HOMOGENEOUS CATALYST MEDIATED GLUCOSE MUTAROTATION STUDIES USING VIBRATIONAL SPECTROSCOPY

By

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and approved by

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The pitfalls of overdependence on fossil fuels are well documented. Current research aims to focus on biomass obtained from renewable cellulose for the production of fuels and chemicals. In that regard, with cellulose as source, 5-hydroxymethylfurfural (HMF) is a versatile platform chemical for the production of chemicals like levulinic acid. The isomerization of glucose to fructose is one of the steps in the synthesis of HMF from cellulose. Glucose exists in several anomeric forms and it has been shown that the isomerization reaction is anomer specific. This work focuses on the study of mutarotation in glucose in the presence of homogeneous Lewis acid catalysts.

A combination of spectroscopic tools: ATR-IR and Raman spectroscopy are used to study the vibrational modes of glucose in aqueous solution. At room temperature, changes in vibrational modes can be attributed to the mutarotation reaction. This work compares the rate of mutarotation in different concentrations of AlCl₃, CrCl₃ and SnCl₄. The influence of metal salts in solution, pH and ionic strength were also probed by comparing with the rates obtained in Brønsted acids. The mutarotation in Lewis acid is faster than that in water. It is fastest in SnCl₄ and increases with increase in concentration of SnCl₄. Results also indicate a lack of glucose-Lewis acid interactions. However, rates vary depending on the nature of metal salts in solution indicating that the mutarotation is influenced by the nature of Lewis acid-water interactions.
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CHAPTER 1

INTRODUCTION
The search for clean source of energy and chemicals has never been more important considering the vagaries of political and economic consequences of petroleum dependency. In this regard, 5-hydroxymethylfurfural (HMF) offers a versatile and clean platform for the production of a variety of chemicals from biomass.

The formation of biomass can be best summarised as follows:

$$CO_2 + H_2O \rightarrow (CH_2O)_x + O_2$$

Biomass comprises of 3 constituents: cellulose, hemicellulose and lignin. The overall composition of biomass is approximately:

- 77-90% by weight sugar polymers
- 10-25% by weight lignin

The decomposition of the three major constituents yields the same major gases: CO₂, CH₄ and CO indicating similar chemical composition.

Cellulose and hemicellulose can be converted to ethanol directly by fermentation (1) (2) (3) (4) and lignin to chemicals by pyrolysis (5), gasification (6) and more recently by bacterial action (7).

Cellulose undergoes hydrolysis to yield glucose. HMF can be produced directly from glucose via dehydration or through glucose isomerization to fructose followed by dehydration to HMF. The direct conversion of glucose to HMF results in decreased conversions and increased formation of side products (8) (9) (10).

Cellulose possesses a crystalline structure with strong inner beta-1,4-glycosidic bonding and a complex hydrogen bonded network making its hydrolysis difficult (11). The isomerisation of glucose to fructose suffers from difficulties in conversion of glucose which exists predominantly as a 6-membered ring to fructose which exists as a 5-membered ring in solution.
Industrially, the isomerization reaction is carried out by the enzyme *glucose isomerase* (12). The use of enzymes for this step makes the reaction susceptible to limitations of temperature and purity and makes the (enzymatic) reaction unsuitable for one-pot conversion to HMF. Lewis acids have been reported to catalyze the isomerization reaction (13) (14) and consequently have been incorporated with a combination of Brönsted acid for one-pot synthesis of HMF from glucose (15) (16). An aqueous solution of glucose consists of several tautomers: alpha-D-glucopyranose, beta-D-glucopyranose, alpha-D-glucofuranose, beta-D-glucofuranose and the open chain glucose. It has been reported that the presence of specific anomers has an effect on the kinetics of isomerisation. For the enzymatic reaction, conversion rate was 43% higher with alpha-D-glucose compared to equilibrated glucose (34% alpha and 66% beta) and 113% higher compared to beta-D-glucose (17). Hence, greater the percentage of alpha-D-glucose in solution, the more efficient is the isomerization reaction. This makes the study of transformation of glucose anomers (mutarotation) critical which is undertaken in this thesis. Mutarotation is the change in optical rotation because of the change in equilibrium between two anomers. In addition to studying the mutarotation reaction in water, this thesis also focuses on this mutarotation reaction in Lewis acids. Attention is paid to Lewis acids since these compounds have been shown to catalyze the isomerization reaction and significant differences in catalytic activity has been reported for the
isomerization of glucose to fructose in the presence of AlCl₃, CrCl₃ and SnCl₄ (18) with highest selectivity reported for AlCl₃ (82.7%) while highest conversion was reported with CrCl₃ (52.3%). Consequently, based on the calculated rate constants, the rates for isomerization reaction follow the order: CrCl₃ > AlCl₃ > SnCl₄.

1.1 Goals

1. Study variations in mutarotation kinetics of glucose as a function of nature and concentration of different metal salts
2. Shed light on the glucose-water-metal salt interactions.
3. Understand the effect of speciation of metal salts on the mutarotation
CHAPTER 2

BACKGROUND
2.1 Mutarotation in glucose

An aqueous solution of glucose consists of the open chain glucose, alpha and beta anomers in the pyranose (6 membered ring) and furanose (5 membered ring) form. The pyranose form exist predominantly in solution. The conversion of the alpha to the beta form or vice versa is termed as mutarotation.

The study of anomers of glucose in solution was carried out by polarimetry (19). This was based on the different degrees by which the tautomers rotated plane polarized light (+18.7 degrees for beta-glucose and +112.2 degrees for alpha-glucose). An equilibrated glucose solution consists of 66% beta-glucose and 34% alpha-glucose with (+52.7 degrees).

Several mechanisms have been proposed for the mutarotation in glucose. They can be broadly classified into endocyclic oxygen attack and the exocyclic oxygen attack. The most accepted mechanism is the endocyclic oxygen attack which involves the formation of the open chain glucose. The exocyclic oxygen attack involves intramolecular proton transfer and attack on the O1 atom.

Rittenberg and Graff (20) demonstrated that the attack on the O1 atom is 30 times slower than the mutarotation reaction. This indicates that reaction proceeds by exocyclic oxygen attack. The mechanism of glucose mutarotation involves the following steps for the endocyclic route (21) (22):

1. Protonation of the endocyclic oxygen
2. O1-H bond breakage
3. O5-C1 bond breakage leading to the open chain glucose
4. Rotation across C1-C2

Figure 3: Tautomers of glucose in solution
Both chair and boat conformers of alpha-D-glucopyranose exist in equilibrium. The chair form is more stable than the boat form as heavier atoms are farther away from each other. It is for the same reason that beta-D-glucopyranose is more stable compared to alpha-D-glucopyranose.

Due to the ubiquitous nature of sugars, especially glucose, a wealth of literature is available on the effect of solvents, acids and temperature on the mechanism of mutarotation.

Tanret in 1896 demonstrated the presence of anomers of glucose by isolating alpha-anomer, beta anomer and also a third glucose structure, which could not be identified with available methodology, which could be converted to beta-glucose in aqueous media. The differences were established using polarimetry. However it was later established that the third form was merely a combination of the alpha and beta anomers in equilibrium. It should not be overlooked that Tanret’s original analysis does not take into account a reversible reaction that takes place leading to the existence of the two anomers in equilibrium. Crystallization of glucose from an aqueous solution leads to the formation of the alpha-anomer which would not be possible if Tanret’s irreversible reaction hypothesis was true. Further study of mechanism indicated that the conversion would be impossible without the formation of the aldehyde or hydrate as an intermediate. This led to the study of the open chain glucose which was Tanret’s postulated third glucose structure. It should
be noted that several contemporaries of Tanret (Ber., 1896, 29, 203) regarded complete conversion to isomeric aldehyde form (the third anomer).

Lowry (23) studied the effect of acid and alkali on the mutarotation of glucose using polarimetry. The rate of mutarotation was found to be directly proportional to the square root of acid concentration and directly proportional to the concentration of alkali (23). This may indicate that the ionic strength might significantly affect the mutarotation rates. The effect of ionic strength on the mutarotation rates in the presence of Lewis acids has been studied in this work. The results of Lowry’s experiments are described in Figure 6.

Lowry (23) therefore observed slight acceleration in N/100 HCl whereas equilibrium was achieved in 30 minutes. Using N/1000 caustic potash, in comparison, it took 3 hours for equilibrium to be achieved using N/100 HCl solutions.

The rate constants have also been determined for the mutarotation of glucose using several experimental methods—polarimetry (24) (19) (25), spectroscopy and even blood glucometry (26). The conversion of alpha-glucose to beta-glucose follows pseudo first order kinetics with equilibrium percentages at 36% alpha-glucose and 64% beta-glucose. Similar studies have also been undertaken in frozen solutions wherein the acid catalyzed mutarotation was reported to be faster than the same at room temperature (27). Other
studies include the study of mutarotation in mixtures of solvents (28) (29). Moreover, interactions between glucose and water molecules have also been probed in theoretical work by Shinichi Yamabe (30). Shinichi Yamabe assumed two imaginary transition states wherein the alpha-D-glucose underwent intermolecular hydrogen bonding leading to the formation of a network where one alpha-D-glucose molecule bonds to \( n \) number of water molecules (where \( n=1,2 \) and 3 refers to the number of water molecules bonded to glucose) where a four centred reaction centre TS1 is formed. The TS1 transforms to the aldehyde form which is then converted to a four centred transition state TS2 by ring closure leading to the formation of the beta-anomer in the next step. The relative energies of activation are summarized in Table 1. Activation energy decreases as \( n \) increases for both alpha and beta anomers indicating that dimmers and trimers of water bonded very strongly with glucose.

<table>
<thead>
<tr>
<th>Number of water molecules (( n ))</th>
<th>Alpha form bonded to ( n ) water molecules</th>
<th>Transition State 1 (TS1)</th>
<th>Aldehyde bonded to ( n ) water molecules</th>
<th>Transition State 2</th>
<th>Beta form bonded to ( n ) water molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>+50.84</td>
<td>+12.00</td>
<td>+49.19</td>
<td>+2.07</td>
</tr>
<tr>
<td>1</td>
<td>-8.20</td>
<td>+22.62</td>
<td>3.60</td>
<td>+20.97</td>
<td>-7.94</td>
</tr>
<tr>
<td>2</td>
<td>-13.25</td>
<td>+15.98</td>
<td>-2.55</td>
<td>+14.34</td>
<td>-12.09</td>
</tr>
<tr>
<td>3</td>
<td>-21.69</td>
<td>+3.19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Relative activation energies (32)- Negative values indicate greater stability

2.2 Isomerization of glucose to fructose

The conversion of glucose to fructose by isomerisation is considered a very important step for the conversion of cellullosic biomass to chemicals and fuels. Although direct conversion of glucose to HMF can be achieved, synthesis of HMF from fructose is advantageous owing to the fact that fructose predominantly exists in the form of five member rings in sharp contrast to glucose which exist in the form of six member rings. Apart from its use for the synthesis of HMF, fructose is also used to produce sweeteners for the food industry. However, the direct use of fructose to make the aforementioned products possesses an important drawback: the cost of fructose. For this reason, the use of
less costly cellulose derived glucose provides an important alternative for the implementation of the biorefinery concept. Commercial isomerisation of glucose to fructose is achieved by the enzymatic route with glucose isomerase acting as the enzyme (31). Further, continuous conversion of glucose to fructose using immobilized enzymes has also been achieved (32). More importantly, it was observed that the reaction is anomer specific: (33) (31) demonstrated that this reaction is alpha-anomer specific.

The first reported isomerisation of glucose was in 1885 with the reaction catalyzed by a base (34). (35) studied the isomerization of glucose to fructose using sodium hydroxide and calcium hydroxide at room temperature. The drawback of this method was poor yields and the large amounts of acidic by products formed which neutralized the base used. This was replaced by organic amines (triethylamine) (36) with the added advantage being slower degradation of saccharides. Further research on utilization of bases as catalysts for the isomerisation led to the use of borates, boronates and aluminates (37) (38) which were found to considerably increase the yield of this reaction. This was attributed to the greater tendency of formation of complexes between anions of borates (boronates and aluminates) with fructose compared to glucose which in turn stabilized fructose and further shift the equilibrium towards the favourable product. Solid bases resemble soluble bases kinetically with examples including KOH, cation exchanged zeolites and magnesium-aluminum hydrotalcites. The mechanism for base catalyzed isomerization reactions was put forth by Carraher et al (39).

The mechanism is depicted in Figure 7 and can be summarized as follows: 1) Formation of glucose anion due to attack by hydroxide ions leading to the formation of open chain glucose. 2) Abstraction of hydrogen atom from C2 to ene-diol intermediate. 3) Formation of open chain fructose. 4) Protonation of open chain fructose which leads to complete fructose.

The isomerization reaction was carried out in aqueous media. Metal salts coordinate with water leading to deactivation of the catalyst. For this reason, the study of mutarotation reaction catalyzed by Lewis acids lagged behind bases. The coupling of Lewis with Brønsted acids enables one pot synthesis of HMF from glucose which is considered as an advantage. Commonly used metal salts such as CrCl₃, AlCl₃ and SnCl₄ have been reported by Tang et al as effective isomerization catalysts; their catalytic activity
decreases in the order CrCl₃ > AlCl₃ > SnCl₄ (40). Further, the species responsible for isomerisation have also been explored for CrCl₃: [Cr(H₂O)₅(OH)]²⁺ (41) and for AlCl₃:[Al(OH)₂aq]⁺ (40). In addition to soluble Lewis acids, solid catalysts have also been reported for the isomerization reaction. Most common acid catalysts include Sn-beta zeolites and Ti-beta. The mechanism for the acid catalyzed isomerisation reaction is summarized in Figure 8.

2.3 HMF
5-hydroxymethylfurfural (HMF), in recent years, has been touted as a potential platform chemical for the production of fuels and chemicals from biomass. HMF can be used as a building block chemical for the synthesis of among other chemicals, biofuel 2,5 dimethylfuran (DMF) (42) (43) (44) and monomer 2,5 furandicarboxylic acid (45) (46). HMF can undergo reactions pertaining to the hydroxy methyl group, the formyl group and the furan ring. Ketoses, namely fructose is a better starting material for the synthesis of HMF compared to aldoses (glucose). This is due to the fact that glucose forms stable 6-membered rings in solution which is thermodynamically unfavourable to undergo dehydration to HMF directly. Fructose forms less stable ring structures thereby a greater proportion exists in the open chain form which aids the enolisation rate (rate determining step). The conversion of fructose to HMF has been achieved by homogeneous and heterogeneous catalysts. Examples include H$_2$SO$_4$, HCl and H$_3$PO$_4$ (47) (48) in addition to Lewis acid such as ZnCl$_2$ and AlCl$_3$ (49) (50). Examples of heterogeneous catalysts for the dehydration of fructose to HMF include TiO$_2$ (51) and ZrO$_2$ (51) and Ce-phosphates (52). Kuster (53) compiled a list of factors determining the rate of formation HMF from cellulose:

1. Substrate (aldoses or ketoses)
2. Type and concentration of catalyst
3. Time and temperature
4. Presence of solvent
2.4 Lewis acid catalysts

Broadly, Lewis acids are electron acceptors. This definition by Lewis was very general and was postulated based on the kinetics of the neutralisation process. Hence, Lewis classified acids into primary acids and secondary acids (54). Primary acids are those acids which have zero activation energy when reacted with primary bases. This puts together acids whose acid properties appear immediately. Alternatively, secondary acids include those acids which require activation energy before the acid properties are revealed.

Pearson (55) put forth a new concept called Hard and Soft Acid and Bases (HSAB) with hard acids being defined as small sized, positively charged and unpolarizable electron acceptors. They concluded stating that hard acids interact with hard bases and vice versa with respect to soft acids. More recent methods to understand quantitatively, based on the charge controlling effect the Lewis acidity has been based on perturbation molecular theory (56) and density functional theory.

Metal atoms bonded to highly electronegative species are typical examples of Lewis acids. The central metal atom acquires a partial positive charge thereby attracting electron pairs. Further, transition elements are capable of expanding their octet to accommodate electrons. More importantly, the non-metal to which the central metal atom is bonded to decides the acid strength.

Lewis acids are used to catalyze a wide range of reactions including but not limited to:

1. Formation and hydrolysis of acetals
2. Alkene alkylation
3. Alkene dimerization
4. Friedel Craft’s reaction
5. Oxidation reactions

In order to explain the effect of Lewis acids in water, a basic overview of the water molecule is required. Spectroscopy of water in 1977 revealed the presence of water dimers (57). More recent ab-initio calculations predicted the existence of large water
clusters-trimers, tetramers and pentamers of water- each monomer acts as a single acceptor and a single donor (58). The calculations were based on increasing the maximum number of hydrogen bonds possible while simultaneously decreasing the molecular strain. It was observed that for larger clusters-hexamers of water there was a tendency to form 3D structures (59). These structures have been analyzed using a combination of Monte Carlo calculations and far-IR laser vibration tunnelling spectroscopy (60). Addition of Lewis acids to water disrupts this hydrogen bonded structure. Shu Kobayashi described a correlation between catalytic activity in water and hydrolysis constants and exchange rate constants (61). Based on their findings, they proposed a mechanism wherein, metal ions (cations) dissociate in water from corresponding compounds and hydration of these metal ions occurs immediately. At this stage, intermolecular and intramolecular exchange reactions of water molecules occur. It was established that Lewis acids with hydrolysis constants between 4.3 to 10.08 and Water Exchange Rate Constants greater than 3.2X10^6 M^-1s^-1 were best catalysts for the Mukaiyama aldol reaction. This was attributed to the stability of cations. Greater the hydrolysis constant, greater is the stability of cations. WERC is related to (electron)^2/Ionic Radii. A small (electron)^2/Ionic Radii means faster WERC. The bounds for hydrolysis constants were established since high stability corresponds to poor catalytic activity. The effect of addition of alkali metal halides (62) and dissolved halides (63) on hydrogen bond network of water has also been studied. The nature of Lewis acids used- combination of cation and anion present can alter the nature of hydrogen bonding in water in different ways.

The hydration of complex species in solution (64) can be approximately written as:

\[ xM^{2+} + y H_2O \rightleftharpoons M_x(\text{OH})_{y}^{(x+y)^+} + y H^+ \]

Ligand formation with increasing charge to ratio of cation is: H_2O < OH^- < O^2-.

<table>
<thead>
<tr>
<th>Cation</th>
<th>Al^{3+}</th>
<th>Cr^{3+}</th>
</tr>
</thead>
<tbody>
<tr>
<td>WERC (M^-1s^-1)</td>
<td>1.6</td>
<td>5.8 x 10^-7</td>
</tr>
<tr>
<td>Hydrolysis Constant</td>
<td>4</td>
<td>1.14</td>
</tr>
</tbody>
</table>

*Table 2: WERC and hydrolysis constant for Lewis acids under consideration*

### 2.5 Vibrational Spectroscopy
Spectroscopy deals with the interaction of electromagnetic radiation with matter. Electromagnetic radiation is a form of energy comprising of alternating electric and magnetic fields acting in planes perpendicular to each other and the direction of propagation. These radiations are produced as a consequence of oscillators arising from the acceleration of electric charges. There are two theories by which the impact of electromagnetic radiation can be explained: Classical theory and Quantum Theory. Maxwell’s Classical Theory considers radiation as 2 mutually perpendicular electric and magnetic fields in a single plane at right angles to each other in phase propagated as a continuous sine wave.

![Propagation of Electromagnetic wave](image11)

From the classical theory,
\[ c = \lambda \nu \]

- \( c \) is the velocity of propagation which is constant in vacuum (3x10^8 m/s)
- \( \lambda \) is the wavelength of radiation (m)
- \( \nu \) is the frequency (1/s)

Typically, the term wavenumber (\( \tilde{\nu} \)) is used in spectroscopy which is the inverse of wavelength which offers the advantage of being linear with energy.
\[ \tilde{\nu} = \frac{1}{\lambda} = \nu/c \]

![Discrete Energy levels](image12)
However, a series of experiments by Bohr, Plank and Einstein indicated that it would be disingenuous to consider electromagnetic radiation interaction with matter as continuous which led forth to the quantum theory. Quantum theory proposes the existence of discrete energy levels. Every atom or molecule exists in these discrete energy levels and interaction with electromagnetic radiation leads to a quantum increase in energy which exactly fits the energy gap. The corresponding energy (in Joules) according to quantum theory is best expressed by the equation:

\[ E = h \nu \]

Where \( h \) is Planck’s constant \( (6.626 \times 10^{-34} \text{ Js}) \) and \( \nu \) is the classical frequency \( (1/\text{s}) \)

In a molecule, atoms are distributed among various energy levels. Atoms must absorb radiation exactly equal to the energy gap in order to jump to the next energy state. Hence the frequency required for transmission is given by:

\[ \nu = (E_1 - E_0)/h \]

### 2.6 IR Spectroscopy

IR spectroscopy involves impinging matter with Infrared radiations and measuring the quantity of incident radiations absorbed by the sample. Primitive IR instruments were primarily dispersive instruments consisting of prisms which were replaced by dispersive gratings in the 1950’s. However, real progress in the use of IR spectrometers arrived with the use of Fourier Transform Spectrometers and corresponding increase in computational power.

For a molecule to be IR active, the dipole moment of the molecule must change during vibration. This is a necessary condition for IR activity. This renders IR spectroscopy a convenient tool for the study of heteronuclear molecules. Homonuclear molecules are IR inactive. Let us consider two simple heteronuclear molecules: linear \( \text{CO}_2 \) and non-linear depicted in figure 13. Hence for a linear molecule, the number of vibrational modes is given by \( 3N-5 \). Figure 15 depicts the vibrational modes of water. A- Symmetric stretching mode where both OH bonds contract and expand in phase; B- asymmetric stretching mode where one OH bond...
expands while the other contracts; C- (scissoring) bending mode where oxygen and hydrogen atoms move out of plane.

Hence, for a non linear molecule, the number of vibrational modes is given by $3N - 6$. In the above cases, vibrations can be due to change in length of bonds or as a consequence of one atom moving out of its current plane - classified as stretching and bending modes respectively. 4 vibrational modes are possible for carbon dioxide described in figure 14 where: A- symmetric stretching mode where both CO bonds contract and expand in phase; B- asymmetric stretching mode where one CO bond expands as the other contracts; C and D- bending modes where carbon and oxygen atoms move out of the current plane with the difference between the modes only being the direction in which said movement takes place.

It should be noted that variations in dipole moment are profound in asymmetric stretching modes only- hence these are IR active whereas the net dipole moment of symmetric stretching modes is zero and hence are IR inactive.

Moreover, bonds between similar molecules lead to poor absorption (due to lower dipole moments).

The IR source typically emits IR radiation of all frequencies in the range of study. This could be near IR (14000-4000 cm$^{-1}$), mid IR (4000-400 cm$^{-1}$) or far IR (400-10 cm$^{-1}$). IR radiations are absorbed by a molecule if there is a change in the dipole moment. An IR spectrum is a plot between absorbance (reflectance or transmittance) versus the wavenumber. The wavenumber is a measure of the energy difference between the ground state and excited (vibrational) state. The intensity of the IR band measured is proportional to the square of the change in dipole moment. The relationship between absorbance and the concentration of a sample is given by Beer Lambert’s law.
2.6.1 Instrumentation

The source of IR radiation commonly used is Globar or Nernst source. The former is a silicon carbide rod which is electrically heated up to 1650°C whereas the later is a tube consisting of certain oxides of zirconium, yttrium and erbium heated to 2000°C. Michelson interferometer is the most common of its type and consists of two plane perpendicular mirrors- a stationary mirror and a moving mirror. Additionally, it consists of a beam splitter which splits incident beam into two parts. One part passes onto the moving mirror and the other part into the stationary mirror. Half the beams falling on the respective mirrors are reflected back into the beam splitter where they combine. Half the beam from the stationary mirror is transmitted through the beam splitter and the other half to the source. The output beam from the interferometer (perpendicular to incident beam) is used for analysis.

The objective of a moving mirror is to produce a path difference and constructive interference takes place for the reflected beams. The output beam from interferometer
passes through the sample and then onto the detector. The most common detector is the DTGS (Deuterium Tryglycine Sulfate). Greater sensitivity is achieved using an MCT (Mercury Cadmium Telluride) which however requires cooling to liquid nitrogen temperatures.

2.6.2 Attenuated Total Reflectance

ATR is one of two commonly used sampling techniques from reflection- the other being DRIFTS (Diffuse Reflectance Infrared Fourier Transform Spectroscopy). ATR is a contact sampling technique. ATR utilizes a crystal of high refractive index and IR IR radiation falling on a sample can be reflected (I_r), absorbed (I_a), transmitted (I_t) or scattered (I_s). This is given by:

\[ I = I_a + I_t + I_r + I_s \]

Hence any of the above parameters can be used to get sample information.

In ATR, the reflected light provides information at the sample-crystal interface. The choice of material for the crystal is vital to ensure total internal reflectance occurs since the critical angle is a function of refractive index of sample and the crystal. Diamond, Germanium and ZnSe are commonly used crystals. ATR is particularly useful for thick samples as well high absorbing samples.
2.7 Raman Spectroscopy

Raman spectroscopy involves impinging the sample with monochromatic radiation from a laser in the UV, visible or near IR range and measuring the scattered radiation using a spectrometer.

Consider incident radiation of frequency $\nu_L$ on a molecule. This leads to transmission, absorption and scattering of the incident radiation. The incident radiation causes excitation of the molecule to a virtual state. The virtual state can be described as a temporary short lived state after which the molecule returns to the ground state. Most of the scattered radiation has the same frequency as that of the incident radiation. However some of the scattered radiation possesses frequency of magnitude $\nu_L + \nu_m$ or $\nu_L - \nu_m$ where $\nu_m$ depends on the transitions between the vibrational, translational and electronic energy levels of the molecule. If the scattered radiations have the same frequency as incident radiation, the phenomenon is called Rayleigh scattering whereas variations in frequency of incident and scattered radiation are termed as Raman scattering. This scattering occurs in all directions and observed intensity is dependent on direction of observation. Further, frequency of magnitude $\nu_L + \nu_m$ is referred to anti-Stoke’s band whereas frequency of magnitude $\nu_L - \nu_m$ is referred to as Stoke’s band. This is summarized in Figure 20.

Rayleigh scattering is the most probable event and the observed intensity of scattered light for Raman scattering is $10^{-6}$ times smaller. It should be noted that the ground state is more populated than excited levels and hence the Stoke’s scattering is more intense than the Anti-Stoke’s scattering. Further, less than 0.001% of incident radiation
undergoes Raman scattering. Moreover, Stoke’s lines are usually used in the spectra due to its higher intensity.

An oscillating electric field is given by:

\[ F = F_0 \cos (\nu t) \]

This electric field induces a dipole moment in a molecule given by:

\[ \mu = \alpha F_0 \cos (\nu t) \]

Where \( \alpha \) is the polarizability of a molecule and is given by:

\[ \alpha = \alpha_0 + \sum_{m=1}^{M} \alpha_0 \cos (\nu_m t + \Phi_m) \]

\[ P = \alpha F_0 \cos (\nu t) + \sum_{m=1}^{M} \alpha_m \cos ((\nu + \nu_m) t + \Phi_m) + \sum_{m=1}^{M} \alpha_m \cos ((\nu - \nu_m) t + \Phi_m) \]

Hence, for a molecule to be Raman active, the polarizability must change during vibration. Consider a molecule in an electric field- a dipole moment is induced in the molecule as electrons are attracted to the positive pole and positively charged nuclei towards the negative pole. Hence IR and Raman spectroscopy can be used to obtain complementary information about a molecule.

The Raman intensity is dependent on several parameters and can be best summarized by the equation:

\[ I_R = \nu^4 I_0 N \frac{\partial \alpha}{\partial Q} \]

Where \( \nu \) is frequency of laser used, \( I_0 \) is intensity of incident beam, \( N \) is number of scattered molecules, \( \alpha \) is polarizability and \( Q \) being vibrational amplitude.

### 2.7.1 Instrumentation

Raman spectrometers typically consist of a laser excitation source, collection optics, a monochromator (or interferometer) and a detector.

It should be noted that Raman spectrometers must have effective filters to keep out Rayleigh scattered radiation while simultaneously recording much weaker Raman scattered radiation. The laser excitation source can be UV, near IR or visible source. If a visible laser is used for excitation, the Raman scattered radiation will also be in the same
range of visible frequencies. A dispersive Raman spectrometer consists of grating which function as individual radiation sources. Incident polychromatic radiation from a laser falls on the gratings and in-phase radiation is incident radiation is reflected onto the exit slit. The radiation is in phase only for select wavelengths where constructive interference takes place. In the other case, when destructive interference takes place, corresponding radiation does not leave the monochromator. The use of effective Rayleigh filters has allowed the utilization of one grating improving optical throughput.

Acquiring spectral information is a trade off between higher throughput and higher resolution. Detectors commonly used include charge-couple-devices (CCD) or array detectors. A schematic of a Raman spectrometer is found in Figure 21 where RF is a Rayleigh filter, G is the grating and M1 and M2 are spherical mirrors and AD is an array detector.
CHAPTER 3

EXPERIMENTAL
3.1 Reagents

- Tin(IV) chloride pentahydrate SnCl\(_4\)·5H\(_2\)O [Molecular weight 350.6, CAS No. 10026-06-9, Sigma-Aldrich]
- Chromium (III) chloride hexahydrate CrCl\(_3\)·6H\(_2\)O [Molecular weight: 266.45 g/mole, CAS No. 10060-12-5, Sigma Aldrich]
- Aluminum Chloride hexahydrate AlCl\(_3\)·6H\(_2\)O [Molecular weight: 241.43 g/mole, CAS No. 7784-13-6, Sigma Aldrich]
- Glucose (Dextrose, D(+)Glucose) C\(_6\)H\(_{12}\)O\(_6\) [Molecular Weight: 180.16 g/mole, CAS No. 50-99-7, Sigma Aldrich]
- Sulfuric acid (volumetric 5 M) H\(_2\)SO\(_4\) [Molecular weight: 98.08 g/mole, CAS No. 7664-93-9, Fluka Analytical]
- Hydrochloric acid 36% w/w aqueous solution [Molecular weight: 36.46 g/mole, CAS No. 7647-01-0, Alfa Aesar]

3.2 Preparation of reaction mixtures

1) 1.6 M glucose solutions were prepared in water. 20 mM, 100 mM, 200 mM solutions of metal salts were prepared in water. The solutions were mixed such that final concentrations of 0.8 M glucose in 10 mM, 50 mM and 100 mM metal salt solutions were prepared.

2) 1.6 M glucose solutions were prepared and allowed to equilibrate at room temperature with stirring for 5 hours. These solutions were used to prepare 0.8M glucose solutions in 10mM, 25 mM and 250 mM AlCl\(_3\) solutions.

3) 0.8 M glucose solutions were prepared in 0.1 M, 0.05 M and 0.01 M HCl and H\(_2\)SO\(_4\) solutions by dissolving 1.6 M glucose solutions in 0.2 M, 0.1 M and 0.02 M HCl and H\(_2\)SO\(_4\) solutions respectively.

In all the above cases, once reaction mixtures were prepared, spectral acquisition was carried out every fifteen minutes for 4 hours.

3.3 Spectroscopy

3.3.1 Raman Spectrometer

Raman spectra were recorded using Horiba LabRAM HR Evolution spectrometer. It is a high spatial and spectral resolution Raman spectrometer (UV compatible) equipped with a confocal microscope. It contains air cooled open electrode 1024x256 pixels CCD -75°C and a laser source of 532nm. The additional feature is the UV-Vis-NIR macro lens of 40 mm focal length for open geometry Raman configuration. The spectra were recorded in
dark room to avoid interference of light. Laser-safety goggles were used whenever Raman spectrometer was in use.

Since spectra of liquid samples are to be collected, an external sample holder was used. 1 ml borosilicate vials with a cap was used to hold the samples. Spectra was collected every fifteen minutes for four hours.

Acquisition Time: Acquisition time is the actual time over which Raman scattered radiations are counted.

Accumulation Number: The number of distinct acquisition times over which spectra is averaged.

Spectro: If spectrum is collected using spectro, this value is the midpoint of the spectrum obtained.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acquisition time</td>
<td>50 seconds</td>
</tr>
<tr>
<td>Accumulations</td>
<td>12</td>
</tr>
<tr>
<td>Spectro</td>
<td>1050 cm(^{-1})</td>
</tr>
<tr>
<td>Laser Intensity</td>
<td>100%</td>
</tr>
<tr>
<td>Grating</td>
<td>600 /mm</td>
</tr>
</tbody>
</table>

**Table 3: Parameters for Raman spectroscopy**

### 3.3.2 FTIR Spectrometer

FTIR spectra were collected using NICOLET IS50 FT-IR spectrometer. This analytical instrument is a high resolution (~0.9 cm\(^{-1}\)) infrared spectrometer and is equipped with DLaTGS and MCT-A detectors. It contains long lifetime Polaris™ infrared source and gold optics. The FTIR is connected to a Parker-Balston 75-45 purge unit to provide a
purified purge gas and air bearing gas from compressed air. The purge gas generator supplies carbon dioxide free air. This helps in obtaining cleaner background spectra in a shorter period of time and more accurate analysis by improving the signal-to-noise ratio. Attenuated Total Reflectance technology is incorporated with this FTIR.

![Figure 24: Nicolet FTIR-ATR](image)

Specac ATR is a high-performance single reflection monolithic diamond ATR. One of the characteristic features of this equipment is that it can analyze a wide range of materials irrespective of the material’s abrasiveness, hardness, reactivity or corrosiveness. The ATR platform is connected with an electrical heating unit which enables the platform to reach a maximum temperature of 300°C. For the analysis of liquid samples using FTIR-ATR, place a drop of the mixture on the diamond crystal and use the cover slip to prevent surface evaporation during analysis.

Parameters used:

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detector</td>
<td>DTGS</td>
</tr>
<tr>
<td>Beam Splitter</td>
<td>KBr</td>
</tr>
<tr>
<td>Resolution</td>
<td>4 cm⁻¹</td>
</tr>
<tr>
<td>Wavenumber range</td>
<td>400-4000 cm⁻¹</td>
</tr>
<tr>
<td>Aperture</td>
<td>100</td>
</tr>
<tr>
<td>No.of scans</td>
<td>32</td>
</tr>
<tr>
<td>Optical Velocity</td>
<td>0.3165 cm/sec</td>
</tr>
</tbody>
</table>

Table 4: Parameters used for ATR-IR spectroscopy
3.4 Normalization of spectra

Mathematical handling of spectroscopic data is highly subjective and dependent on the dataset as well as the type of analysis being targeted—i.e. whether peaks within a spectrum are being compared or whether two different spectra are being compared. Common normalizing techniques include:

1. Division of entire spectrum by intensity of single peak
2. Normalizing over the entire range from 0 to 1 with the formula:
   \[
   Y = \frac{(y_{\text{max}} - y)}{(y_{\text{max}} - y_{\text{min}})}
   \]
3. Divide the entire spectrum by the area under the spectrum.

In this study, area normalization is followed. The IR spectra is normalized over the region 850-1500 cm\(^{-1}\) and for the Raman spectra, the whole range is used. The importance of normalization especially when comparing different samples, consider figures 25 and 26.

In Figure 25, a comparison is made between raw data and normalized data. In the case of
raw data, the variation in peak intensity for the peak at 1054 cm\(^{-1}\) does not vary uniformly with time. Additionally, the intensity of peak at 1079 cm\(^{-1}\) increases with time indefinitely. In contrast to this, when normalized data is used, the variation in the peak intensity at 1054 cm\(^{-1}\) is decreases with time and becomes constant after a certain time. Moreover, the peak at 1080 cm\(^{-1}\) increases in intensity with time and eventually reaching a constant value. The information obtained from normalized data allows for determination of equilibrium times. Moreover, knowing that the absorbance is a measure of concentration, the peak at 1057 cm\(^{-1}\) and 1080 cm\(^{-1}\) cannot increase or decrease indefinitely with time. In the case of raw data, the increase in intensity of a particular vibrational mode is a consequence of an increase in area of the spectrum. In figure 26, comparing the raw values of intensities from 50 mM CrCl\(_3\) and water, there is a vast difference in the intensity of Raman scattered radiation. This makes any direct comparison futile. This difference in intensity can be attributed to differences in scattering volume and absorption of radiation by solution. This can be overcome by taking into account the same absorption volume—i.e., normalizing with respect to area of the spectrum. The scales are comparable after normalization. Hence, all data presented in the following section will be from normalized spectra.
CHAPTER 4

RESULTS
4.1 Bands of glucose

Assignments of vibrational modes were made from literature. Several discrepancies were found among assigned vibrational modes. Over fifteen publications were cross checked and tables 5 and 6 summarize the assignments for vibrational modes of glucose obtained from Raman and IR spectroscopy respectively.

Assignments for Raman bands (65) (66) (67) (68) (69) (70) (71) (72) (73) (74):

<table>
<thead>
<tr>
<th>Raman shift</th>
<th>Vibrational assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1625</td>
<td>(HOH) bending mode</td>
</tr>
<tr>
<td>1455</td>
<td>(CH2) bending mode</td>
</tr>
<tr>
<td>1360</td>
<td>(CH2) wagging mode of beta anomer</td>
</tr>
<tr>
<td>1327</td>
<td>(CH2) wagging mode of alpha anomer</td>
</tr>
<tr>
<td>1258</td>
<td>(CH2) torsion mode</td>
</tr>
<tr>
<td>1120</td>
<td>(COH) bending mode</td>
</tr>
<tr>
<td>1080</td>
<td>(C1-O1) stretching mode of beta anomer</td>
</tr>
<tr>
<td>1060</td>
<td>(C1-O1) stretching mode of alpha anomer</td>
</tr>
<tr>
<td>910</td>
<td>(C1-H1) stretching mode of beta anomer</td>
</tr>
<tr>
<td>890</td>
<td>(C1-C2) stretching mode of beta anomer</td>
</tr>
<tr>
<td>860</td>
<td>(C1-H1) stretching mode of alpha anomer</td>
</tr>
<tr>
<td>842</td>
<td>(C1-C2) stretching mode of alpha anomer</td>
</tr>
<tr>
<td>770</td>
<td>(C5-C1-O1) bending mode of beta anomer</td>
</tr>
<tr>
<td>710</td>
<td>(O5-C1-O1) bending mode of alpha anomer</td>
</tr>
<tr>
<td>585</td>
<td>(C6-C5-O5) bending mode</td>
</tr>
<tr>
<td>540</td>
<td>(C2-C1-O1) bending mode of alpha anomer</td>
</tr>
<tr>
<td>518</td>
<td>(C2-C1-O1) bending mode of beta anomer</td>
</tr>
<tr>
<td>450</td>
<td>Endocyclic (CCO) bending mode</td>
</tr>
<tr>
<td>424</td>
<td>(CCC) bending mode</td>
</tr>
<tr>
<td>355</td>
<td>(COC) bending mode</td>
</tr>
</tbody>
</table>

Table 5: Assignments for vibrational modes obtained from Raman spectroscopy
Assignments for IR bands (66) (65) (68) (69) (70) (75) (72) (73) (74) (76) (77) (78):

<table>
<thead>
<tr>
<th>Wavenumber (cm$^{-1}$)</th>
<th>Vibrational assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1422</td>
<td>(CH$_2$) scissoring mode</td>
</tr>
<tr>
<td>1333</td>
<td>(CH$_2$) wagging mode</td>
</tr>
<tr>
<td>1273</td>
<td>(CH$_2$) twisting mode</td>
</tr>
<tr>
<td>1203</td>
<td>(CH$_2$) twisting mode</td>
</tr>
<tr>
<td>1150</td>
<td>(CO) and (CC) stretching mode</td>
</tr>
<tr>
<td>1121</td>
<td>(CC) and (CO) stretching mode and</td>
</tr>
<tr>
<td></td>
<td>(COH) in plane bending mode</td>
</tr>
<tr>
<td>1080</td>
<td>(C1-OH) stretching mode of beta anomer</td>
</tr>
<tr>
<td>1060</td>
<td>(C1-OH) stretching mode of alpha anomer</td>
</tr>
<tr>
<td>1032</td>
<td>(CC) and (CO) stretching mode and</td>
</tr>
<tr>
<td></td>
<td>(COH) in plane bending mode</td>
</tr>
<tr>
<td>1001</td>
<td>(CCH) and (CCO) in plane bending</td>
</tr>
<tr>
<td>915</td>
<td>(CCH) and (CCO) in plane bending</td>
</tr>
<tr>
<td>844</td>
<td>(CC) stretching mode</td>
</tr>
</tbody>
</table>

Table 6: Assignments for vibrational modes obtained from IR spectroscopy

Figures 27 and 28 summarize the IR and Raman spectra obtained for glucose in water. The peak positions in water were used as reference for comparing samples with glucose in Lewis acid. From figure 27, it can be observed that the peak at 1054 cm$^{-1}$ decreases in intensity with time while the peak at 1076 cm$^{-1}$ increases in intensity with time. The two peaks are assigned to the (C1-OH) stretching mode of the alpha and beta anomer respectively. It should be kept in mind that hydrogen bonding via glucose-water and
glucose-glucose interactions exists and thus the peak positions are affected by this. Any significant variation in nature of bonding would lead to red or blue shifts depending on the comparative strength of new bonds with respect to original interactions. The variation in intensity of the (C1-OH) stretching mode of beta anomer with respect to time is studied in greater detail in the following section to comment on the formation of beta-D-glucose. Moreover, the ratio of the peaks at 1054 cm\(^{-1}\) and 1076 cm\(^{-1}\) is used for the determination of percentages of anomers in solution. Figure 28 displays the Raman spectra of glucose in water. The ratio of intensities of peaks at 512 cm\(^{-1}\) and 540 cm\(^{-1}\), the (C2-C1-O1) bending mode of alpha and beta anomers respectively, are used for determination of relative percentage of anomers in solution. The peak at 890 cm\(^{-1}\), which corresponds to C1-C2 stretching mode, is used from the Raman spectra to study the formation of the beta anomer. Therefore, once assignments are made, analysis of peak position as well as the variation in intensity is studied in greater detail in the following sections.
4.2 In-situ Raman and IR spectroscopy of mutarotation

The peak at 1076 cm\(^{-1}\) is of interest from the IR spectra and the variation in its intensity is plotted as a function of time in Figure 29. This peak, assigned to (C1-OH) stretching mode of the beta anomer and this is used to track the relative amount of the anomer in solution. This is of significance because the variation in this peak can be used to compare the rate of formation of beta anomer in different Lewis acids. The rate of formation of beta anomer in the presence of AlCl\(_3\) is the same as water whereas this rate is faster in the presence of CrCl\(_3\) and SnCl\(_4\). Moreover, the rate increases as concentration of CrCl\(_3\) increases. The time taken to reach equilibrium is summarized in Table 7. It is apparent that the time to reach equilibrium is not uniform as a function of Lewis acid concentration. It is unclear if this is a consequence of the metal atoms present and this variation is probed in the absence of metal salts using Brönsted acids in later sections. Similar information can be obtained from Raman spectrum. Figure 31 shows variation in (C1-C2) stretching mode as a function of time in the presence of Lewis acids. It is clear that the rate of mutarotation is not greatly altered as a function of AlCl\(_3\).
However the rate of mutarotation is faster in CrCl$_3$ and SnCl$_4$ compared to water. Changing concentration of CrCl$_3$ does not produce any change in the rate of formation of beta anomer. However, rate of formation of beta anomer increases as concentration of SnCl$_4$ increases. There is no change in equilibrium time due to concentration of CrCl$_3$.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time taken to reach equilibrium (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>165</td>
</tr>
<tr>
<td>10 mM AlCl$_3$</td>
<td>150</td>
</tr>
<tr>
<td>50 mM AlCl$_3$</td>
<td>125</td>
</tr>
<tr>
<td>100 mM AlCl$_3$</td>
<td>165</td>
</tr>
<tr>
<td>10 mM CrCl$_3$</td>
<td>105</td>
</tr>
<tr>
<td>50 mM CrCl$_3$</td>
<td>90</td>
</tr>
<tr>
<td>100 mM CrCl$_3$</td>
<td>90</td>
</tr>
<tr>
<td>10 mM SnCl$_4$</td>
<td>60</td>
</tr>
<tr>
<td>50 mM SnCl$_4$</td>
<td>75</td>
</tr>
<tr>
<td>100 mM SnCl$_4$</td>
<td>150</td>
</tr>
</tbody>
</table>

Table 7: Time taken to reach equilibrium from IR spectroscopy
It is unclear as to why the reaction is fastest in SnCl$_4$. This may be attributed to pH, ionic strength or speciation of metal salts which are individually probed in later sections. It should be noted that this is conflicting with results from IR spectroscopy.

Vibrational modes from IR spectroscopy are littered with interferences and as a consequence overlapping from neighboring vibrational modes. Therefore, results from Raman spectroscopy are used. On the other hand, rather than following the decomposition of alpha anomer or the formation of beta anomer, we can use isosbestic points to determine anomic pairs and calculate the relative percentage of alpha and beta anomers at equilibrium.

### 4.3 Effect of Lewis acids and Brönsted acids on equilibrium

**Isosbestic points refer to those wavelengths where at least two chemical species have the same molar absorption coefficient which remains constant as reaction proceeds.** The presence of an isosbestic point is proof that the stoichiometry of a reaction remains unchanged during the reaction. Figure 33 depicts isosbestic points in the 350-600 cm$^{-1}$ range from the Raman spectra. 4 isosbestic points are observed at 408 cm$^{-1}$, 506 cm$^{-1}$, 516 cm$^{-1}$, 530 cm$^{-1}$. The isosbestic points are preceded and followed by species consumed or
formed in reaction. These clearly correspond to the alpha-D-glucose and beta-D-glucose respectively. For the determination of equilibrium percentages, the peaks surrounding the isosbestic point at 530 cm\(^{-1}\) are used. Similarly, figure 34 shows the isosbestic point from IR spectra at 1063 cm\(^{-1}\). In both cases, the vibrational assignments are known from literature as follows:

1. 520 cm\(^{-1}\): (C2-C1-O1) bending mode of beta anomer
2. 543 cm\(^{-1}\): (C2-C1-O1) bending mode of alpha anomer
3. 1063 cm\(^{-1}\): (C1-OH) stretching mode of beta anomer
4. 1080 cm\(^{-1}\): (C1-OH) stretching mode of alpha anomer

The equilibrium percentages are calculated as follows:

\[
(\% \text{ alpha} - D - \text{glucose})_{\text{Raman}} = \left[ \frac{I_{543}}{I_{543} + I_{520}} \right] \times 100
\]

\[
(\% \text{ alpha} - D - \text{glucose})_{\text{IR}} = \left[ \frac{I_{1055}}{I_{1055} + I_{1080}} \right] \times 100
\]

The calculated equilibrium percentages are compared in Figure 35. Moreover, the relative % alpha anomer is obtained as a function of time from IR and Raman spectroscopy in Figures 36 and 37 respectively.
The final equilibrium percentages (around 39%) from Raman spectroscopy matches more closely with values reported in literature compared to 45% from IR spectroscopy. Moreover, the equilibrium percentages are almost uniform across water and Lewis acids indicating that the presence of Lewis acids has no effect on the equilibrium.

Figures 37 and 38 can be used to compare the rate of mutarotation in Lewis acids. The reaction with AlCl₃ is faster than that water. However there appears to be no great difference with change in concentration. It is a similar story for CrCl₃ which is almost the same as that in water. However the reaction is fastest in SnCl₄ and rate increases with increase in concentration of SnCl₄. Therefore in summary, for a given concentration of Lewis acid,
Having determined that the presence of Lewis acids alters the rate of mutarotation, it is important to determine the cause of this difference. There are several possible reasons which could explain the variation: presence of metal ions, pH, ionic strength etc.

To analyze the effect of presence of metal salts, mutarotation was studied in the presence of Brönsted acids namely $H_2SO_4$ and $HCl$. The relative equilibrium percentages were determined and summarized in Figure 38. The rate of variation in %alpha-D-glucose was also determined as a function of concentration of the Brönsted acid (Figures 39 and 40).

From figure 38 it is clear that the equilibrium percentage remains fairly constant across concentrations and nature of Brönsted acids used. Analogous to results from Lewis acids, equilibrium percentages
calculated from Raman spectra are better fit to values reported in literature. More significantly, from figures 39 and 40, it is observed that the mutarotation is faster as concentration of acid increases. This is true for H$_2$SO$_4$ and HCl. Hence, to summarize mutarotation in Brönsted acids, for a given concentration of acid:

**Mutarotation rate:** H$_2$SO$_4$ > HCl > water

To be sure that the equilibrium percentages are independent of the Lewis acid used, another set of experiments were performed. Known concentrations of equilibrated glucose were added to 10 mM, 50 mM and 250 mM AlCl$_3$ and the Raman and spectra was collected. The relative percentage alpha-D-glucose was determined and the results from this experiment are presented in figure 41. It becomes apparent that there is no change in equilibrium as a result of addition of Lewis acid. As a result, we clearly show herein that the presence of homogeneous Lewis acid or Brönsted acid catalyst does not influence the equilibrium of glucose anomers but based on the discussion in previous paragraphs, only the kinetics. This important result can decouple these two effects and provide useful insights into reaction modelling. The rate of mutarotation can also be affected by interactions between glucose-metal salts. Metal atoms could bind to the intermediate or to one of the anomeric forms thereby altering the stability. These glucose-metal salt interactions are discussed in the next section.

### 4.4 Insights into Glucose-Metal salt interactions

Analysis of peak position from IR and Raman spectra provides information on the nature of bonding. It should be kept in mind that hydrogen bonding via glucose-water and glucose-glucose interactions exist and thus the peak positions are affected by this.
Any significant variation in nature of bonding would lead to red or blue shifts depending on the comparative strength of new bonds with respect to original interactions. Figures 42 and 43 show IR and Raman spectra of glucose in metal salts at 1 min and 240 min respectively. There is no variation in peak position due to the addition of Lewis acid. This indicates the absence of any long time glucose-metal salt interactions. It should be noted that unstable complexes with lifetimes too low to be picked up using spectroscopic techniques cannot be studied using this approach. Moreover, the absence of new peaks also highlights the absence of an IR or Raman active intermediate. There appears to be variations in the peak at 350 cm\(^{-1}\) in the Raman spectra. However, these variations are less than 5 cm\(^{-1}\). In figure 44, the Raman spectra of Lewis acid in water is shown. Attention must be paid to the peak at 339 cm\(^{-1}\) which increases as concentration of metal salt increases. Therefore, the variations in the peak at 350 cm\(^{-1}\) can be attributed to interference from the spectra of Lewis acid.
To conclude, the presence of metal salts influences rates of mutarotation with no visible long time interactions with glucose molecules. This result holds true for AlCl₃, CrCl₃ and SnCl₄. However, metal salts can form a plethora of complex species with water and this is discussed in later sections. The glucose-water interactions, on the other hand, can be studied by determining the ratio of intensity between CH₂ scissoring mode and HOH bending mode i.e the ratio obtained from bands at 1420 and 1640 cm⁻¹ respectively and monitoring the change in this ratio with time. Reduced glucose-water interactions are depicted by an increase in this ratio (66). Figure 45 indicates that the ratio remains fairly constant as a function of time with the only exceptions being 50 mM SnCl₄ and 100 mM SnCl₄ in which an increase in this ratio is observed. Additionally, with the exception of the two tin samples, the ratio is lower than water indicating enhanced interactions between glucose and water. There is significant difference for the two tin samples with time indicating strong glucose-water interactions at low times but subsequent weakening of these interactions. This could explain rapid mutarotation in SnCl₄ at the start of the reaction. Since metal-salt interactions are still unknown, the next section will focus on the species formed as a result of metal salt-water interactions.
4.5 Speciation of metal salts

To study Lewis acid-water interactions, equilibrium speciation software, Minteq is used. Minteq is used to determine the nature and concentration of species formed as a consequence of Lewis acid-water interactions. The concentration of the metal salt (in molal) is the input and the mass and charge balance model is used. This model is based on the premise that the quantity of all species in a solution containing a particular atom (or a group of atoms) must be equal to the amount (or group) delivered to the solution and that in any solution the sum of positive charges is equal to the sum of negative charges.

Results from Minteq are expressed in the form of % total concentration of individual species with respect to

Figure 46: Water-metal salt complexes from MINTEQ
concentration of metal salt in figure 46. Minteq utilizes activity coefficients to determine speciation. Therefore, information on speciation is obtained under the condition zero ionic strength. At this condition, the activity of ions is equal to the concentrations as activity coefficient is 1. Simulations fail when run without fixing ionic strength at high concentrations of chromium and tin. Therefore, for uniformity in comparison, the ionic strength is fixed at zero.

Minteq predicts 6 different species of aluminum chloride which exist at concentrations of interest- $\text{Al}^{3+}$ denotes free aluminum ions in solution, $\text{AlCl}_2^{2+}$ indicates partially undissociated $\text{AlCl}_3$ and $\text{Al(OH)}_2^{+}$, $\text{AlOH}^{2+}$, $\text{Al}_2(\text{OH})_2^{4+}$ and $\text{Al}_3(\text{OH})_4^{5+}$ indicate aluminum-water complexes. At low concentrations of $\text{AlCl}_3$, most of the aluminum ions exist in the free state in solution i.e as $\text{Al}^{3+}$ ions. As the concentration of metal salt increases, relative percentage of free $\text{Al}^{3+}$ ions decreases. The $\text{Al(OH)}_2^{+}$ exists only at low concentrations of salt. Further, increase in concentration of salt also leads to a decrease in the aluminum-water complexes although the rate of decrease is not linear. Although increasing concentration of aluminum salt decreases the free $\text{Al}^{3+}$ in solution, this does not lead to an increase in aluminum-water complexes: relative percentages of $\text{AlOH}^{2+}$, $\text{Al}_2(\text{OH})_2^{4+}$ and $\text{Al}_3(\text{OH})_4^{5+}$ decrease with increasing concentration of the aluminum salt and that of $\text{Al(OH)}_2^{+}$ becomes zero. This is offset by an increase in $\text{AlCl}_2^{2+}$ is observed. This can be attributed to an increase in salt-salt interactions rather than salt-water interactions.

Variation in chromium chloride speciation with respect to concentration of $\text{CrCl}_3$ is also obtained. Minteq predicts 5 different species that exist in this range. $\text{Cr}^{3+}$ which is the free chromium in solution, complexes with water $\text{Cr(OH)}_2^{4+}$, $\text{Cr}_3(\text{OH})_4^{5+}$ as well as $\text{Cr(OH)}_2^{2+}$ in addition to the chloride $\text{CrCl}_2^{2+}$. Increase in concentration of salt leads to an increase in free chromium until a maximum is reached beyond which there is a slight decrease in relative percentage of free chromium. The percentage of complexes with water decreases with increasing concentration of salt which leads to a corresponding increase in the chloride form. This variation is very similar to aluminum.

For tin chloride, minteq predicts only 2 species; free tin species $\text{Sn}^{4+}$ and the complex with water $\text{Sn(OH)}_6^{2-}$. Interestingly, tin is the only salt capable of forming anions on
complexation with water. This can be attributed to increased electronegativity of tin compared to chromium and aluminum.

One uniform observation from speciation using MINTEQ is that the percentage of $\text{M(OH)}_x^{y+}$ species i.e., water-metal complexes decrease as concentration increases. Further, percentage of free cations (metal ions) in solution remains constant for tin, increases with concentration for chromium and decreases as concentration increases for aluminum. On the other hand, the nature of species formed is significantly different for tin compared to aluminum and chromium. Kinetics of mutarotation obtained from earlier sections indicate that the rate of mutarotation reaction increases as the concentration of $\text{SnCl}_4$ increases but remains fairly uniform with changes in concentration of $\text{CrCl}_3$ and $\text{AlCl}_3$.

Results on Brønsted acids however show uniform variations with respect to acid concentration. It is important to know whether increased rates with increased concentrations are a function of pH, ionic strength or a combination of both.

### 4.6 pH and ionic strength considerations

Since there is no correlation between individual species of metal salts and rate of mutarotation, ionic strengths were calculated to determine if the variation in this rate is dependent on the number of ions in solution rather than the nature of ions. Calculated ionic strength in mmoles/litre takes into account concentration and charge of the cation and anion under consideration.

\[
I = \frac{1}{2} \sum C_i Z_i^2
\]

where $C_i$ is the concentration and $Z_i$ is the valency of component $i$.

The ionic strength of $\text{SnCl}_4$ is greater than $\text{AlCl}_3$ and $\text{CrCl}_3$ of the same concentration. Mutarotation was found to be faster in $\text{SnCl}_4$ compared to $\text{AlCl}_3$ and $\text{CrCl}_3$. A reaction can be sped up or slowed down by controlling the ionic strength depending on the
mechanism. This is of great importance especially in cases where in the intermediate formed is between two cations or anions. In this example, reaction can be sped up by increasing ionic strength. On the other hand, an intermediate formed between a cation and an anion is stabilized at higher ionic strengths which would slow down the reaction. Lowry (23) demonstrated the proportional effect of acid and base concentration on the rate of mutarotation. The rate of mutarotation was found to be directly proportional to the square root of acid concentration and directly proportional to the concentration of alkali. This indicates in addition to ionic strength, other factors also play a role in the rate kinetics. Lowry further concluded that the mutarotation remains unchecked by neutralization and remains unaffected by acidic or basic impurities. In keeping with these results, the mutarotation of glucose was probed in three concentrations of HCl and H₂SO₄ solutions whose ionic strengths along with those of Lewis acids used are summarized in figure 47.

<table>
<thead>
<tr>
<th>Concentration (M)</th>
<th>pH of AlCl₃</th>
<th>pH of CrCl₃</th>
<th>pH of SnCl₄</th>
<th>pH of H₂SO₄</th>
<th>pH of HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
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<td>2.172</td>
<td>3.063</td>
<td>1.496</td>
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<td>2.347</td>
<td>3.106</td>
<td>1.699</td>
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<td>3.827</td>
<td>2.762</td>
<td>3.206</td>
<td>2.206</td>
<td>2.045</td>
</tr>
</tbody>
</table>

Table 9: pH of Lewis and Brönsted acids used

![Comparison of mutarotation rates as a function of pH for H₂SO₄](image)

![Comparison of mutarotation rates as a function of pH for HCl](image)

Figure 48: Variation in rates as a function of pH for the same acid

Further, the ionic strength of metal salts is much higher than acids for the same concentration in water. However, taking into account the lack of metal salt-glucose interactions, the comparison of mutarotation kinetics with respect to pH would be more pertinent. pH takes into
consideration only the free hydrogen ions in solution in contrast to ionic strength which takes into account all ions and their valencies. Table 9 indicates the pH of acids and metal salts used in this study.

Comparing rate of mutarotation for systems consisting of the same species (Figure 48), rate increases with lower values of pH and increasing values of ionic strength for Brönsted acids. This is indication that when the solution contains no metal ions, there exists monotonic variation in rate with respect to the acid concentration. This is further indication that the nature of metal-water species formed despite not bonding to glucose contribute to the kinetics. The same variation does not hold true for high ionic strength. AlCl$_3$ and CrCl$_3$ have the same ionic strength but different pH values. Figure 49 shows that at constant ionic strength, the mutarotation rates are identical.

Figure 50 compares the rate of mutarotation in 0.01 M H$_2$SO$_4$, 0.01 M HCl and 0.1 M CrCl$_3$: 3 solutions of comparable pH and water. From this figure, it is clear that the mutarotation reaction is faster in solutions whose pH is lower than that of water. On the other hand, this consumption rate is not a linear function of the pH when different species are involved as. The mutarotation is fastest in 0.01 M H$_2$SO$_4$ despite this solution
having a higher pH than 0.01 HCl and 0.1 M CrCl₃. On the other hand ionic strength varies as follows for the acids compared: CrCl₃ > H₂SO₄ > HCl but mutarotation is fastest in H₂SO₄.

In conclusion, for Brönsted acids, the rate of mutarotation increases as acid strength (lower pH and higher ionic strength) increases. In the case of Lewis acids, at low ionic strengths, mutarotation rates are identical for different species. However, this fails when different species exist in solution (figure 51). This may also be attributed to differences in electronegativity between Al (1.61) and Cr (1.66) becoming more significant at higher concentrations.
CHAPTER 5

CONCLUSIONS
It has been shown that vibrational spectroscopy is a very capable tool for the in-situ study of mutarotation in glucose in homogeneous catalysts. Compared to IR spectroscopy, Raman spectroscopy is a superior tool for analysis of such systems due to less interference from neighboring bands. Results from IR and Raman spectra indicate that there is no interaction between glucose and metal salts. However, the presence of metal salts, namely CrCl$_3$, AlCl$_3$ and SnCl$_4$ aid the mutarotation reaction leading to formation of beta-D-glucose from alpha-D-glucose. The relative equilibrium percentages of alpha-D-glucose and beta-D-glucose obtained from Raman spectra match well with values reported in literature (36%-64% respectively).

Rate of mutarotation is fastest in SnCl$_4$ wherein the rate increases with concentration of SnCl$_4$. The rate of mutarotation does not vary significantly with concentration of AlCl$_3$ and CrCl$_3$ but in both cases the reaction is faster than in water. Differences in nature of glucose-water interactions from ratio of intensities of CH$_2$ bending mode to OH bending mode of water with time for tin chloride might offer an explanation for increased mutarotation rates wherein glucose-water interactions are strong initially but become weaker with time. Since glucose-metal salt interactions were not visible from spectroscopy but the presence of the metal salt alters the rate of mutarotation, it can be concluded that the presence of metal salt has a significant effect on glucose-water and water-water interactions. Although increasing ionic strength leads to faster mutarotation for SnCl$_4$, a similar observation is not seen for aluminum and chromium chloride. In order to further highlight the influence of metal atoms in solution, mutarotation was studied in Br"{o}nsted acids. In the case of Br"{o}nsted acids, there is uniform variation with respect to ionic strength. Increasing acid concentrations led to faster mutarotation rates for Br"{o}nsted acids. It has been shown that water-water interactions can be substituted by glucose-water interactions (79). This leads to an increase in hydrogen bonding in a glucose-water system compared to a system containing pure water since the number of water molecules that can bond to a glucose molecule ranges from 4 to 9. A decrease in the highly reactive OH groups of water on addition of glucose has been reported (79). On the other hand, Minteq studies have shown that the nature of species formed is significantly different for Sn compared to Al and Cr which could contribute to
faster mutarotation rates and in all cases, metal species are formed with varying number (OH) groups. The tendency of metal salts to form such species leads to breaking of water-glucose bonds thereby influencing the mutarotation reaction.

Glucose-water complexes with 8 or 9 molecules of water bonded to glucose were studied (80). Addition of metal salts to glucose-water systems causes variations in this bonded network by metal salts combining with the highly reactive OH groups of water. The mutarotation which is carried out by protonation is aided by metal salts combining with OH groups of water. The greater the number of OH groups a metal salt is capable of binding with, faster is the mutarotation rate. This could offer an explanation as to why the reaction is fastest in SnCl$_4$ and remains fairly uniform in CrCl$_3$ and AlCl$_3$ and faster than water. The variation in rate of mutarotation is uniform with respect to concentration of Brönsted acids due to uniform increase in protons in solution.

Although this work addresses rate of mutarotation in Lewis acids, no information on possible short lived interactions between glucose and metal salt were obtained. More metal salts can be studied to determine if there is a definitive variation in mutarotation rates with respect to the number of OH groups to which the metal salt can bind with. This would also enable the determination of generic equations that describe variations in mutarotation rates depending on position of a metal cation in the periodic table. Once effects of Lewis acids on mutarotation rates are known and the impact of acidity understood, solid catalysts with desired structures and Lewis acidities can be used in
place of liquid catalysts. This reaction can also be studied at lower temperatures as this would slow down mutarotation rates making it easier for analysis. The speciation of metal salts varies with temperature and hence the effect of temperature on mutarotation rates can also be studied in greater detail as a function of the metal salt. This work remains a frontrunner in the study of homogeneous Lewis acid catalyzed mutarotation using vibrational spectroscopy. Several pertinent questions still remain unanswered which makes the study of this system vital if the dream of the biorefinery concept is ever to be fulfilled.
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