonation at even 20% H_2O. As we increased the percent of water further we began to see precipitation of NBA, which

would be more substantial at the cryogenic temperature range we aim to study. Finding a mixture of methanol and water where we could observe proton transfer to the acetate would complicate our experiment that is directed towards biological pathways where the solvent is overwhelmingly water. The problem of precipitation also affects our aim to have equivalent amounts of acid and base. At this point in the study our goal was to have a working system with as few variables as possible.

3.b. Gated Proton Transfer Reactions in D_2O at Room Temperature, and Between 20K and 150K.

3.b.i. Results

The proton transfer reaction occurs as expected at room temperature, and the question is can we slow down the reaction at cryogenic temperatures. Here we present the LvD spectra of NBA and ammonium acetate in D_2O at room temperature and compare the spectrum to those recorded from 20K to 150K.

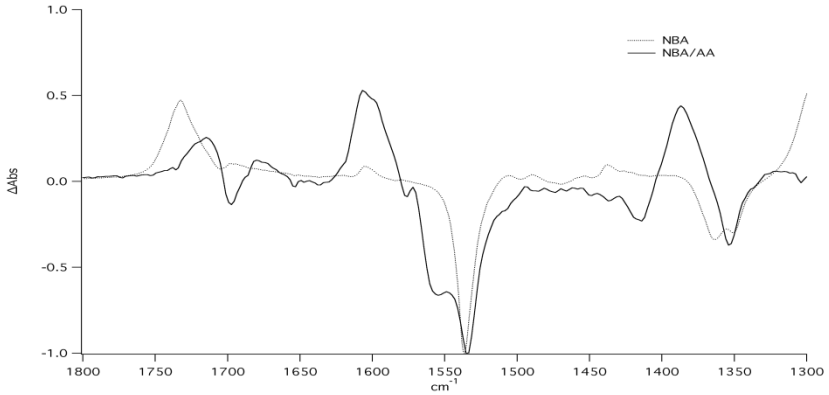

Figure 4. Light versus dark difference spectrum of NBA (dashed) and NBA and AA (solid) in D_2O recorded at 295K.

The room temperature LvD spectrum of NBA in D_2O has two prominent features (**Fig. 4)**: a bleach at 1530 cm^{-1} and absorption at 1730 cm^{-1}. For the sample

containing NBA and AA, there is a bleach at 1560 cm^{-1} in addition to the bleach at 1530 cm^{-1}. Also, there is an absorption at 1710 cm^{-1} and 1600 cm^{-1}.

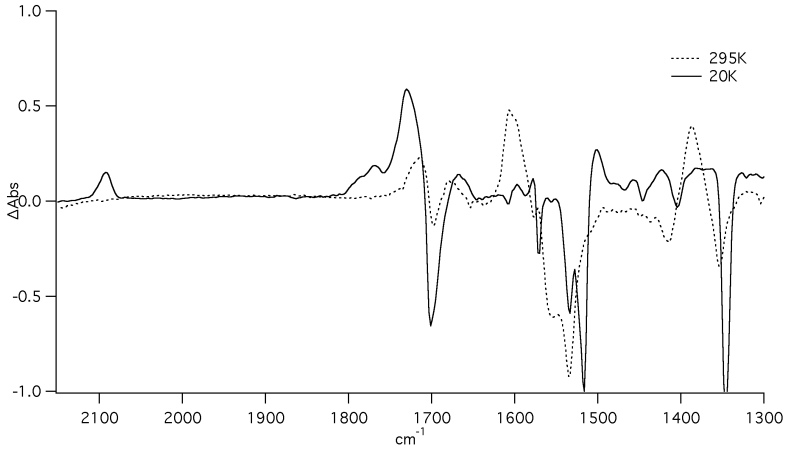

Figure 5. Light versus dark difference spectrum of NBA and AA in D_2O at 20K and 295K.

The spectrum recorded at 20K (**Fig. 5)** has a sharp 1730 cm^{-1} absorption and two bleaches present between 1510 and 1530 cm^{-1} and has some features of the spectrum recorded at 295K. There is a prominent absorption at 2100 cm^{-1} present in the 20K spectrum.

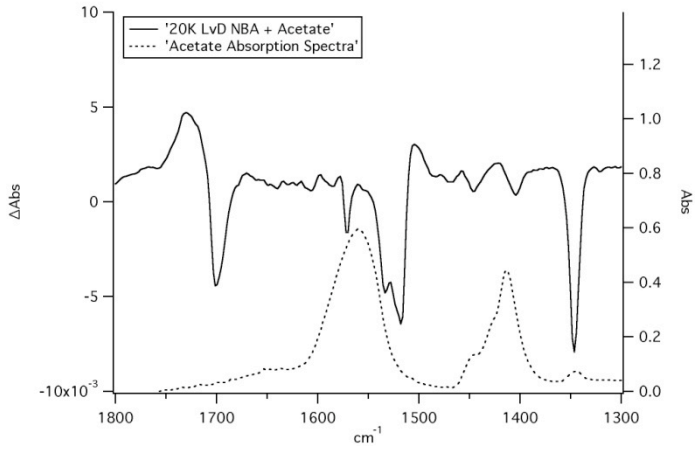

Figure 6. Light versus dark difference spectrum of NBA and Acetate (left ordinate) and acetate absorption spectrum (right ordinate) both recorded at 20K.

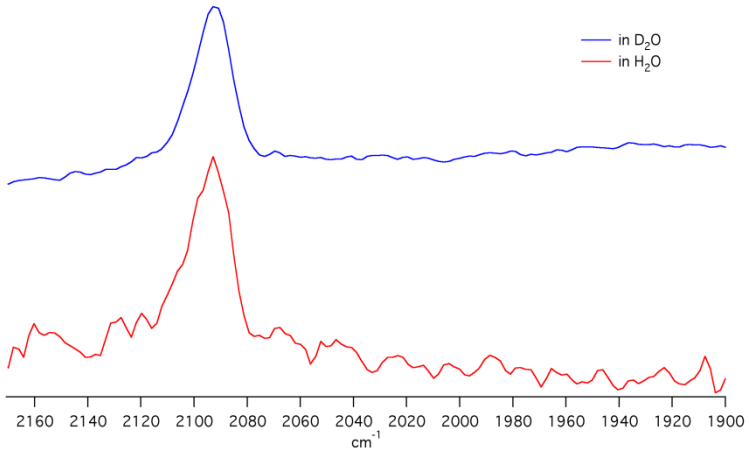

Figure 7. At 2100 cm^{-1} in the light versus dark difference spectrum for NBA and AA in D_2O (dashed) and H_2O (solid).

The LvD spectra in both D_2O and H_2O at 20K (**Fig. 7**) contain a similar absorption at 2100 cm^{-1}. The spectrum recorded in a solution of H_2O has more noise due to broad and intense IR absorption of H_2O in this region.

3.b.ii Discussion

In the light versus dark spectrum of NBA recorded at 295K there is a bleach at 1530 cm^{-1} characteristic of the disappearance of the C=O from the aldehyde, and absorption at 1730 cm^{-1} characteristic of the carboxylic acid produced (**Fig. 5**). When ammonium acetate is present in the photochemistry reaction, additional spectral features were observed. Once again there is a bleach at 1530 cm^{-1} signifying that NBA has converted into NB acid, additionally there is second bleach at 1560 cm^{-1}, attributed to the carboxylate group in ammonium acetate. The 1730 cm^{-1} peak has shifted towards 1710 cm^{-1} and there is a strong absorbance around 1600 cm^{-1}. 1710 cm^{-1} is the asymmetric stretching frequency of acetic acid's carbonyl group, and 1600 cm^{-1} represents a deprotonated NBacid. The light versus

dark spectrum shows that acetic acid and a deprotonated photoproduct are present after illumination. In other words, following excitation with light and photochemical rearrangement of NBA, a proton is transferred from the NB acid photoproduct to the acetate acceptor, forming nitrobenzoate and acetic acid as the final products.

At 20K, several of the features seen at 295K are present, although most have shifted down in frequency, probably as a result of the decrease in temperature [14]. The 1530 cm^{-1} and 1560 cm^{-1} bleaches are not present at 20K, but two peaks similar in ratio appear at 1510 and 1530 cm^{-1}. At first, it would appear that the 1530 cm^{-1} peak is from the disappearance of the carboxyl group of ammonium acetate, however we recorded (**Fig. 6)** an absorption spectrum of ammonium acetate in D_2O at 20K, and it is clear there is no significant overlap between the bands in this region, meaning AA has not been protonated. We can infer that the photoproduct is retaining the proton and is in the form NB Acid by the absorption at 1730 cm^{-1}.

Another distinguishable feature of the 20K light versus dark spectrum is the absorption at 2100 cm^{-1}. Theoretical predictions for the H_3O^+ Eigen core vibration are around 2600 cm^{-1}, and for a D_3O^+ Eigen core it has been observed at 1850 cm^{-1}. If we consider our photoacid system, we are releasing protons into a D_2O solution, and if we assume proton transfer to proceed via Grotthus mechanism, there could be a distribution of H_2DO^+ and HD_2O^+ species which would have vibrations somewhere in between 1850 and 2600 cm^{-1}. Alternatively, this could be a proton solvated by the photoacid and a neighboring D_2O. The same experiment was performed in a H_2O and this peak was observed in the same location. Therefore it cannot result from any of the proposed transient species.

What then is the origin of this new peak at 2100 cm^{-1}? We found the answer in a paper on the photochemistry of NBA studied with femtosecond vibrational spectroscopy [13]. One of the earlier intermediates is a carbene, which absorbs at 2100 cm^{-1}. A carbene is an unstable structure; however we have succeeded in "capturing" this species at low temperature. We were encouraged by this result, because it suggested that we might be able to trap intermediates in the proton transfer reaction using this same approach.

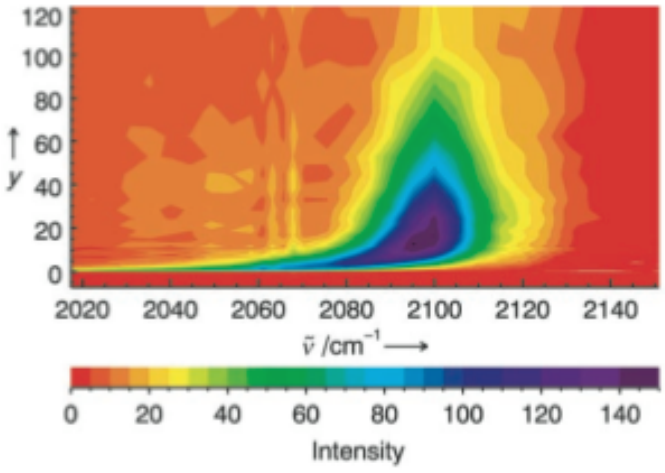

Figure 9. Picosecond time resolution (y-axis) of the photochemistry of NBA. Taken from reference [15]

We can be sure NBA is being converted into its final product by the feature at 1730 cm^{-1}, because one of the last steps is the formation of the carboxylic acid. This feature was observed in all of the spectra, despite the presence of a population of transient intermediates. Proton transfer to the acetate has still not occurred at the current temperature range studied, at least not after several hours of illumination.

The proton must be trapped on the photoproduct at these temperatures, and only transfer to the acceptor at some temperature between 20K and room temperature.

3.c. Gated Proton Transfer Reactions in D_2O Between 170K to 200K

3.c.i. Results:

Proton transfer was not observed at the cryogenic temperatures. From the stong absorption at 1730 cm^{-1} (**Fig. 6**) we can infer the proton is remaining on the photoproduct in the form NB acid. We continued to collect LvD spectra at higher temperatures to allow the proton transfer reaction to occur.

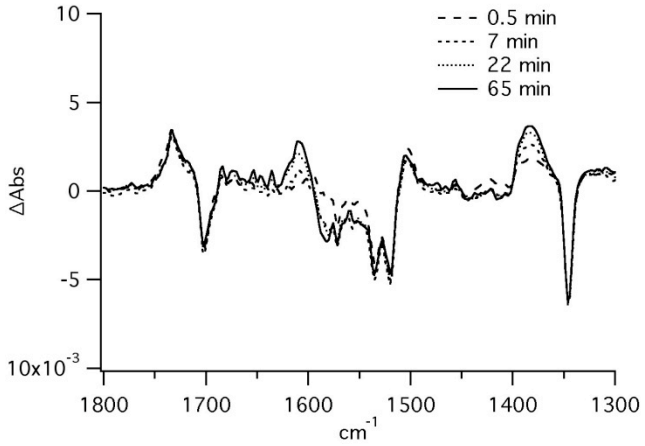

Figure 10. Light versus dark spectrum of NBA and ammonium acetate at 190K recorded over a period of time.

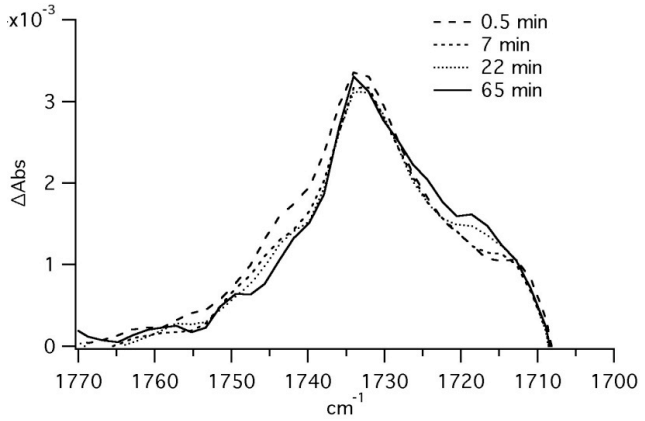

Figure 11. Light versus dark spectra of asymmetric carboxylic acid stretching region at 190K over a period of time.

When the light versus dark spectra was performed at 190K, a striking observation was made over an hour. Over time, we observed both a bleach at 1560 cm^{-1} and the formation of a peak at 1600 cm^{-1}. A closer look at the region around 1730 cm^{-1} reveals the development of a peak at 1715 cm^{-1} and the disappearance of the 1730 cm^{-1} peak.

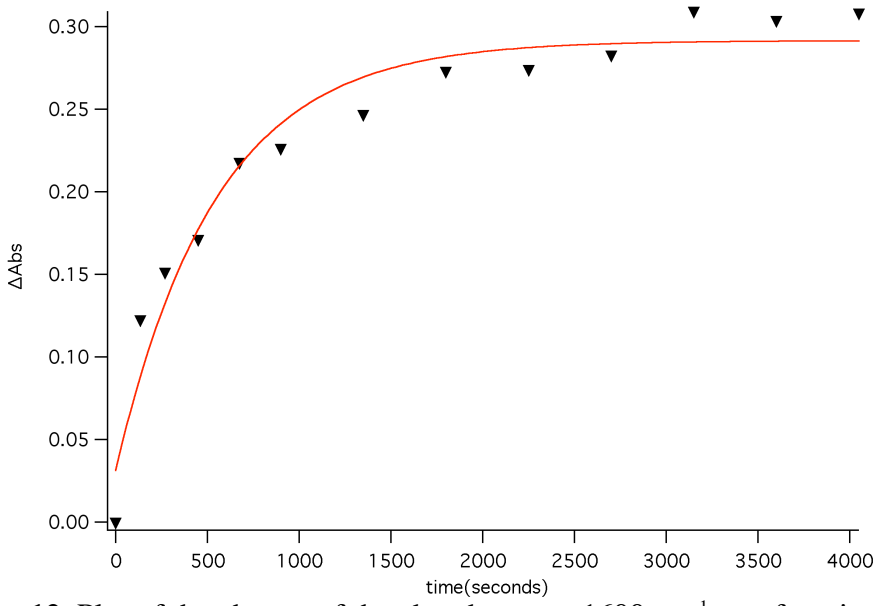

Figure 12. Plot of the change of the absorbance at 1600 cm^{-1} as a function of time at 190K.

The increase of the peak at 1600 cm^{-1} was fit to an exponential with a time constant of 547 ± 91 seconds.

Figure 13. Light versus dark spectrum of NBA and ammonium acetate solution in D_2O at 200K

3.c.ii. Discussion

A striking observation was made at 190K. After only ten minutes, there was a notable change in the spectra: a bleach at 1560 cm^{-1} and a growing absorption at 1600 cm^{-1}, which continued to develop over the next hour. There is a strong correlation between these peaks and those in the 1710 cm^{-1} to 1750 cm^{-1} region. The increasing intensity of a 1710 cm^{-1} peak at the expense of the 1730 cm^{-1} peak indicates that protons are leaving NBAcid and protonating acetate.

Upon warming the solution to 200K we see the prominent 1590 cm^{-1} peak as most of the NBA is in the form NBA^-, with some population of NB acid. Acetic acid's carbonyl stretch is not present because there is some overlap with a bleach from NBA at 1700 cm^{-1}. This bleach is easier to observe at 20K where we see a sharpening of peaks in response to the temperature decrease. Based on the bleach at 1560 cm^{-1}, we concluded that acetate is protonated at this temperature.

We were able to obtain some kinetics data from the light versus dark spectrum at 190K. Light versus dark spectra were then recorded at 175K, 180K, and 185K. We observed marginal changes in the spectra recorded at 175K and 180K; however, the period of collecting data was almost that of the time it takes the MCT detector to warm up. The response on the detector was monitored over time by recording spectra sequentially over five hours. The noise in the baseline remained approximately the same, except when there was an influx of water vapors which could be subtracted out. The response did vary linearly and each spectrum's baseline was corrected with subtraction.

The quantitative data that may be obtained from our kinetic data at 190K (**Fig. 13)** is not too reliable, as in our time constant we have an error of 20%. However, we did

observe a saturation of the 1600 cm^{-1} band after approximately 2000 seconds, or 30 minutes. At 185K we observed proton transfer complete in an hour. Spectra at 175K and 180K (not shown) were recorded for nearly five hours had not slowed down. Because we have found a temperature range where the reaction occurs at several rates, we can estimate the activation energy. Whether it is the activation energy for the transfer of the hydrogen to the water, which is monitored by the growth of the 1600 cm^{-1} and decay at 1500 cm^{-1}, or perhaps the transfer of the charge defect through the water network, we are unsure.

Mohammed (et al.) reported a transient vibration due to a dueterated eigen cation (D_3O^+) on a picosecond time scale using ultrafast IR spectroscopy, and the frequency of the vibration was at 1850 cm^{-1}. As NBAcid is releasing a proton into a solution of D_2O, several different eigen species may be present: H_3O^+, HD_2O^+, H_2DO^+, and D_3O^+. However, because the amount of dueterium is far greater than that of hydrogen, the two most likely species to be observed are HD_2O^+ and D_3O^+, assuming proton transfer occurs in a Grotthus-like mechanism. Further investigation of the spectra did not reveal the transient Eigen cation species.

4. Conclusion

We have reported the infrared spectra for a proton transfer reaction at a broad range of temperatures, from 20K to 200K, and at room temperature. The spectra indicate that no proton transfer has occurred over several hours at temperatures below 175K. However, we did observe the transfer at 190K over a time scale of half an hour and it would be interesting to test which is the rate limiting step: the transfer of the hydrogen or transfer of the charge defect. A future

experiment would be to do vary the concentration of ammonium acetate at 190K. By increasing the concentration, we are decreasing the distance between the photoacid and base. If the rate is concentration dependent, then we may infer the charge transfer is the rate-limiting step.

In conclusion, we have narrowed down a temperature range that proton transfer occurs at and may be observed over minutes to hours. Rates were obtained from the protonation of ammonium acetate by the development of a bleach from acetate and a positive absorbance from the formation of the deprotonated photoproduct. These rates were used in an Arrhenius plot and gave an approximation for the activation energy as 66 kJ/mol.

5. References

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