A STUDY OF THE EFFECT OF SALT SOLUTIONS ON THE KINETICS OF SUCROSE INVERSION AS MONITORED BY POLARIMETRY

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DECLARATION

I declare that this dissertation entitled *"***A STUDY OF THE EFFECT OF SALT SOLUTIONS ON THE KINETICS OF SUCROSE INVERSION AS MONITORED BY POLARIMETRY"** is the result of my own work except as cited in the references. The dissertation has not been accepted for any degree and is not concurrently submitted in canditure of any other degree.

Signature :

Name of candidate : Tlou Auguston Makwakwa

Date :

DEDICATION

This Research Dissertation is dedicated to the Will of God of Mount Zion-

You have caused wonders into my life that cannot be forgotten;

‘’Your word is a lamp to guide me and a light for my path’’

My loving parents Mr. Obed Makwakwa and Mrs. Margaret Makwakwa; no words can describe the love and support you have shown me ever since I was born. I thank God to have blessed me with such wonderful parents like you. God bless you!

My brothers, Freddy and Xanda, and my niece Oratilwe

My two Evas in my life,

Eva the Sister

You are so wonderful my Sister. Your encouragement and motivation kept me going when I felt down and out. Keep it up and God bless you!

Eva the wife;

Oh my paragon of beauty! The solace and comfort I find in you really soothe my heart. Remain in my life as I will remain in yours!

My cute son Bohlale and my gorgeous Angel Lebogang; My bundle of joy! You are the reason why I keep on smiling every day. You have brought so much joy and happiness into my life. Daddy loves you guys!

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Above all**,** I would like to thank The Almighty God who bestowed upon me the power and the blessings for accomplishing this work. Glory and Praises unto You My Lord!

ABSTRACT

The acid-catalyzed inversion of sucrose is often taken as an example of a first order reaction. It is, however, influenced by many factors such as temperature, type of acid used, concentration of sucrose, and the concentration of acid. What has received little attention so far is the influence of addition, in particular, other salts to the reacting solution. In this study, the influence of different salt solutions on the kinetics of sucrose inversion rate was studied at 29 °C by use of optical rotation measurements. The salts chosen for this study are readily soluble in sucrose solution and they provide an opportunity to study the interaction of electrolytes in aqueous solution of sucrose as well as their effects on the inversion of sucrose kinetics. The rates are found to be influenced by the concentration of the salts. No significant differences was measured when the salt were dissolved either in the sucrose or in the acid solutions.

The influence of added salts to saccharide solutions was determined by evaluating the difference between the rotation of pure saccharides solutions and the rotation of pure saccharide solutions with salts. The changes in optical rotation were compared to the Hofmeister series.

The saccharide-salt systems containing acidic salts (Na_2HPO_4 or NaH_2PO_4) were found to be dependent on the pH. Changing the molar ratio of sucrose and salt added also had an influence of the change in optical rotation.

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LIST OF ACRONYMS

IUPAC	International Union of Pure and Applied Chemistry
c	Concentration
Bx	Brix
l	Levorotatory
d	Dextrorotatory
DST	Density Functional Theory
ISS	International Sugar Scale
ICUMSA	International Commission for Uniform Methods of Sugar Analysis
I	Ionic Strength (mol kg^{-1})
k	Rate Constant
SDS	Salt Dissolved in Sucrose
SDA	Salt Dissolved in Acid
ND	Not Determined/ Not Dissolved
NMR	Nuclear Magnetic Resonance
HPLC	High Performance Liquid Chromatography
CAD	Charged Aerosol Detector
GC	Gas Chromatography
MS	Mass Spectrometry

CHAPTER ONE

INTRODUCTION AND GENERAL CONSIDERATIONS

This chapter describes in detail the background theory, the project aims as well as the project approach.

1.1 Project background.

1.1.1 Structure and properties of sucrose.

Sucrose, common table sugar, also called saccharose (Figure 1a and 1b), is a non-reducing dimer composed of two sugar monomers, α-D-glucose and β-D-fructose. Its systematic (IUPAC) name is α-D-glucopyranosyl β-D-fructofuranoside, and it has the molecular formula $C_{12}H_{22}O_{11}$.

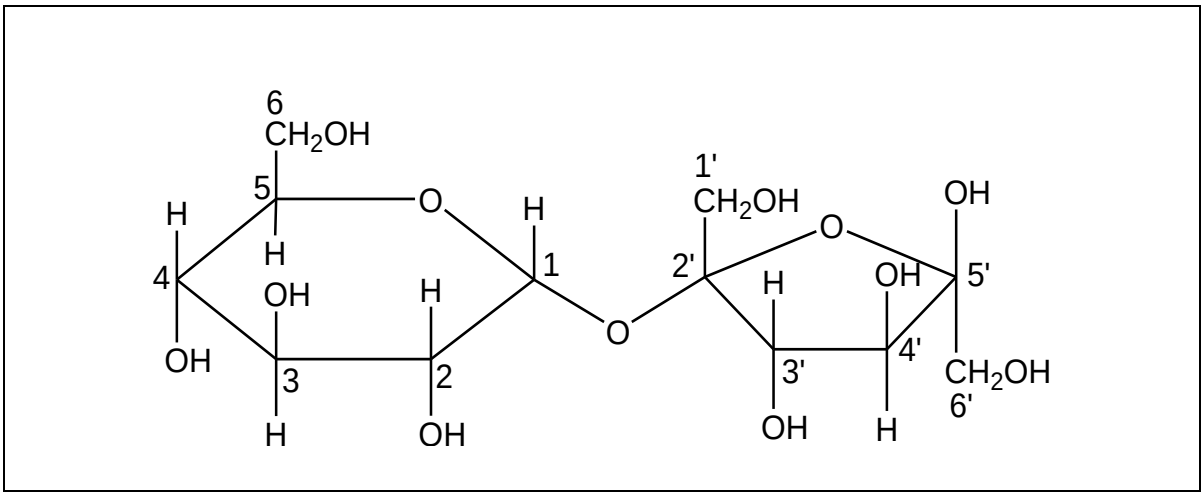

Figure 1 a Fisher structural representation of sucrose in a solution form.

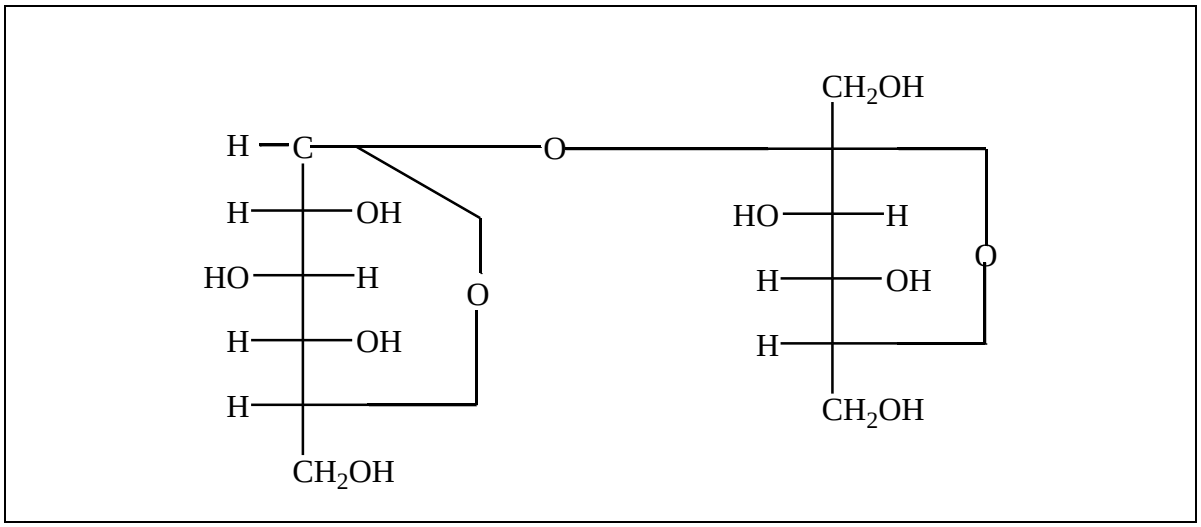

Figure 1 b Haworth structural representation of sucrose in a solution form.

The IUPAC name was derived from the observation that the pyranose and furanose notations in the name of a sugar indicate its ring(s) type. The six-membered ring sugars are known as pyranoses (after the compound pyran). Five-membered-ring sugars are called furanoses (after the compound furan). The notations + (or right hand) and – (or left hand) in the name of a sugar indicate the rotation of polarized light in Polarimetry. Generally, right hand sugars rotate polarized light clockwise, while left-hand sugars rotate polarized light to anticlockwise. The alpha (α) and beta (β) notations of glucose and fructose molecules indicate the position of the OH on the carbon atom that participates in glycosidic bonding to form sucrose. The glucose molecule is in α–form and the fructose molecule is in β–form, so the sucrose molecule is the combination of α–glucopyranosyl and β-fructofuranose rings. The notation D in the sucrose molecule is determined by the OH group attached to the farthest asymmetric (interior) carbon atom from the C-1 in its formula. When the OH group is positioned on the right side of this carbon when the molecule is presented in Haworth projection, the D isomer is meant [1]**.** The physical properties of sucrose are summarised below in Table 1.1.

Table 1.1 Physical properties of sucrose [1].

Name	Quantity
Molecular formula	$C_{12}H_{22}O_{11}$
Molecular mass	342.3 g/mol
Density	1587.9 kg/m^3
Melting point	160-186 ºC
Specific rotation at 20 ºC (589 nm, Sodium D line)	+66.53 º
Solubility at 20 ºC	204 g of sucrose/100 g of water
Specific heat-Crystalline	415.8 J/mol at 20 ºC
Specific heat-amorphous (noncrystalline sucrose)	90.2 J/mol at 22 ºC
Heat of formation	-2.26 MJ/mol
Heat of combustion	-5.79 MJ/mol
Normal entropy	360.5 J/(mol.k)^o, Entropy of solid at standard conditions (1 bar)
Crystallization Enthalpy	105 kJ/mol at 30 º

The specific rotations of glucose and fructose are +52.5º and -92.4º, respectively.

1.1.2 Sources and applications.

Sucrose is by far the most abundant carbohydrate found in the sap of land plants. It is formed as a result of photosynthesis and occurs in abundance in sugarcane (*Saccharum officinarum*, a perennial tropical grass) and sugarbeets (*Beta vulgaris*, a hearty biennial) in amounts ranging from 12-15 and 13-20% by mass, respectively [1]. Commercial quantities are provided only from these two sources. Other sources include sorghum (*Sorghum vulgare*), the sugar maple (*Acer saccharum*), and the date palm (*Phoenix dactylifera*). Sugar consumption in all forms from beet and cane, is an important factor in the diet of a man due to its richness as a source of energy. In food industry, sucrose plays a major role in the production of many food products from cure meats through preserves and frozen fruits to confections [2]. Pharmaceutical industries, cosmetics, and other non-food users use sucrose as a

starting material to produce, among others, fermentative products such as ethanol, butanol, glycerol, citric acid, levulinic acids and other products in the plastics and cellulose industry [3]. In biological systems, it can be used to prepare density gradients for cell/organelle separation [4]. In addition, sucrose can also be used as a supplement in plant, insect, and bacterial culture media as well as in various enzymatic assays [5].

1.1.3 Reactivity and kinetics.

Structurally and functionally, sucrose is a complex molecule, highly oxygenated, and chemically sensitive. It is a molecule with five stereo centres and many sites that are reactive or can be reactive. It contains eight hydroxyl groups, three of which are primary (C-6, 6', 1') and the remaining five are secondary (C-2, 3, 3', 4, 4'). The primary hydroxyl groups react preferentially, in particular the hydroxyl groups at C-6 and C-6' positions [1]. Part of its extraordinary versatility is that it can be hydrolysed partially or fully into glucose and fructose [6]. The reaction during which sucrose is converted into glucose and fructose is known as inversion. Sucrose is important in the determination of adulteration in honey, quantification of the sweetness of a product or simply for the determination of total sugars [7]. Historically, the study of sucrose inversion by homogeneous acid catalysis has been important in the development of chemical kinetics [8-11], because it serves as a primary example of a pseudo-first order kinetic reaction. It has been studied by a variety of techniques such as Polarimetry; Spectroscopy; Chromatography; Colorimetry and Spectrophotometry; Nuclear Magnetic Resonance; Isotope Dilution; Titrimetry; Enzymatic Analysis; and Enzyme Electrodes [12]. The Polarimetry technique continues to be an active and convenient method to research the determination of sucrose and has therefore been used in the present study.

The Polarimetry technique exploits the optical properties of compounds by measuring the polarisation of light as it appears in solutions of chiral molecules such as sucrose and other macromolecules. Sucrose is thermodynamically unstable with respect to hydrolysis to form glucose and fructose according to the following formula:

$$C_{12}H_{22}O_{11} + H_2O \xrightarrow{H^+(aq)} C_6H_{12}O_6 + C_6H_{12}O_6 \quad (1.1)$$

The reactant sucrose and the products glucose and fructose are optically active [13], and consequently rotate the plane of polarized light by characteristic amounts. Sucrose is dextrorotatory (rotates the plane of polarization clockwise) and the equimolar mixture of glucose and fructose is levorotatory (rotates the plane of polarization anticlockwise). As a result, as the hydrolysis of sucrose proceeds the optical rotation of the solution changes from positive to zero to negative. Because of this sign change the hydrolysis of sucrose is also referred to as the inversion of sucrose and the product as inverted sugar [14]. The change in optical rotation provides a convenient means of monitoring the course of the reaction.

Generally, the reaction of sucrose inversion proceeds too slowly to be measured in pure water, but it is catalyzed by H^+ (*aq*). In biological systems, the same reaction of sucrose hydrolysis is catalyzed by an enzyme called invertase [15-16]. In a dilute hydrochloric acid solution, the hydrolysis of sucrose into single sugars also occurs in the human stomach [17].

The differential rate law for the inversion of sucrose is in the form [18]:

$$\frac{d[sucrose]}{dt} = k[sucrose]^m\,[H_2O]^n\,[H^+(aq)]^p \quad (1.2)$$

This expression indicates that the reaction is of order *m* with respect to sucrose, order *n* with respect to water, and order *p* with respect to hydronium ion. It is clear from the equation (1.2) that the rate of inversion depends on the concentration of sucrose, water and acid. Since water is the solvent, it is in large excess, so that its concentration does not change appreciably. If the concentration of the H^+ (*aq*)

catalyst is also high, it can be regarded to be in excess and can therefore be considered as constant.

It can be included in the effective rate constant k_{eff} based on the assumption that the stoichiometry is presented as one mole of sucrose giving one mole each of glucose and fructose; and that the mutarotation of glucose and fructose is negligible. So equation (1.2) can be re-written to isolate the dependence on the concentration of sucrose only [19]:

$$-\frac{d[sucrose]}{dt} = k_{eff}[sucrose]^m \tag{1.3}$$

where

$$k_{eff} = k[H_2o]^n[\,H^+(aq)]^p \tag{1.4}$$

Under the specified conditions, water is in excess (55 M) and the acid concentration is of the order of 2.5 M (This work), the inversion of sucrose can be considered as a pseudo first order reaction in sucrose [20]. This can be verified by fitting the experimental data to a first-order integrated rate law.

The integrated form of the first-order reaction differential equation is then:

$$c = c_o e^{-k_{eff}t} \tag{1.5}$$

where c_o is the initial concentration of sucrose, c is the concentration of sucrose at time t, and k_{eff} is the reaction rate constant. By taking logarithms on both sides of the equation (1.5), gives:

$$\ell n\left({}^{c}/_{c_o}\right) = -k_{eff}t \tag{1.6}$$

However, in this work the concentration of sucrose is not directly measured. Instead the optical rotation of sucrose and its dissociation products are measured during the course of the reaction. The angle of rotation is determined at the beginning of the experiment (α_0) and after completion of the reaction (α_∞), and the algebraic difference (α_0-α_∞) is a measure of the original sucrose concentration. The optical rotation (α) of a solution is directly proportional to the change in concentration of sucrose in the reacting solution. Thus, c is proportional to (α_t-α_∞) and c_0 is

proportional to (α_0-α_∞). The relationship between optical rotation and concentration allows equation (1.6) to be re-written in the form [21-23]:

$$\ell n \frac{\alpha_t - \alpha_\infty}{\alpha_o - \alpha_\infty} = -k_{eff} t \qquad (1.7)$$

If the plot of $\ell n(\alpha_t - \alpha_\infty / \alpha_0 - \alpha_\infty)$ versus t is a straight line, its slope will be $-k_{eff}$ and the reaction is pseudo first-order.

The inversion rate of sucrose hydrolysis depends on the following general factors [24-26]:

I. The concentration of sucrose
II. Temperature
III. The concentration of H^+ (aq)

Some previous investigations and basic concepts on the study of these factors affecting inversion rate of sucrose are briefly discussed below.

Effect of Initial concentration of sucrose

The first substantial quantitative study of the rate of a reaction was performed by L. Wilhelmy, who in 1850 studied the inversion of an acidic aqueous solution of sucrose into its constituents, glucose and fructose [27]. He found empirically that the rate of decrease of concentration of sucrose was simply proportional to the concentration of sucrose remaining unconverted, i.e. following first order reaction [28]. At higher sucrose concentrations, it is expected that the kinetics may deviate from first order as the assumption that the water concentration is in large excess may not be valid. However, under conditions of high acid and water concentrations, the reaction follows pseudo-first order kinetics. A study of varying concentrations of sucrose was performed by Nelson and Schubert [29]. They found that the rate of hydrolysis by invertase decreased when the sucrose concentration is greater than 10%, the relation between sucrose concentration and reaction rate being approximately linear between 10% and 70% sucrose. The decrease in reaction rate was attributed to the falling off in water content.

Temperature effect.

For many reactions it is found that the rate constant varies with temperature according to:

$$k = Ae^{-\frac{E_A}{RT}} \tag{1.8}$$

where *A* and *R* are constants. A plot of Ink vs. 1/T should thus yield a straight line. Equation (1.8) is known as the Arrhenius law after Arrhenius, who demonstrated its validity by simple thermodynamic arguments that are applicable to elementary reactions [30]. It is known that by raising the temperature of the reacting solution, the rate of molecular collision increases and so does the rate of reaction. Table 1.2 shows the values of inversion constants (K_0) which are used to calculate the quantity of invert sugar formed per unit of time from an initial sucrose concentration at different temperatures ranging from 50-100 °C; unfortunately no information about the acid and the sucrose concentrations as well as the rate constants is available for this data**.**

Table 1.2 The values of inversion constants (K_0) for the inversion of sucrose at different temperatures [31].

Temperature (° C)	*k*
50	0.12
60	0.38
70	1.18
89	3.30
90	8.92
100	26.80

Ward [32] compiled a summary of rate constants for inversion of 0.0584 M sucrose in 0.57 M HCl at temperatures ranging from 0-40 °C. Table 1.3 shows the data analyzed by using a polarimetry technique reproduced from Buchanan *et al*. [33]. The reaction is pseudo first-order.

The values of rate constants were converted to $k_{H_3O^+}$ per seconds and by dividing the observed rate constants (k_{obs}) by the concentration of HCl measured in Molar units.

Table 1.3 Summary of rate constants for inversion of 0.0584 M sucrose in 0.57 M HCl [33].

Temperature (° C)	k (M^{-1} s^{-1} x10^5)
10	1.609
15	3.444
20	6.928
25	16.07
30	32.69
35	67.79
40	129.30

Effect of H^+ (aq).

The influence of the common acids as catalysts has been investigated in many reactions for both low and high concentrations. The catalytic ability of various acids (Table 1.4) on the inversion of sucrose may be characterized by a quantity called Inversion Ability, which is defined as a ratio of kinetic constants related to hydrochloric acid catalysis as 100% [34].

Table 1.4 Catalytic ability of various acids in the inversion of sucrose [34].

Acid	Inversion Ability (%)
HBr	111.4
HCl	100
HNO_3	100
H_2SO_4	53.6
H_2SO_3	30.4
Oxalic acid	18.6
H_3PO_4	6.2
Citric acid	1.72
Maleic acid	1.27
Lactic acid	1.07
Acetic acid	0.5

Studies indicate that for dilute solutions of acids the most useful measure of acidity is pH. In solutions of strong acids more concentrated than about 0.1 M, however, the acidity is more satisfactorily measured by the Hammett acidity function, H_0, or other acidity functions [20]. By extending the concentration of hydrochloric acid to 8 N (Normality) and sulfuric acid to 10 N (Normality), Krieble [35] correlated the rate constants (velocity constants) for the hydrolysis of sucrose with the activity of H^+ (aq) of the catalysts. With all variations of strong and dilute acids studied on the inversion of sucrose, the inversion rate was found to increase with increasing the concentration of the acid used.

1.2 Project aim.

1.2.1 Objective and justification.

The hydrolysis of sucrose, generally known as the inversion of sucrose, has been studied for centuries [10, 36]. Although the inversion of sucrose has often been taken as a ‘classical’ example of pseudo-first order reaction, this is by no means unambiguously the case [20, 37-38]. There are many studies where various aspects of the sucrose inversion reaction have been investigated such as rate constants [17, 24], temperature dependence [26], and mutarotation [39]. Many parameters influence the rate of sucrose inversion. In general, studies of rate of sucrose inversion in the literature cover a large variety of conditions, as it is outlined in Section 1.1.3 of this dissertation.

The details of and influences on the sucrose inversion reaction are still relevant and of interest. This is evident in an article about the study of the sucrose inversion mechanism by analyzing products formed during the hydrolysis of sucrose catalyzed by dilute acids [7], or the recent publication [40], where the most likely mechanism of the hydrolysis of sucrose has been computed using Density Functional Theory (DFT) methods.

Because of the availability of an improved polarimeter with automated, temperature controlled measurements set at predetermined time intervals, it was attempted to reinvestigate the rate of sucrose inversion using polarimetry. Some basic aspects of sucrose solutions and the acid catalyzed sucrose inversion have been tested. The presence of salts in many naturally occurring sucrose inversion reactions may have a considerable influence on the rate of hydrolysis; therefore the addition of salts to the acid catalyzed sucrose inversion was studied.

1.2.2 The project approach.

The approach employed to satisfy the objective of the study was to use polarimetry technique as the main analytical tool to:

a) Study the angles of rotation of saccharide solutions, and of salts added to the saccharides. The distinct difference between the optical activity of saccharides and the optical inactiveness of salt solutions allow the measurements of change in rotation when these salts are added to the saccharide solutions; and the evaluation of their effects on modifying the optical rotation.
b) Test the rate of acid catalyzed sucrose inversion at constant temperature; study the influence of added salts.
c) Consider the reproducibility of the results and the error of measurement involved in the kinetics study.
d) Apply different techniques of evaluating the results. Techniques such as Infinite Time, Guggenheim, and Kezdy-Swinbourne (to be described in Chapter 2) were used to determine reaction rate constants from the optical rotation of the inversion reaction as monitored over time by using polarimeter.
e) Apply scientific theories to various aspects of the results. The effect of anions on saccharide-salt solutions was examined according to the Hofmeister Series (Section 2.4). The addition of different salt solutions in the inversion reaction was studied considering the kinetic salt effect (Section 2.3) which can aid in providing qualitative and quantitative information about the mechanism of the reaction.

CHAPTER 2

THEORETICAL CONSIDERATIONS

This chapter describes the theory of Polarimetry. In the initial section, the principle of polarization and two kinds of polarized light are discussed. A distinction is made between the types of polarized light associated with Polarimetry instruments. A detailed overview of the phenomenon of optical rotation and the theory of optical rotation based on quantum mechanics is explained. The basic components of a Polarimeter, including its operation principle and application in sugar industry are also presented. In the last sections, the evaluation methods for treatment of the kinetic data, the theory of the kinetic salt effect as well the Hofmeister series are discussed.

2.1 Theory of polarimetry.

2.1.1 Introduction to Polarimetry.

Polarimetry is a technique which measures the extent to which a substance interacts with plane polarized light. When light passes through a polarizer, the vibrations of the polarized light propagate only in one direction. This process is known as polarization. There are basically two main types of polarization, i.e. linear and circular. Each of these two types can be achieved by using different devices. Both linearly polarized and circularly polarized light have important chemical applications [41]. Circularly polarized light is applied in various techniques of circular dichroism [42], which are used for biomolecular and nanostructural analyses. Polarimetry uses linearly polarized light to measure the rotations of optical activity of molecules [43].

When a beam of plane-polarized light propagates through a transparent sample of a crystal or a liquid sample, the emerging beam will also be plane-polarized. However, for specific classes of substances (These are substances that contain an asymmetric

center i.e. chiral atom or chiral center), the plane of polarization is rotated by an angle (α) with respect to the direction of the plane of polarization of the incident light. This phenomenon is called optical activity and is determined by measuring the angle of rotation of the polarized light. The optical activity of molecules manifests itself through the Law of Malus [44], which states that when a perfect polarizer (which exhibits 100% transmission of the desired state of polarization) is placed in a polarized beam of light, the intensity of light, *I*, varies according to $I = I_0 cos^2\theta_i$, where I_0 is the initial intensity of light and θ_i is the angle between the light's initial polarization direction and the axis of the polarizer in the Polarimeter. The phenomenon of optical rotation is explained in detail in the next section.

2.1.2 Optical activity.

Linear polarized light is produced when a polarizer is placed into an incident beam and waves of the emerging beam oscillate in only one plane (Figure 2.1). Optical rotation is the property displayed by chiral substances in which the direction of linearly polarized light is changed. The plane of polarization could rotate clockwise or counterclockwise due to the molecular conformation of an optically active compound. Molecules possessing the ability to rotate light to the left or counter-clockwise are denoted as levorotatory (designated as *l*) and those rotating light to the right or clockwise are referred to as dextrorotatory (designated as *d*) [41]. The usual explanation given in introductory organic chemistry [45] indicates that an asymmetric center or chirality is required to characterize the optical activity of a substance. The most common feature though not the only one that lends chirality is a carbon atom that is bonded to four different groups.

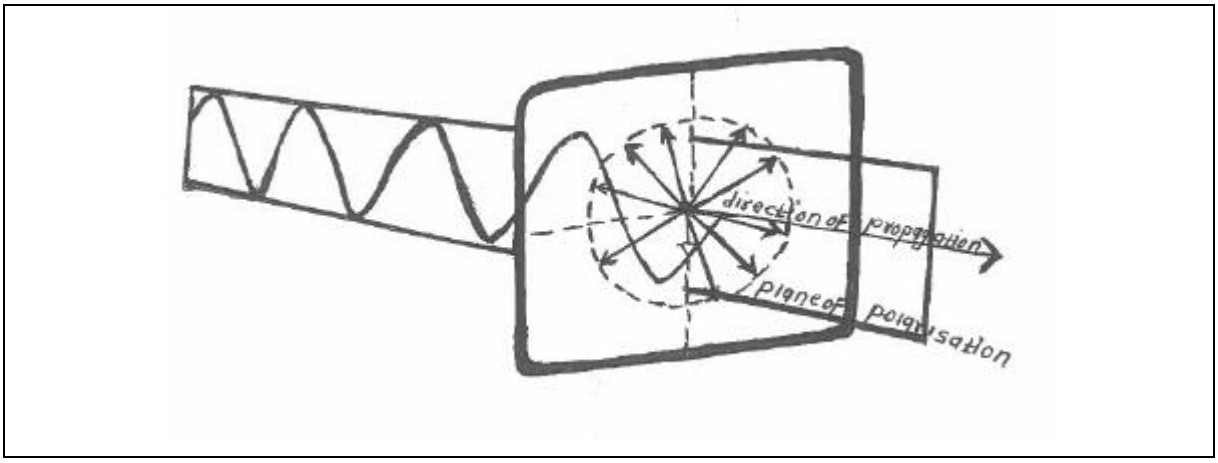

Figure 2.1 A linear polarizer conversion of an unpolarized beam into one with a single linear polarization. The vertical components of all waves are transmitted, while the horizontal components are absorbed and reflected. This figure was redrawn from reference [46].

On the other hand, the theory of optical activity can also be explained in terms of quantum mechanics of molecules and quantum theory of radiation. The quantum mechanical theory of optical rotation was first formulated in 1928 by Rosenfeld [47], and it focuses on semi-classical argument to calculate the interaction between an electromagnetic wave and a molecule. In 1937 Kirkwood [48] formulated a quantum mechanical theory of natural optical activity which relates optical rotator power to molecular structure. This theory is the result of quantum mechanical formulation of the Born-Kuhn theory [49].

2.1.3 Basic principle of polarimetry.

A typical conventional polarimeter is shown in Figure 2.2, and it consists of

a) A monochromatic light source.
b) A polarizer.
c) A sample cell.
d) Polarization analyzer ,and
e) A light detector.

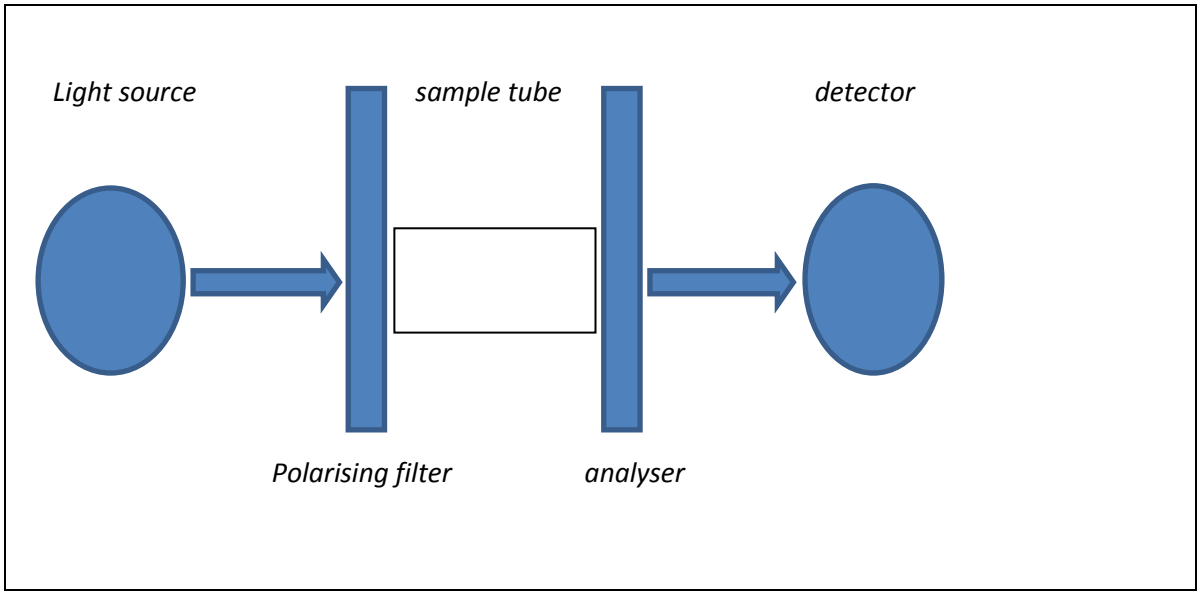

Figure 2.2 A schematic representation of a typical Polarimeter. This figure was redrawn from references [50-51].

Unpolarized light from the light source is first polarized. This polarized light passes through a sample cell (usually 10 cm). If an optical active substance is in a sample tube, the plane of the polarized light waves is rotated by an amount which is characteristic of the test solution. The analyzer is used to determine the rotation due to sample of an optically active substance. Generally, the analyzer is oriented perpendicular to the polarizer. If no optically active substance is present, theoretically no polarized light can emerge from the analyzer to the observer. If an optically active sample is introduced into the system, the component of the polarized light is transmitted through the analyzer and the intensity of this part of light is proportional to the amount of rotation, which depends on the concentration of the optically active medium if the path length is kept constant. Thus, the concentration of the medium can be calculated using polarimetric measurements.

The sodium wavelength of 589 nm is by far the most common light source used in polarimetry and most experimental methods and published data are based on this wavelength. This is because; the classical performance of polarimetry used an instrument where the degree of optical rotation is estimated by visual matching of the intensity of split fields. Split-fields polarimeters were previously designed and constructed to provide simultaneous image pairs in a single frame, differing only in

the direction of linear polarization, i.e. the direction in which one half of polarization occurs at right angle to the other [52]. Consequently, the emission D-line of the sodium lamp at the visible wavelength of 589 nm was commonly used [53]. Other popular light sources are mercury at 546 nm, Helium-Neon Laser at 632 nm, and tungsten-halogen Near Infrared (NIR) at 882 nm.

The angle (α) of rotation of the plane of polarized light by an optically active substance depends on:

a) Concentration of optically active compound [45].
b) Optical length of solution through which the polarized light passes [54].
c) Temperature of the measured solution [54].
d) Wavelength of used polarized light [54].
e) Structure of optically active compound [54]
f) The kind of the solvent used [54].

The optical rotation caused by a given solution is proportional to the concentration of that sample. The relationship between the angle of rotation (α) and the concentration (*c*) of optically active compounds is described by equation [45]:

$$\alpha = [\alpha]_{\lambda} lc \tag{2.1}$$

where *l* is the length of the solution container, $[\alpha]_{\lambda}$ is the specific rotation, and *c* is the concentration in grams per mL. Specific rotation characterizes optically active compounds. It is a constant for every given compound, temperature and wavelength of light. Since optical rotation is both temperature and wavelength dependent [23], one works with monochromatic radiation and at a specified temperature. This monochromatic light is usually provided by the yellow light of a sodium lamp. The optical rotation of a solution, *α*, for a concentration of *c* expressed in grams/100 mL of solution, gives the specific rotation $[\alpha]$ with a sodium D line light source at 25°C according to the following equation:

$$[\alpha]_D^{25} = 100\alpha/lc, \tag{2.2}$$

where *l* is the length of the Polarimeter tube in centimeters. The specific rotation is determined as the rotation exhibited by 1 gram of an optically active substance in 100 mL of solution having an optical path length of 10 cm.

2.1.4 Application of polarimetry in sugar industry.

Polarimetry is frequently used for determining the yield and quality of the final sugar products obtained from sugar cane or beets. Measurements are made using polarimeters or saccharimeters with the scale in angle degrees (°) or sugar degrees (°Z). Similar to any optically active compound, the angle of rotation depends linearly on the concentration of sugar in the solution while other parameters such as temperature, light source, length of the tube are as described above. The International Sugar Scale (ISS) [54] measures sugar solutions in °Z units. It was introduced in the sugar industry by International Commission for Uniform Methods of Sugar Analysis (ICUMSA). According to ICUMSA, 100.00 °Z units correspond to a Normal Sucrose Solution. A normal sucrose solution is defined as 26.000 g of pure sucrose weighed in vacuo and dissolved in water at 20.00°C to a final volume of 100.000 cm^3. This corresponds to 26.000 g weighed in air under normal conditions (1013 mbar, 20°C, 50% relative humidity) and dissolved in water to a final volume of 100.000 cm^3 [55].

In general, the process of sugar manufacture involves extraction, evaporation and crystallization steps [56]. The impure sucrose solutions are tested by the addition of a clarifying reagent to the sample before they are tested by using a polarimeter. The purpose of clarifying reagents is to:

a) Precipitate colloids and pigmented substances, which interfere with polarimetric tests.

b) Produce a clear sample, which is a prerequisite for polarimetric tests.

Impure sucrose solutions form part of in-process products such as juices, syrups etc. The amount of impurities (non-sucrose substances), may affect the results obtained from polarimetric measurements since many compounds other than sucrose are also optically active and change the direction of polarized light. Clarifying reagents, such as aluminium chloride, are used by sugar laboratories to reduce the effects of these interfering substances that precipitate non-sugar samples [57]. Since these clarifying reagents precipitate active substances in the manufacturing process, after the precipitation step the precipitate is filtered, and the percentage of sucrose in the filtrate is measured by using a polarimeter. In impure solutions, the polarimetric sucrose denoted PS represents only the approximate sucrose content. The exact sucrose is measured by the inversion method and it is called true sucrose, denoted TS. The inversion method has found many applications in research [26], and it is generally used as a basis for the development of kinetics studies [58].

2.2 Measurement of kinetic data.

The usual procedure to analyse kinetic data is to plot a function of measured concentration versus time, according to the integrated rate law for the order of reaction. For a first order reaction that would be ℓn (C_t-C_∞/C_0-C_∞) or in the present case ℓn (α_t-α_∞/α_0-α_∞) versus time (see equation 1.3), which is called 'infinite time method' in this dissertation. Besides the infinite time method for first order integrated law, there are two other methods for establishing a rate law and evaluating first order kinetic data which were found relevant to this study. These two methods were developed by Guggenheim and Kezdy-Swinbourne, and are described below. Cornish-Bowden [59] indicated that the results of these two graphical methods differ slightly but can be used to estimate the reaction rate constants.

2.2.1 The Guggenheim method.

The Guggenheim method allows the estimation of the rate constants from measurements without the need of making the infinite-time measurement and without utilizing curve fitting techniques [60]. The method is described as follows:

If times t_1, t_2, t_3 and $t_1+\Delta$, $t_2+\Delta$, $t_3+\Delta$ are selected, where Δ is a constant increment, the following holds true:

$$(\alpha_1 - \alpha_\infty) = (\alpha_0 - \alpha_\infty)\, e^{-kt_1} \tag{2.3}$$

$$(\alpha'_1 - \alpha_\infty) = (\alpha_0 - \alpha_\infty) e^{-k(t_1+\Delta)} \tag{2.4}$$

where α_1 and α'_1 are the optical rotations at t_1 and $t_1+\Delta$, respectively. From equations (2.3) and (2.4), the following is obtained:

$$(\alpha_1 - \alpha'_1) = (\alpha_0 - \alpha_\infty) e^{-kt_1} (1 - e^{-k\Delta}) \tag{2.5}$$

$$kt_1 + \ell n(\alpha_1 - \alpha'_1) = \ell n(\alpha_0 - \alpha_\infty)(1 - e^{-k\Delta}) \tag{2.6}$$

$$kt_1 + \ell n(\alpha_1 - \alpha'_1) = constant \quad (2.7)$$

Thus, a plot of $-\ell n(\alpha_1 - \alpha'_1)$ versus time has a slope *k* [61].

2.2.2 Kezdy-Swinebourne method.

The Kezdy-Swinebourne method also allows the calculation of the values of *k* and it is claimed by Bobrovnik [62] to be an improvement on the Guggenheim method. In addition, the Kezdy-Swinbourne method allows determination of α_∞ as shown below:

Given that the equations

$$(\alpha_\infty - \alpha_t) = (\alpha_\infty - \alpha_0)e^{-kt} \quad (2.8)$$

and

$$\alpha_\infty - \alpha_{t+\tau} = \alpha_\infty - \alpha_0 e^{-k(t+\tau)} \quad (2.9)$$

hold true, where α_t is the optical rotation at time t and $\alpha_{t+\tau}$ is the optical rotation at time t+τ, then by division, it follows that

$$(\alpha_\infty - \alpha_{t+\tau})/(\alpha_\infty - \alpha_t) = e^{-k\tau} \quad (2.10)$$

This further rearranges into

$$\alpha_t = \alpha_\infty(e^{k\tau} - 1) + \alpha_{t+\tau}e^{-k\tau} \quad (2.11)$$

Plot of α_t vs. $\alpha_{t+\tau}$ gives the slope = $e^{-k\tau}$. The linear plot of equation 2.11 also yields alpha infinity (α_∞).

2.3 The kinetic salt effect.

Kinetic salt effect is defined as the general effect of an added electrolyte on the observed rate constant of a reaction in solution [63]. The foundation of the theory of primary kinetic salt effects was laid by Bronsted [37]. Bronsted's fundamental theory for the kinetic salt effect predicts the influence of ionic concentration and charges on the reaction rate in dilute solutions according to the following equation:

$$k = k_0 \gamma_A \gamma_B / \gamma_{\ddagger} \tag{2.12}$$

where k_0 is the limiting value of the rate constant as ionic strength tends to zero and γ_A, γ_B, and $\gamma_{\ddagger}$ are the activity coefficients for reagents *A* and *B*, and activated complex, respectively. If the solutions are dilute, then the activity coefficient of a given ion *i* of charge z_i is given by the Debye-Huckel limiting law [64]:

$$\ell og \gamma_i = -A z_i^2 / I^{1/2} \tag{2.13}$$

The above equation presents the relationship between the ionic strength and the activity coefficients (γ_i) of the reacting ions in the solution, where z_i is the ionic charge and *A* is a constant that has the value 0.509 in aqueous solution at 298 K [64]. Considering that an activated complex is formed from the reagents, and expressing the concentration of this activated complex in terms of the concentrations of the reagents and activity coefficients, an equation expressing the kinetic salt effect can be derived by making use of the Debye-Huckel law'

$$\ell ogk = \ell ogk_0 + 2Az_A z_B I^{1/2} \tag{2.14}$$

Thus, a plot of log *k* versus $I^{1/2}$ should be linear with the slope and intercept equal to 1.02 $z_A z_B$ and log k_0, respectively. The slope represents the product of charges on the species involved in the rate-limiting step. If the rate-limiting step is between the species of like charges, a positive slope is expected. When the reaction is between opposite charges, it results in a negative slope [64].

The kinetic salt effect has been investigated for the inversion of sucrose in dilute solutions by Guggenheim *et al*. [37] and it has been found that the addition of univalent, bivalent and trivalent salts follows Bronsted's principle of specific interaction which showed a negative salt effect of the multivalent cations that increased with their valency. The negative salt effect was attributed to the principle of specific interaction which became detectably inaccurate at higher ionic strengths. Jonnalagadda *et al*. [65] were able to make conclusions about the mechanisms of sulfite reduction taking place in dilute solution. Although in the present study solutions were not dilute some qualitative observations could be interpreted using kinetic salt effect.

2.4 The Hofmeister series.

The Hofmeister series or lyotropic series is a classification of ions in order of their ability to salt out or salt in proteins. It was first investigated with the aim to characterize the ability of salts to precipitate proteins from aqueous solution [66]. It was originally described by the capacity of various ions to "make" or "break" bulk water structure [67]. The Hofmeister series [68], which is more pronounced for anions than for cations, orders anions by their decreasing hydrophobicity therefore increasing the degree of aqueous solvation. The series is in the following sequence:

> Organic anions > ClO_4^- > SCN^- > I^- > salicylate$^-$ > NO_3^- > Br^- > Cl^- > HCO_3^- > $H_2PO_4^-$ > F^- > SO_4^{2-} > HPO_4^{2-}

The ions at the right end of the series are referred to as chaotropes and usually act to dissolve salt protein molecules into solution, while the ions on the left are called kosmotropes and cause proteins to salt-out of solution. The Hofmeister effect is related to many phenomena besides the protein solubility, for example, surface tension of electrolyte solutions, the electrolyte activity, pH measurements [69]. The Hofmeister sequence for the optical rotation of sucrose when salts are added to sucrose solutions, and for the kinetics of sucrose inversion has not yet been investigated and is presented in the present study.

CHAPTER 3

EXPERIMENTAL SECTION

In this chapter, a detailed description of all the experiments covered in this research is presented. In the first section, the preparation of systems involving aqueous mixtures of sucrose with various salts such as NaCl, Na_2HPO_4, NaH_2PO_4, and Na_2SO_4 is described. The limiting solubility of the salts in sucrose solutions was tested. The addition of salts to glucose and fructose solutions has also been studied to pinpoint with which moiety of the sucrose the salts have the most influence. In the final section, the application of sucrose-salt and acid-salt solutions to kinetic studies is described.

3.1 Instruments and reagents.

All reagents used were either analytical reagent grade or the purest available commercially and were used without further purification. Mass measurements were taken using Mettler Toledo, AB204-S/Fact (South Africa) balance. Conductivity and refractive index measurements were taken using, Conductivity TDS/SAL/Resistivity and Rudolph Research J257 Automatic Refractometer (Lasec SA (Pty) Ltd) instruments. Measurement of pH was made with Metrohm 632-pH meter with a combination electrode. All spectra were recorded on a Bruker MultiRam FT-Raman spectrometer fitted with a Nd-YAG laser and Germanium diode detector. A Rudolph Autopol II/IIZ Polarimeter equipped with sodium lamp at the nominal wavelength of 589 nm, sensitive to 0.01°, was used to measure optical rotation while Rudolph Research Analytical/ Rudolph PC-Software was used to record the data. The Polarimeter sample tube was 100 mm in length, made of quartz and jacketed with water for temperature control.

The temperature of the reaction was controlled with a thermostated water bath which allows the constant temperature water to circulate as shown in Figure 3.1.

Figure 3.1 Experimental Set-up. Left hand side: Polarimeter; middle: computer; right hand side: waterbath.

The temperature of the solution contained in the sample tube was measured by inserting a built-in temperature probe of the Polarimeter in the reacting solution. There was no detectable difference between the temperature registered on a thermometer in the waterbath and the temperature of the solution inside the sample tube. The reaction temperature for all the experiments conducted was 29 °C. This temperature was found to be the most suitable temperature set-up as it equilibrated well with the water jacketed polarimeter tube and the reacting solutions, because the radiation from the sodium source caused the temperature to rise to about 29 °C. The concentration of 10% (mass/volume) sucrose was chosen to give manageable measurement times of the order of 1 hour, as has been suggested for experiments for students [19].

3.2 Procedure.

3.2.1 Preparation of salt solutions for optical rotation measurements.

Pure salt solutions of NaCl, Na_2SO_4, Na_2HPO_4 and NaH_2PO_4 were prepared by dissolving accurately 1 g of salt in 10.00 mL volumetric flask using deionised water.

3.2.2 Preparation of sucrose-salt, glucose-salt, and fructose-salt mixtures.

10 g of sucrose was dissolved in a small quantity of deionised water and the amount of salt (by mass) to give concentration in M was then added and made up to 100.00 mL of solution. This addition of salt in sucrose solution was monitored in terms of how the optical rotation changes by varying the concentration of salt. Most suitably, the extent to which NaCl, NaH_2PO_4 and Na_2SO_4 dissolved in sucrose solution covered the range 0.100 to 5.000 M, 0.100 to 1.500 M and 1.000 to 1.500 M, respectively. Details of the calculated and weighed masses are given in Appendix 1. Generally, the difference between the calculated and weighed amounts is of the order of 0.01% and can therefore be neglected. A similar procedure was followed for the preparation of glucose-salt and fructose-salt systems. For further investigations on how the salts influence the saccharides, systems of sucrose, glucose and fructose were prepared in the Molar ratio of 1:1 with the acidic salts NaH_2PO_4 or Na_2HPO_4 by varying the pH of the saccharide-salt solutions. The preparation of molar ratios was further extended to 1:2 and 1:3 (sucrose: salt) for sucrose. The pH of these systems was maintained by adding drops of NaOH or acid using dropping pipettes. The selected pH values were adjusted and measured according to the distribution of phosphate anions in speciation diagram of H_3PO_4. The application of H_3PO_4 speciation diagram can provide valuable information on the study of saccharide-phosphate systems. It predicts that at pH 7 both HPO_4^{2-} and $H_2PO_4^-$ are present in equal proportions, with HPO_4^{2-} being more prevalent in slightly alkaline conditions at pH 10 and $H_2PO_4^-$ dominating in slightly acidic environment at pH 4 as illustrated in Figure 3.2 below.

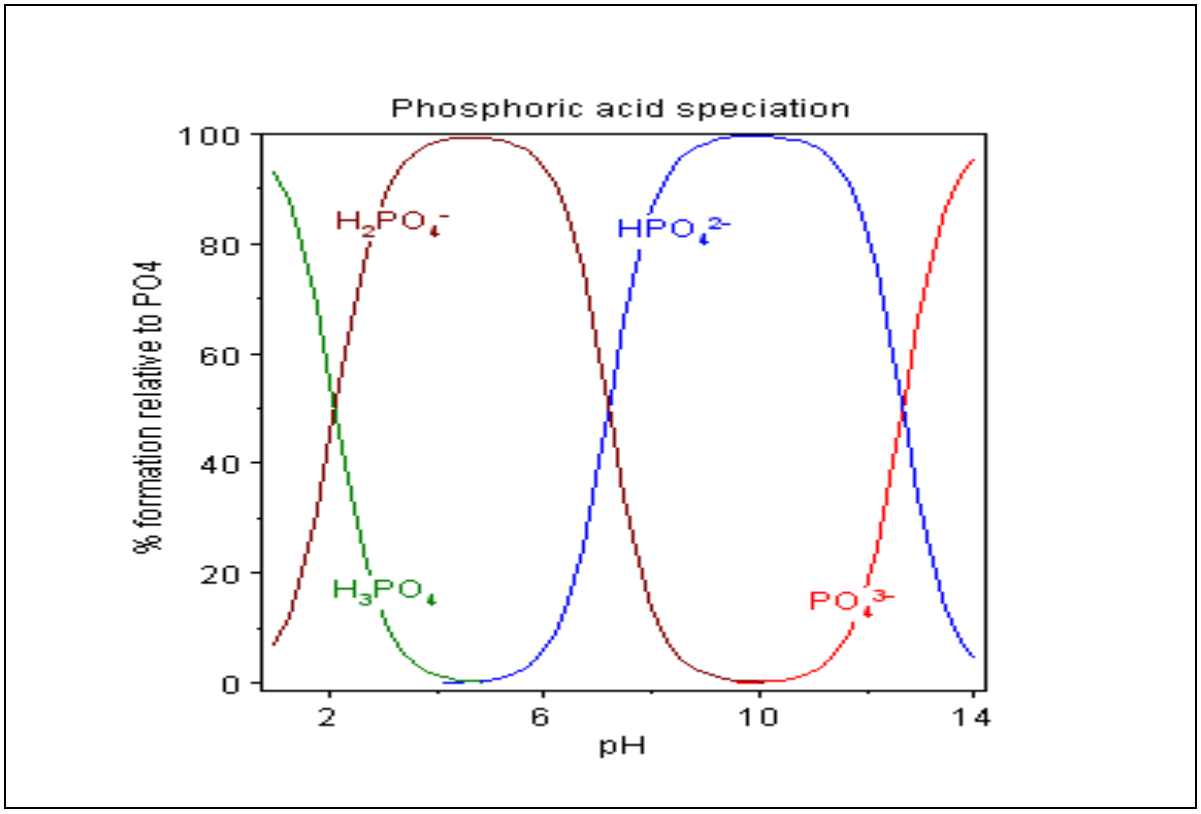

Figure 3.2 A distribution diagram showing the percentage composition of phosphoric acid species as a function of pH [70].

All measurements of sucrose-salt systems were performed immediately after preparations. This could not be done for the glucose and fructose systems. It is well known that freshly prepared water solutions of glucose and fructose gradually change in optical rotatory power [71]. This mutarotation is most commonly accounted for by considering that the dissolved glucose and fructose undergo transformation from the α-1 to the β-1 form as shown in Figure 3.3a and 3.3b**.**

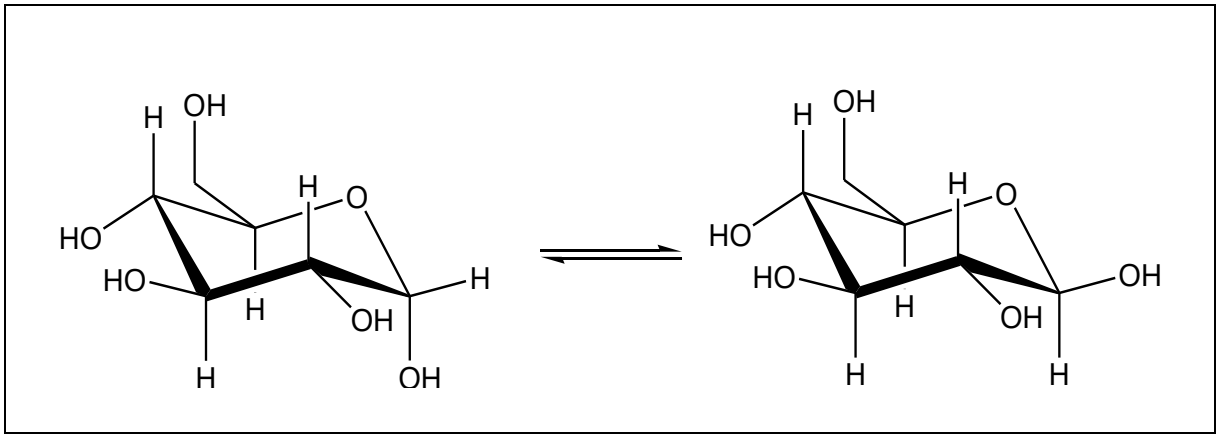

Figure 3.3 a Structural representation of glucose mutarotation in solution, redrawn from [72].

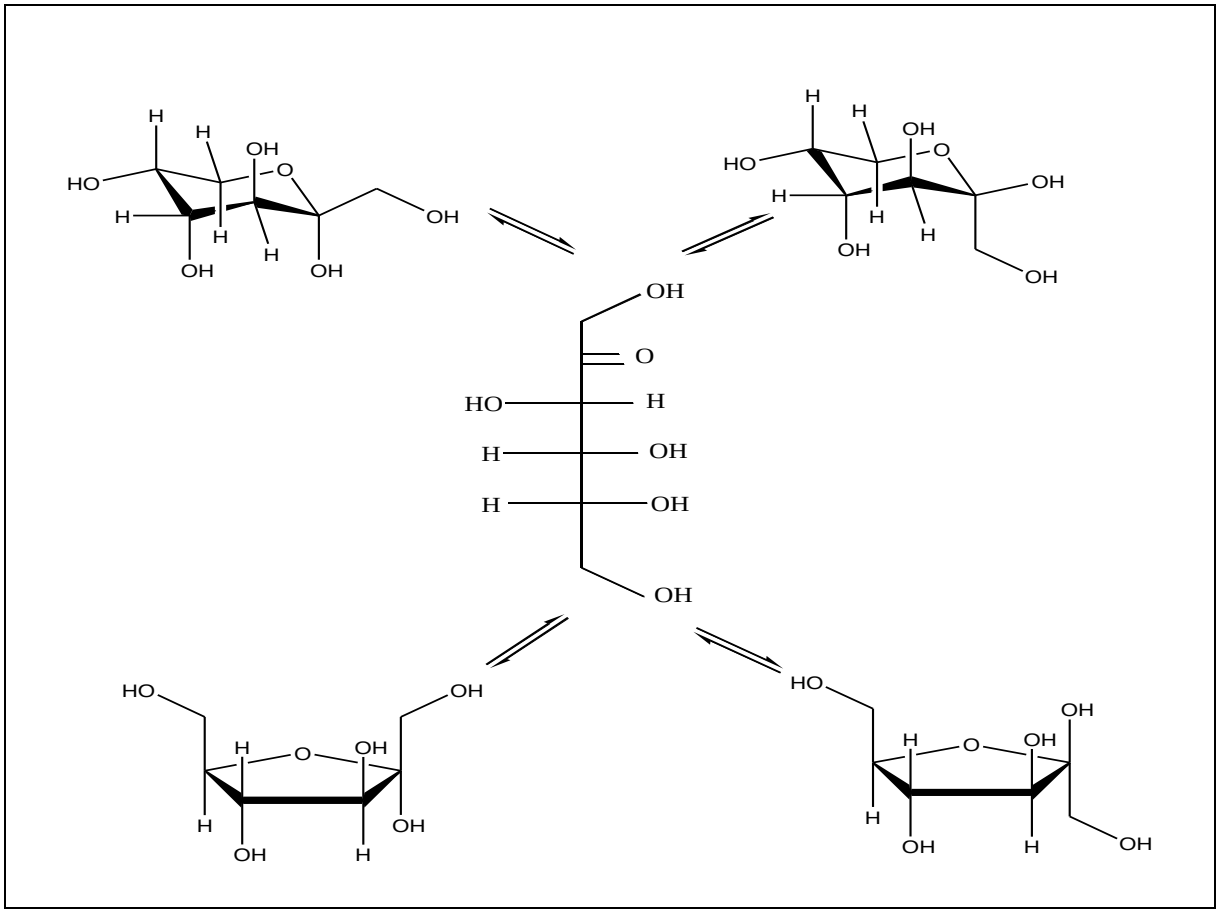

Figure 3.3 b Structural representation of fructose mutarotation in solution, redrawn from [73].

For salt mixtures of glucose and fructose, pure solutions of these saccharides were prepared at least 20 hours prior the addition of salts to allow complete mutarotation to take place [74].

3.3 Inversion of sucrose kinetics.

3.3.1 Preparation of acid solutions.

Stock solutions of 5.000 M HCl and 5.000 M H_2SO_4 were separately prepared in 2000.00 mL volumetric flasks using deionised water. The solutions were standardised using dilution and titration with standard NaOH and methyl red indicator. The exact molar concentration of HCl and H_2SO_4 solutions were found to be 5.014 M and 5.012 M, respectively.

3.3.2 Procedure for reaction systems of sucrose-NaCl mixture + HCl and sucrose-Na_2SO_4 mixture + H_2SO_4.

The procedure for reaction systems was as follows: For each hydrolysis reaction, 20 g of sucrose was dissolved in a small quantity of deionised water and the requisite amount of salt (by mass) was then added and made up to 100.00 mL of solution. Calculated and weighed masses are given in the Appendix 2. 25.00 mL of 20% sucrose solution and acid solution were separately pipetted into clean dry beakers and allowed to reach the required temperature in a water bath maintained at 29° C. The acid solution was then poured into the sucrose solution to initiate the reaction and at the same time the clock was started to record the time spent for mixing the reacting solution, rinsing the Polarimeter tube as well as transferring the reacting solution for monitoring with the Polarimeter. With some practice, this could be done in two minutes for all reactions. The polarimeter wavelength was set at 589 nm. The readings of the optical rotation were recorded in 60 s intervals using the Rudolph Polarimeter Software. The first reading was therefore taken after 180 s of the reaction (120 s added to every 60 s interval as monitored by the instrumental software).

The reaction was allowed to continue until a constant reading was obtained, i.e. about at least five constant values which were recorded. This was taken as the final reading denoted α_∞ of the optical rotation which has been proven to be a repeated value at infinite time denoted t_∞.

3.3.3 Procedure for reaction systems of HCl-NaCl mixture + sucrose and H_2SO_4-Na_2SO_4 mixture + sucrose.

The procedure for acid-salt mixture is similar to the sucrose-salt mixture with the exception that a requisite amount of salt was dissolved and made up to 100.00 mL of acid solution. 25.00 mL of this acid-salt mixture was then reacted with 25.00 mL solution of the 20% (mass/volume) solution of sucrose. All other conditions were retained so that the results obtained would be comparable. The exact mass of sucrose and salt weighed are given in the Appendix 3.

3.3.4 Precision measurements: Repeatability and Reproducibility.

The precision of the instrument and the reproducibility of the method were determined by monitoring the inversion of sucrose catalyzed by HCl (5.014 M) in the presence of the added 0.500 M NaCl for both sucrose-NaCl and HCl-NaCl. The concentration of NaCl was randomly chosen. Repeatability was examined by evaluating the same reaction mixtures of sucrose-NaCl and HCl-NaCl, each ran successively in triplicate (n=3), on an intra-day (same day) analysis, under the same experimental conditions according to the procedure as outlined in Section 3.3.2. The reproducibility of the method was assessed by carrying out the reaction mixtures on an inter-day analysis (three different days), under the same experimental conditions. The examination for each mixture was performed after an interval of at least three days between consecutive days according to the procedure as outlined in Section 3.3.2.

The amount of salt mentioned above did not dissolve in acid solutions and therefore no results were obtained for these mixtures. The overall precision measurement between the addition of sucrose-NaCl and HCl-NaCl solutions in the inversion reaction was assessed by checking the reproducibility conditions at 95% confidence level (n=16).

3.3.5 Procedure for the addition of more than one salt in a single inversion reaction.

The procedure for the addition of more than one salt in a single inversion reaction was followed by monitoring only two test runs. Stock solutions containing 1 M NaCl + 1 M Na_2SO_4 + 20 g of sucrose and 1 M NaCl + 1 M Na_2SO_4 + 1 M NaH_2PO_4 + 20 g of sucrose were prepared separately in 100.00 mL volumetric flasks. For each test run, 25.00 mL from each of the stock solutions was then reacted with 25.00 mL of 5.014 M HCl under the specified reaction conditions as outlined in Section 3.3.2. The molarities above were determined to 4 significant figures.

3.4 Treatment of kinetics data.

The rate constants of the inversion reactions were determined by Infinite Time, Guggenheim and Kezdy-Swinebourne methods. These methods are described in details in Section 2.2. In all the experiments, the optical rotations were recorded at intervals of 60 s. Data of optical rotation as a function of time for all reaction systems are given in Appendices 4-13. The interval of 60 s was used to calculate the rate constants of Infinite Time and Guggenheim methods while 240 s was used for Kezdy-Swinebourne method. The former time interval is approximately a quarter of the total reaction time according to the requirement of the method description [75].

CHAPTER 4

RESULTS AND DISCUSSION

In this chapter, all the results obtained in this research work are presented. The chapter is divided into sections which correspond to a sequence as presented in the experimental chapter. The discussion part of each section is directly combined with the results.

4.1 Influence of added salts in sucrose solutions.

4.1.1 Measurements of optical rotation, refractive index, and conductivity.

Analysis of sucrose-salt solutions for three systems (Sucrose + NaCl + Water, Sucrose + Na_2SO_4 + Water, and Sucrose + NaH_2PO_4 + water) involved the measurements of optical rotation, refractive index, and electrical conductivity. In each case, the concentration of sucrose was 10% (mass/volume). The optical rotation measurements of pure salt solutions of NaCl, Na_2SO_4, Na_2HPO_4 and NaH_2PO_4 were also taken to assess their contributions on the effects of the change in optical rotation when these salts were dissolved in sucrose, glucose or fructose solutions. Pure solutions of these salts were measured at 29 °C and were found to be optically inactive i.e. measuring zero optical rotations. The results of the above mentioned physical properties for the addition of salts in sucrose solutions are presented in Table 4.1.

Table 4.1 Values of optical rotation, conductivity, and refractive index for the solutions of sucrose containing added NaCl, Na_2SO_4, or NaH_2PO_4.

NaCl (M) + 10 % sucrose	Optical Rotation (Degree, °)	Conductivity (m S/cm)	Refractive Index
0.100	6.770 (0.06)	8.05	1.3485
0.500	6.670 (0.16)	35.0	1.3522
1.000	6.570 (0.26)	64.2	1.3568
2.000	6.340 (0.49)	107.9	1.3651
3.000	6.140 (0.69)	141.1	1.3727
4.000	5.930 (0.90)	165.7	1.3797
5.000	5.740 (1.09)	177.3	1.3860
Na_2SO_4 (M) + 10% Sucrose	Optical Rotation (Degree, °)	Conductivity (m S/cm)	Refractive Index
0.100	6.740 (0.09)	11.98	1.3496
0.300	6.640 (0.19)	30.3	1.3534
0.500	6.570 (0.26)	45.6	1.3570
0.750	6.500 (0.33)	59.8	1.3611
1.000	6.400 (0.43)	72.6	1.3650
1.250	6.290 (0.54)	83.5	1.3687
1.500	6.220 (0.61)	89.4	1.3720
NaH_2PO_4 (M) + 10% Sucrose	Optical Rotation (Degree, °)	Conductivity (m S/cm)	Refractive Index
0.100	6.73 (0.10)	4.95	1.3490
0.300	6.60 (0.23)	12.49	1.3518
0.500	6.48 (0.35)	18.88	1.3546
0.750	6.34 (0.49)	25.8	1.3578
1.000	6.23 (0.60)	32.7	1.3608
1.250	6.06 (0.77)	37.8	1.3637
1.500	5.97 (0.86)	38.6	1.3663

The optical rotation of pure sucrose solution at 29 °C is 6.83°. The optical rotations of pure salt solutions are zero.

The influence of added salts in sucrose solution can be determined when the concentration of the salt is the only variable. It was determined by assessing the difference between pure sucrose solution and then with a given concentration of salt. By inspecting Table 4.1, it becomes clear that the change in rotation (values in brackets) due to the effect of salt is much smaller for NaCl and Na_2SO_4 than NaH_2PO_4 when like concentrations are compared within the range of 0.100-1.500 M and becomes noticeably bigger beyond 1.5 M of NaCl due to higher concentrations of this salt within the range of 4.000-5.000 M.

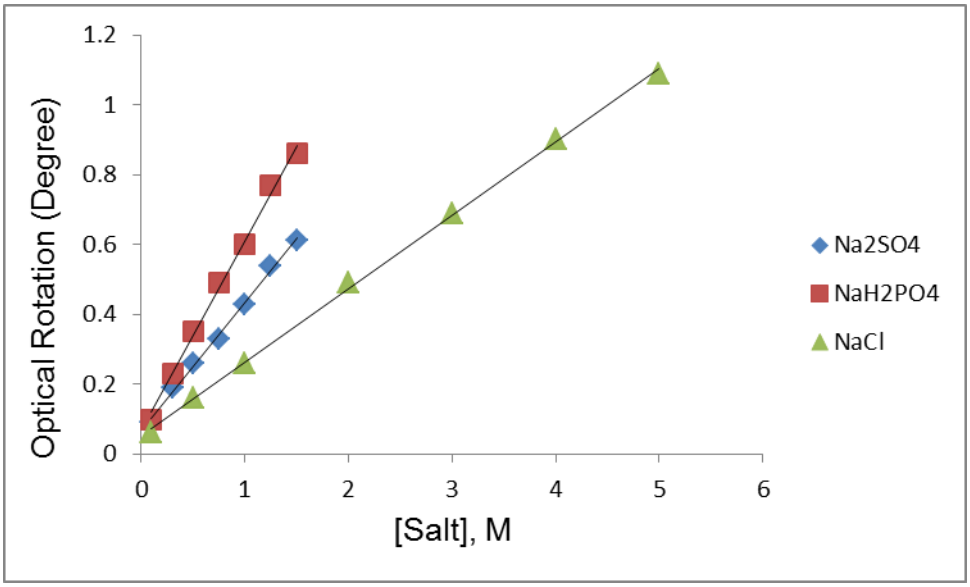

Figure 4.1 A plot of the optical rotation as a function of the concentration.

Figure 4.1 shows the relationship between the optical rotation and the concentrations of the added salts. The concentration ranges shown were due to the solubility of the salts in the sucrose solution. The change in optical rotation of sucrose increases with increasing the concentration of salts. The pronounced effects of the change in optical rotations when the amount of salt is added to sucrose solution suggest an important role for anions in depressing the rotation. These effects of the anions on optical rotation are possibly the results of the association between sucrose and salt molecules. The effectiveness of the added salts on change in optical rotation of pure sucrose and with a given concentration of salt is in the order: $H_2PO_4^-$ > SO_4^{2-} > Cl^- within 0.100-1.500 M concentration range.

The relationship between the refractive index and the concentration of added salts is shown Figure 4.2. The refractive index increases most for Na_2SO_4, least for NaCl, with concentration.

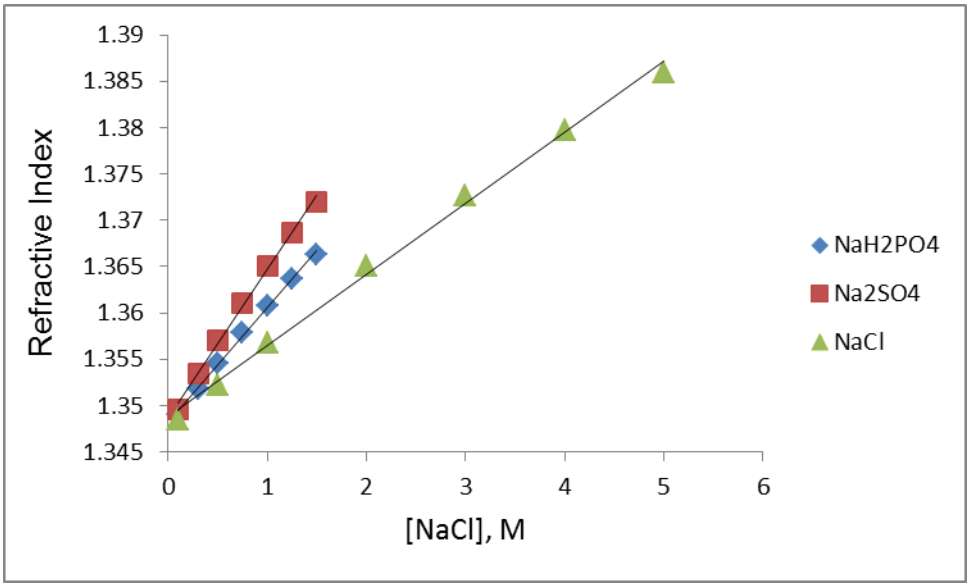

Figure 4.2 A plot of the refractive index as a function of the concentration.

On the other hand, the conductivity of sucrose-salt mixtures was significantly influenced by the addition of both salts (NaH_2PO_4, Na_2SO_4 or NaCl. Figure 4.3 shows the relationship between the conductivity of sucrose-salt solutions and the concentration of the added salts.

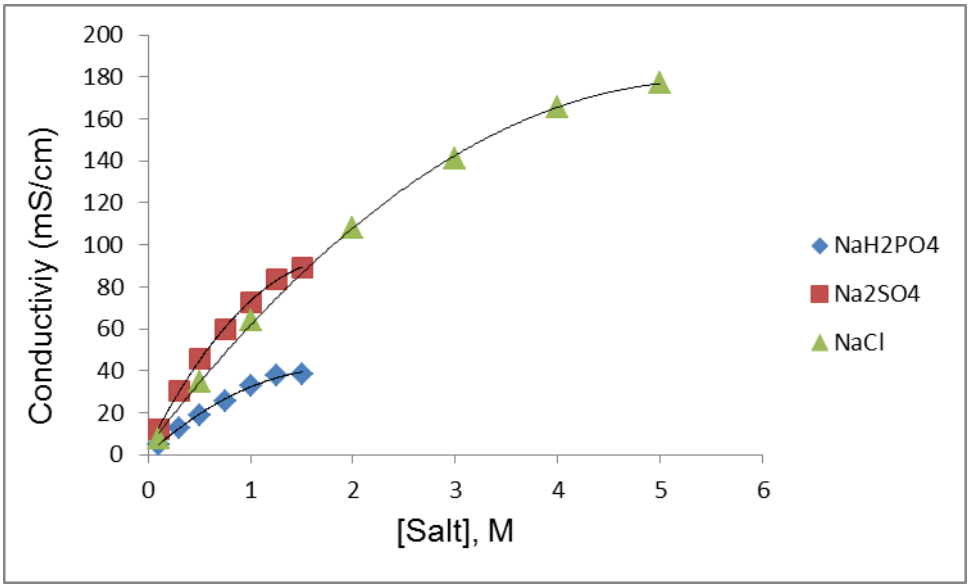

Figure 4.3 A plot of the conductivity as a function of the concentration.

The conductivity of all salts increases with concentration but the behavior of salts is quite different as illustrated in Figure 4.3. However, the salts show a trend of polynomial curves with near maximum turning points, but at different concentrations. The results are consistent with those found in theory [76] which indicates that the difference in turning points are the results of different salts in saccharide solutions which cause conductivity to fall with increasing concentration due to the close proximity of oppositely charged ions involved in the solutions.

4.2 Effect of added salts on saccharide solutions in different molar ratios.

Sharareh and Wilkins [77] analysed glucose complexes by using IR technique and asserted the formation of weak complexes between glucose and barium or copper ions. The results presented in this section were measured by monitoring change in optical rotation of the saccharides sucrose, glucose, and fructose in systems containing added NaCl, Na_2SO_4, Na_2HPO_4 or NaH_2PO_4, prepared in a 1:1 Molar ratio (saccharide: salt). The effect of 1:2 and 1:3 Molar ratios was further studied for sucrose-NaH_2PO_4 systems. The temperature of the saccharide-salt systems was kept constant at 29 °C. The pH was controlled for all systems containing acidic salts (Na_2HPO_4 and NaH_2PO_4). The values of optical rotation were obtained in triplicate measurements from individual preparations of saccharide-salt solutions for each measurement. Table 4.2 presents the values of optical rotation resulting from saccharide-salt solutions with added NaCl or Na_2SO_4.

Table 4.2 Optical rotation values of Sucrose, Glucose, and Fructose containing added NaCl and Na_2SO_4 in a 1:1 Molar ratio at 29 °C.

NaCl + Saccharide	Sucrose + NaCl Optical Rotation (°)	Glucose + NaCl Optical Rotation (°)	Fructose + NaCl Optical Rotation (°)
	6.70	5.39	-8.79
	6.72	5.38	-8.80
	6.72	5.38	-8.79
Mean	6.71(0.12)	5.38(0.61)	-8.79(0.03)
Na_2SO_4 + Saccharide	Sucrose + Na_2SO_4 Optical Rotation (°)	Glucose + Na_2SO_4 Optical Rotation (°)	Fructose + Na_2SO_4 Optical Rotation (°)
	6.67	5.40	-8.90
	6.65	5.42	-8.89
	6.64	5.40	-8.88
Mean	6.65(0.18)	5.40(0.18)	-8.89(0.13)

Pure Sucrose = 6.83°, Pure Glucose = 5.22°,(After mutarotation), Pure Fructose = -8.76°(After mutarotation).

The values in brackets are the change in optical rotation. They were determined by evaluating the difference between the rotation of pure saccharide solutions and of saccharide-salt solutions with salts added to saccharide solutions after complete mutarotation. The rotation of pure saccharide solutions are given in the foot note of Tables 4.2-4.4. The addition of NaCl and Na_2SO_4 caused the greatest change in optical rotation of glucose solution as compared to sucrose and fructose. NaCl had almost no influence on fructose solution with only 0.03° change in optical rotation between pure fructose and fructose-NaCl solutions. Overall, Na_2SO_4 had more influence in changing the optical rotation of saccharides than NaCl across all saccharide-salt systems. The pH was controlled for the measurements of optical rotations of saccharide solutions containing added NaH_2PO_4 and Na_2HPO_4**.** Table 4.3 and 4.4 show the average results of rotations obtained at pH 4, 7 and 10 with the values of the change in rotation shown in brackets.

Table 4.3 Optical rotations of Sucrose, Glucose, and Fructose containing added NaH_2PO_4 in a 1:1 Molar ratio at 29 °C.

Sucrose + NaH_2PO_4	pH 4 Rotation(°)	pH 7 Rotation(°)	pH 10 Rotation(°)
	6.63	4.67	4.10
	6.62	4.67	4.10
	6.60	4.67	4.10
Mean	6.62(0.21)	4.67(2.16)	4.10(2.73)
Glucose + NaH_2PO_4	pH 4 Rotation(°)	pH 7 Rotation(°)	pH 10 Rotation(°)
	5.29	3.31	3.06
	5.29	3.31	3.06
	5.29	3.31	3.06
Mean	5.29(0.07)	3.31(1.91)	3.06(2.16)
Fructose + NaH_2PO_4	pH 4 Rotation(°)	pH 7 Rotation(°)	pH 10 Rotation(°)
	-8.55	-5.83	-5.20
	-8.56	-5.83	-5.19
	-8.55	-5.83	-5.19
Mean	-8.56(0.20)	-5.83(2.93)	-5.19(3.57)

Pure Sucrose = 6.83°, Pure Glucose = 5.22°,(After mutarotation), Pure Fructose = -8.76°(After mutarotation).

Table 4.4 Optical rotations of Sucrose, Glucose, and Fructose containing added Na_2HPO_4 in a 1:1 Molar ratio at 29 °C.

Sucrose + Na_2HPO_4	pH 4 Rotation(°)	pH 7 Rotation(°)	pH 10 Rotation(°)
	5.60	6.07	6.30
	5.60	6.08	6.29
	5.60	6.07	6.29
Mean	5.60(1.23)	6.07(0.76)	6.29(0.54)
Glucose + Na_2HPO_4	pH 4 Rotation(°)	pH 7 Rotation(°)	pH 10 Rotation(°)
	3.98	4.34	4.76
	3.99	4.34	4.76
	3.98	4.34	4.76
Mean	3.98(1.24)	4.34(0.88)	4.76(0.46)
Fructose +Na_2HPO_4	pH 4 Rotation(°)	pH 7 Rotation(°)	pH 10 Rotation(°)
	-6.58	-7.74	-8.08
	-6.59	-7.74	-8.08
	-6.56	-7.71	-8.07
Mean	-6.58(2.18)	-7.73(1.03)	8.08(0.68)

Pure Sucrose = 6.83°, Pure Glucose = 5.22°,(After mutarotation), Pure Fructose = -8.76°(After mutarotation).

The relationship between the optical rotations of saccharide solutions containing NaH_2PO_4 and Na_2HPO_4 with the controlled values of pH is shown in Figures 4.4 (a) and (b).

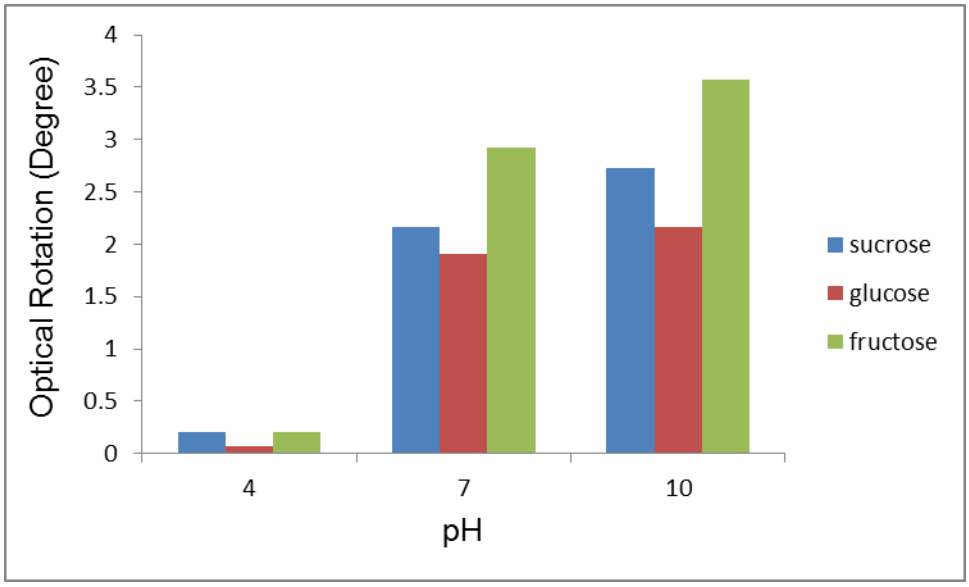

Figure 4.4 (a) The effect of pH variation on the change in optical rotation of sucrose, glucose, and fructose solutions containing added NaH_2PO_4 at 29 °C.

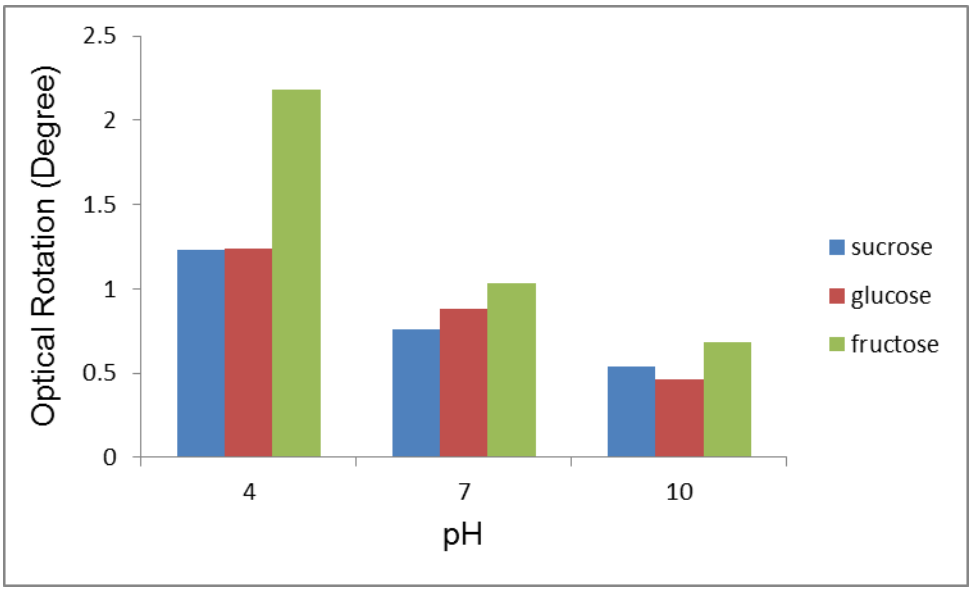

Figure 4.4 (b) The effect of pH variations on the change in optical rotation of sucrose, glucose, and fructose solutions containing added Na_2HPO_4 at 29 °C.

The optical rotation of saccharide-NaH_2PO_4 solutions were found to increase with increasing pH. By contrast, the optical rotation of saccharide-Na_2HPO_4 solutions decreases with increasing pH. Both NaH_2PO_4 and Na_2HPO_4 caused the greatest change in optical rotation when these salts were dissolved in fructose solutions. On the whole, the sequences of changes in optical rotation of saccharide-salt systems were found to be as follows:

Sucrose

$Cl^- < SO_4^{2-} < H_2PO_4^-$ (pH 4) < HPO_4^{2-} (pH 4)

Cl- < SO_4^{2-} < HPO_4^{2-} (pH 7) < $H_2PO_4^-$ (pH 7)

Cl- < SO_4^{2-} < HPO_4^{2-} (pH 10) < $H_2PO_4^-$ (pH 10)

Glucose

$H_2PO_4^-$ (pH 4) < SO_4^{2-} < Cl^- < HPO_4^{2-} (pH 4)

SO_4^{2-} < Cl^- < HPO_4^{2-} (pH 7) < $H_2PO_4^-$ (pH 7)

SO_4^{2-} < HPO_4^{2-} (pH 10) < Cl^- < $H_2PO_4^-$ (pH 10)

Fructose

$Cl^- < SO_4^{2-} < H_2PO_4^-$ (pH 4) < HPO_4^{2-} (pH 4)

Cl^- < SO_4^{2-} < HPO_4^{2-} (pH 7) < $H_2PO_4^-$ (pH 7)

Cl^- < SO_4^{2-} < HPO_4^{2-} (pH 10) < $H_2PO_4^-$ (pH 10)

The qualitative sequence of the Hofmeister series has been described in Section 2.4 and it is shown below:

Organic anions > ClO_4^- > SCN^- > I^- > salicylate$^-$ > NO_3^- > Br^- > Cl^- > HCO_3^- > $H_2PO_4^-$ > F^- > SO_4^{2-} > HPO_4^{2-}

Not one of the sequences follows the Hofmeister series. What is interesting is that for sucrose and fructose, the change in rotation follows $Cl^- < SO_4^{2-}$, for glucose it is the other way around. Again sucrose and fructose have the same sequences for the phosphates; the sequence for glucose is different and for glucose the change in

rotation is also the smallest. For sucrose and fructose $H_2PO_4^-$ has the largest influence on the rotation at pH 7 and pH 10, although it is, according to the speciation diagram, not the most common ion. However, at pH 4 it would be the most common species, yet its influence on the optical rotation is smaller. Again the opposite sequence is observed for the rotations of glucose when the salts are added.

It was decided to investigate further, if varying the Molar ratio of the saccharide-salt systems would have an effect on optical rotation of the solutions. Therefore, experiments were performed in a Molar ratio of 1:2 and 1:3 (sucrose:NaH_2PO_4) at pH (pH = 4, 7 and 10). Their results were compared with those obtained from 1:1 Molar ratio. The differences in optical rotation values between the pure sucrose solution and sucrose-NaH_2PO_4 are recorded in Table 4.5.

Table 4.5 Optical Rotation values of sucrose-NaH_2PO_4 in a Molar ratio of 1:1, 1:2, and 1:3 at 29 °C.

p H	4	7	10
Optical Rotation (1:1)	6.62°	4.67°	4.10°
Pure sucrose	6.83°	6.83°	6.83°
Difference	0.21°	2.16°	2.73°
Optical Rotation (1:2)	6.85°	6.18°	6.09°
Pure sucrose	6.83°	6.83°	6.83°
Difference	0.02°	0.65°	0.74°
Optical Rotation (1:3)	6.07°	5.28°	5.18°
Pure sucrose	6.83°	6.83°	6.83°
Difference	0.76°	1.55°	1.65°

The analysis of the results obtained from the preparations of these systems show the increase in change in optical rotation as the pH increases. The difference in rotation was observed to be higher for 1:3 than 1:2 Molar ratio, but both lower than the 1:1 ratio, except at pH 4 which shows higher change in rotation for 1:3 ratio. These results indicate that changes in optical rotation vary with pH and are directly affected by changing the concentration of added NaH_2PO_4 causing different degree of rotation at fixed concentration of sucrose. These values are also plotted in Figure 4.5 below showing the same trend as discussed above.

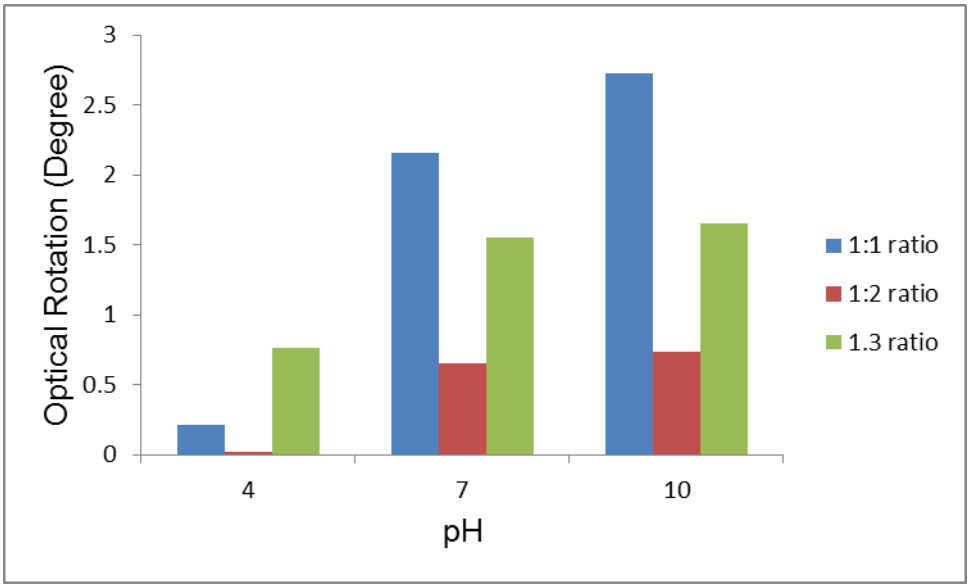

Figure 4.5 The plot of difference in optical rotation of sucrose-NaH_2PO_4 in a Molar ratio of 1:1, 1:2 and 1:3) at different pH.

4.2.1 Analysis of sucrose-salt, glucose-salt, and fructose-salt mixtures by Raman spectroscopy.

Longinotti and co-workers [78] have shown that borate ion adds to trehalose or sucrose in solution to form borate-disaccharide (sucrose or trehalose) ester ions. Both borate and phosphate ions are polyanions and have similar chemical structure [79], and so does the sulfate ion. Based on the close similarities of their chemical structures, it is possible that the sulfate and phosphate anions may form complexes with sucrose in aqueous solution.

Evidence of formation of sucrose and trehalose complexes with borate salt as indicated by Longinotti has prompted the study of the analysis of sucrose-salt, including glucose and fructose salt mixtures in this work. The salts studied were NaCl, Na_2SO_4, and NaH_2PO_4.

Measurements of carbohydrates in aqueous solutions by vibrational spectroscopy are not observed in infrared radiation due to strong absorption by the solvent, water. Raman spectra may be obtained for molecules in aqueous solutions, since water shows only a weak Raman spectrum. The flexibility of Raman spectroscopy is great in the sense that it can be used to analyze both liquid and solid samples with no or minimal pretreatment [80]. Analysis of the Raman spectra in the range from 980 to 970 cm^{-1} should provide information on the new bond formed (C-O-P vibration) as a result of phosphate ions interacting with sucrose thereby forming a complex in solution [61, 81-82]. The most prominent Raman peak (at 1060 cm^{-1}) when sugars interact with sulfate anion is attributed to the symmetrical vibration of the S=O groups [83], and for degrees of sulfation of carbohydrates the intensity of this band is higher than that of the carbohydrate band (C-O stretch) in the 1050-1000 cm^{-1} region. Analysis of Raman spectra on the effects of binary mixtures of salt + sucrose on the OH peaks of water have shown a shift towards lower frequencies by approximately 8 cm^{-1} if the interaction between sucrose and salt is prevalent as compared to the spectra of pure sucrose solution [84]. All spectra of sucrose-salt systems, including glucose-salt and fructose-salt systems investigated in this study were taken and analyzed, but the weak Raman bands of water were not investigated. The bands were there, but the Raman scatter was too weak for analysis. Typical Raman spectra of pure sucrose, glucose, fructose, and NaH_2PO_4 solutions and the mixtures of the three saccharides with NaH_2PO_4 are shown in Figures 4.6-4.8.

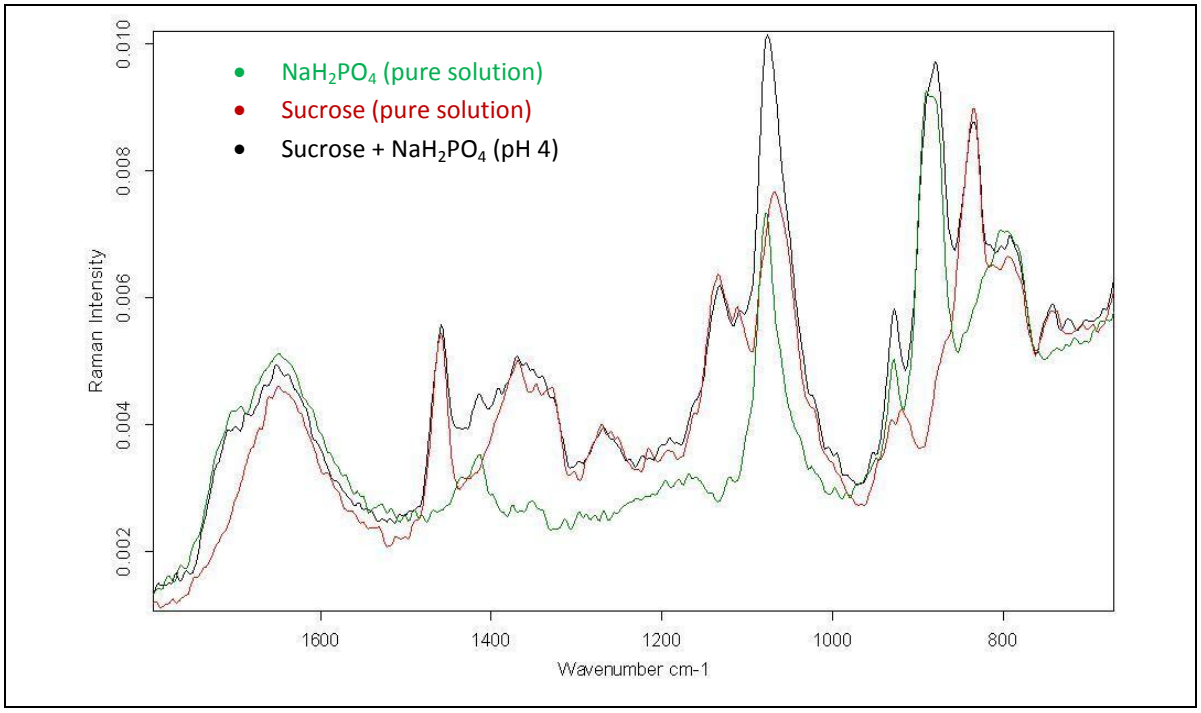

Figure 4.6 Raman spectra of pure NaH_2PO_4 solution, pure sucrose solution, and sucrose + NaH_2PO_4 solution (1:1 molar ratio, pH 4).

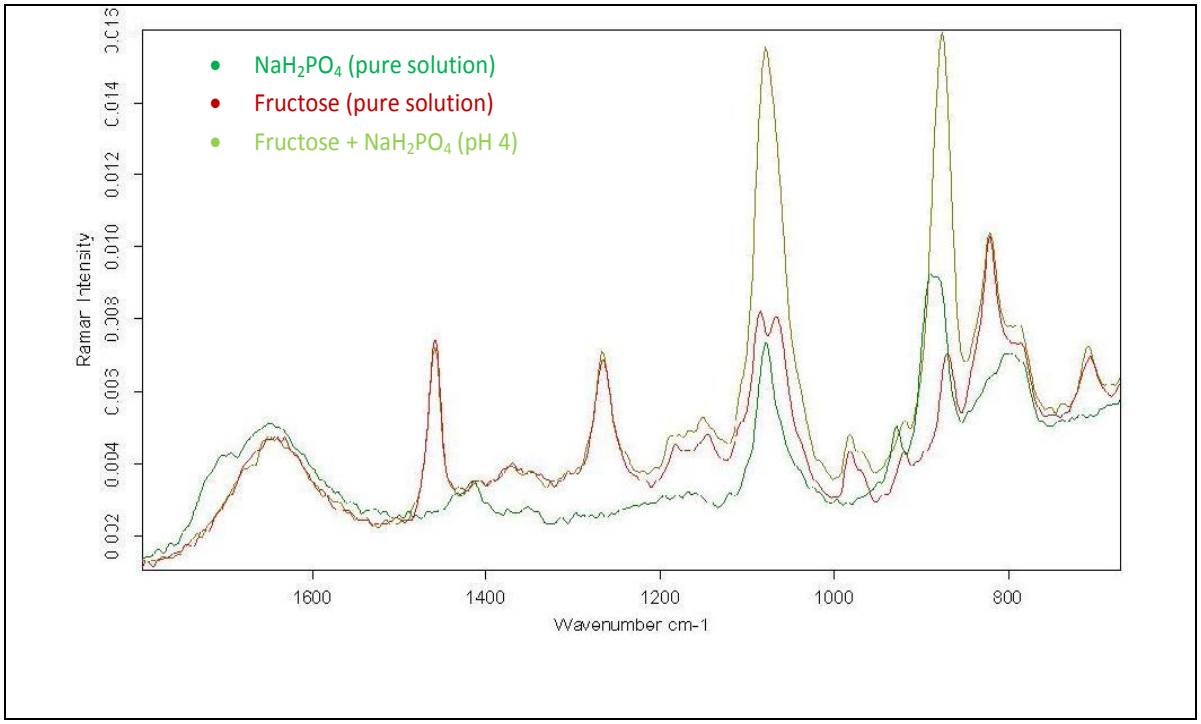

Figure 4.7 Raman spectra of pure NaH_2PO_4 solution, pure fructose solution, and fructose + NaH_2PO_4 solution (1:1 molar ratio, pH 4).

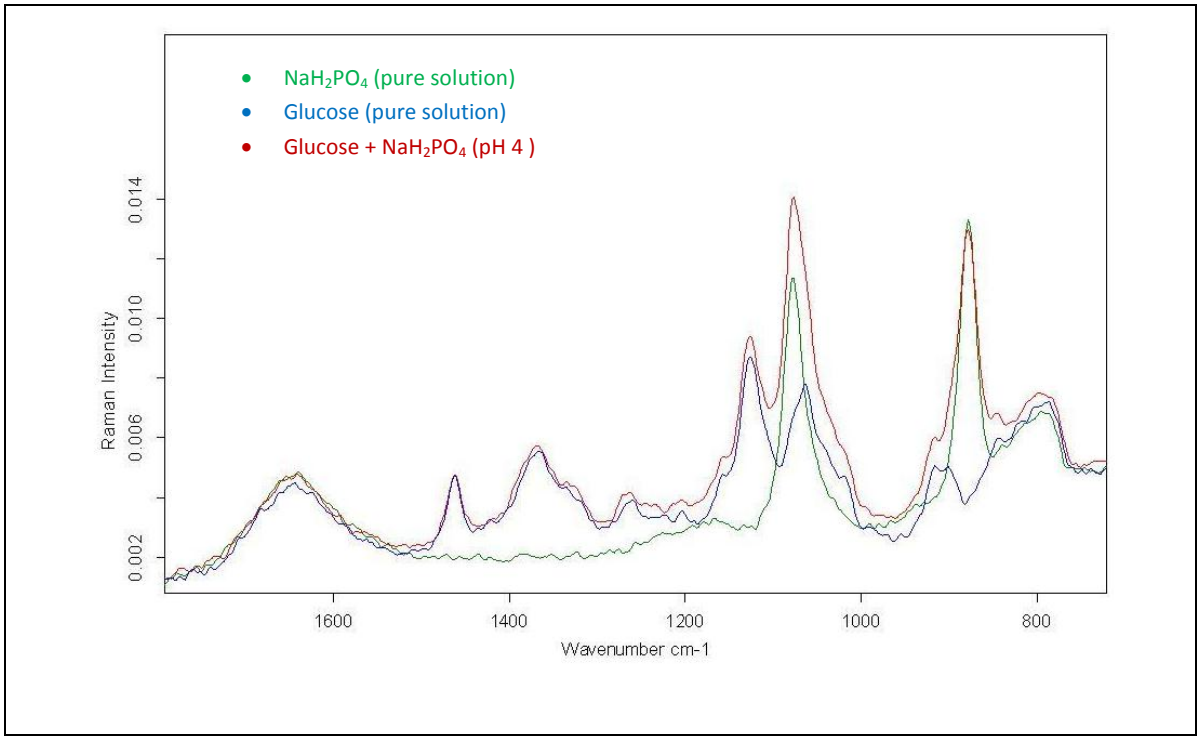

Figure 4.8 Raman spectra of pure NaH_2PO_4 solution, pure glucose solution, glucose + NaH_2PO_4 solution (1:1 molar ratio, pH 4).

The wavenumber position of the bands is of importance. The key difference in detecting the formation of complexes in solutions is by observing shifts in wavenumbers and appearance of new bands in the spectra. However, based on the information deduced from the spectra in Figures 4.7-4.9 clearly no such particular shifts in wavenumber and appearance of new bands were observed.

If complexes are formed in aqueous solution, they should also exist in the solid form. Therefore, the decision was made to precipitate the solids from the sucrose-salt mixtures in solution through crystallization at room temperature. The process of crystallization was very slow and lasted for months and eventually precipitates were obtained although some were sticky. Figure 4.9 shows some typical precipitates formed from crystallization process.

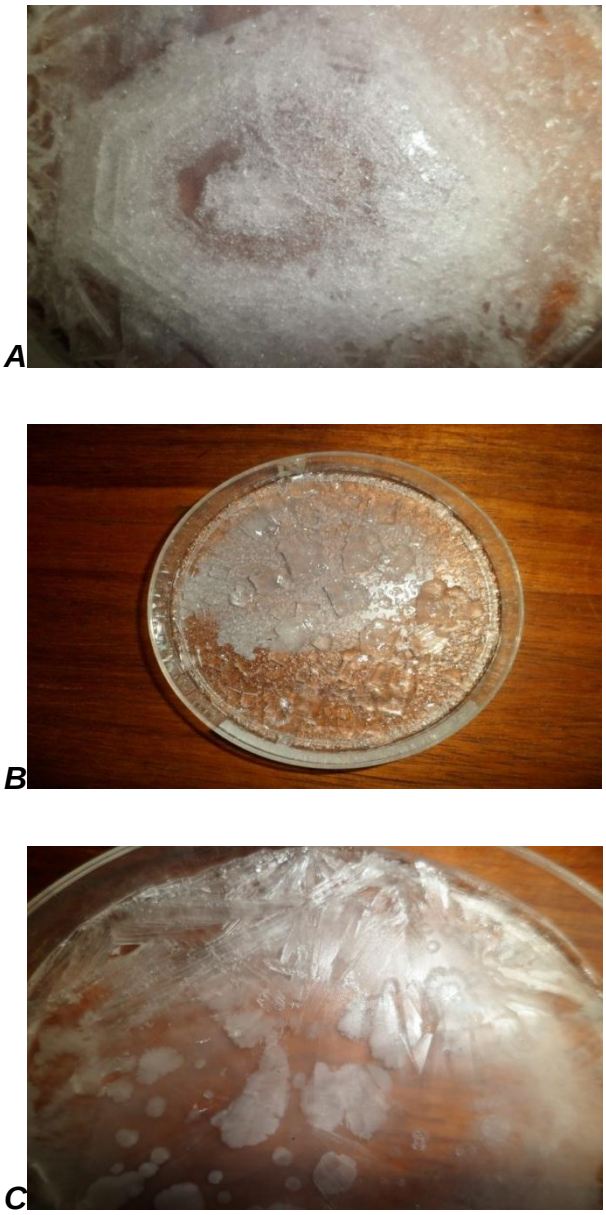

Figure 4.9 Formation of precipitates after crystallization at room temperature. Precipitate A (sucrose-Na_2SO_4), Precipitate B (sucrose-NaCl), Precipitate C (sucrose-NaH_2PO_4).

For Raman spectra of solids the intensities of bands often differ because of the different sizes and shapes of the crystals in the solid powder. Raman spectra of NaH_2PO_4 just showed peaks that were observable also in the spectra of the pure sucrose and salts. However, some unusual new bands were observable for the sucrose-NaCl (Figure 4.10) and sucrose-Na_2SO_4 (Figure 4.11) precipitates. These bands occurred only in the O-H stretching (Shown below) and O-H bending vibrational regions (Appendix 14), and no other bands due to sucrose or the salts were affected or shifted.

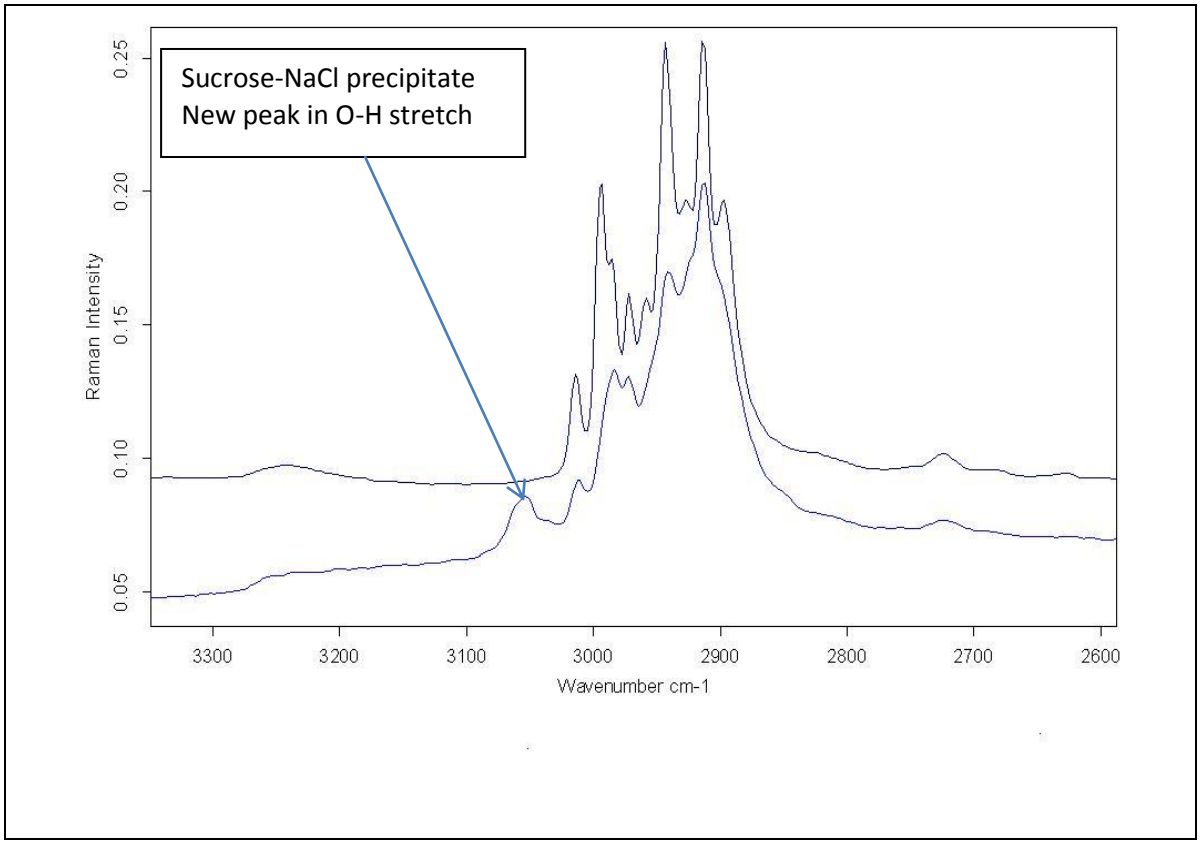

Figure 4.10 Raman spectra of sucrose-NaCl precipitate (lower) and crystalline sucrose (upper) in the range 3300-2600 cm^{-1}. Sodium chloride does not have a band in the shown region.

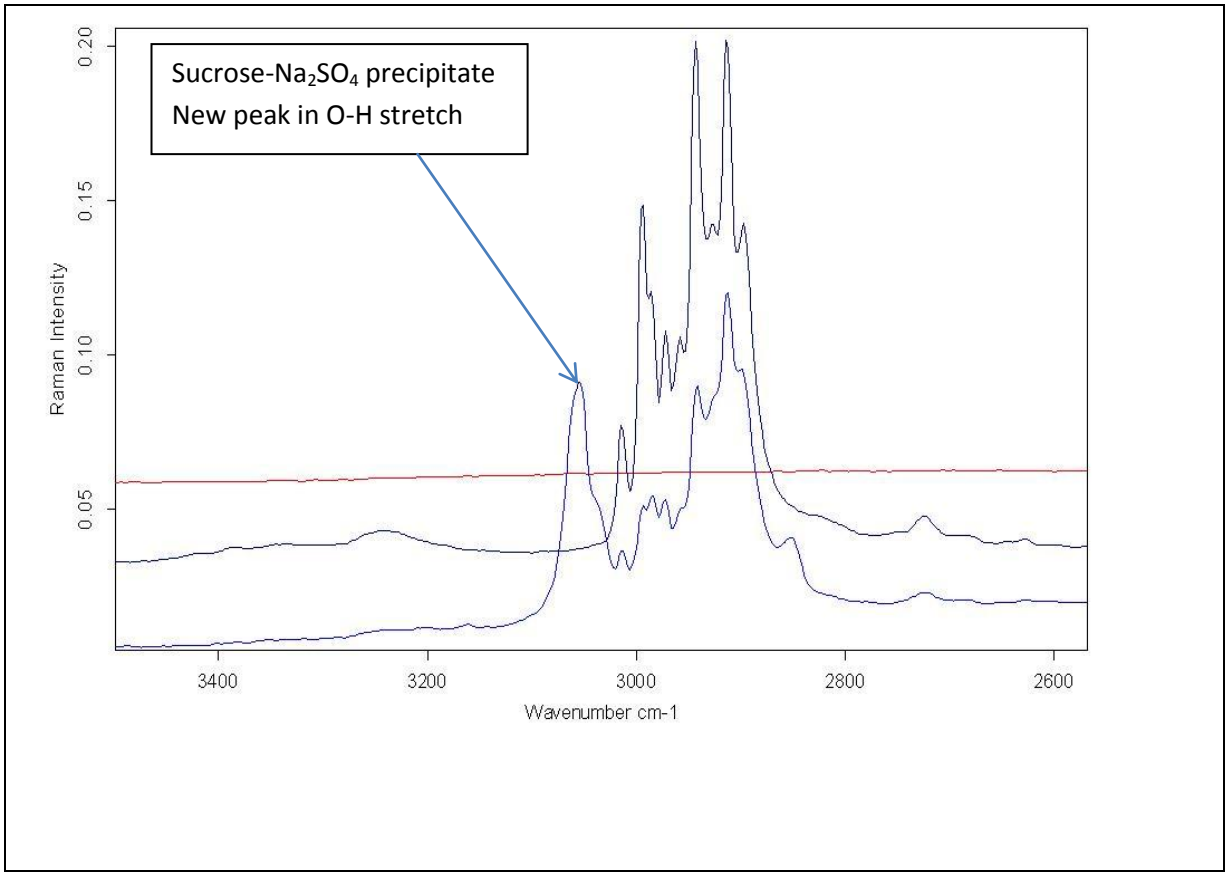

Figure 4.11 Raman spectra of sucrose-Na_2SO_4 precipitate (lower) and crystalline sucrose (upper) in the range 3400-2600 cm^{-1}. Sodium Sulphate (red spectral line) shows no band in the wavenumber region shown above.

Based on these observations, it was presumed that the new bands may be due to some trapped water in the crystal lattice of the precipitates. It is therefore apparent from the present studies that no new sucrose-salt complexes have been formed.

4.3 Inversion of sucrose kinetics.

4.3.1 Influence of added salts.

The inversion of sucrose by addition of different salts in acidic medium has been studied by polarimetry technique at various concentrations of NaCl and Na_2SO_4, keeping sucrose, HCl, or H_2SO_4 concentrations and the temperature constant. The effect of the added salts was investigated at different concentrations in the range of 0.050-0.750 M. Figure 4.12 shows a typical graph of optical rotation plotted against time obtained for sucrose inversion (NaCl (0.250 M)-sucrose (10% mass/volume)-HCl (2.507 M mixture)) at 29 °C. This graph shows that the optical rotation decreases as time increases. This is in good agreement with the exponential decay of a pseudo-first order reaction.

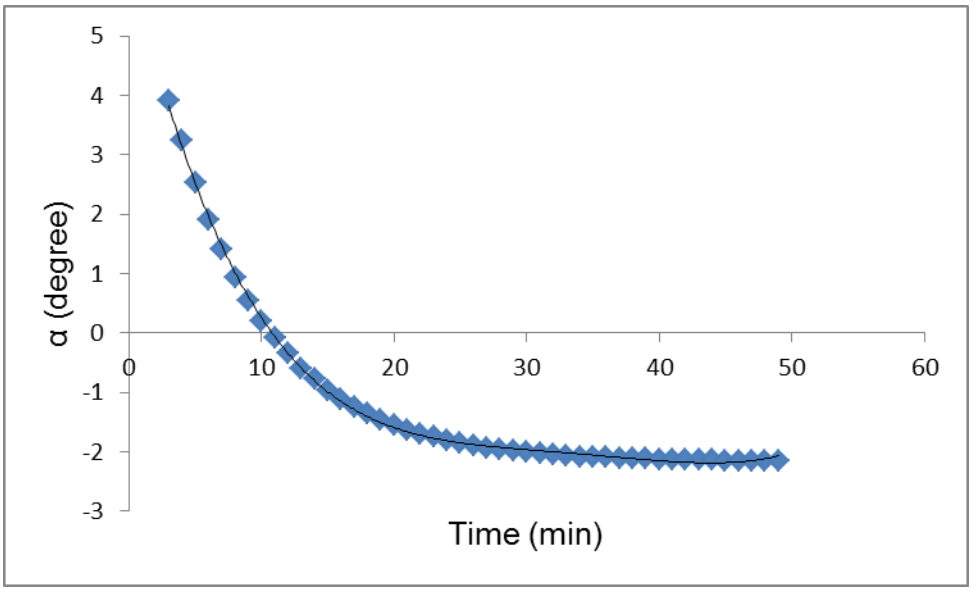

Figure 4.12 A typical graph of optical rotation plotted against time obtained for sucrose inversion (NaCl (0.250 M)-sucrose (10% mass/volume)-HCl (2.507 M mixture)) at 29 °C. Sucrose-NaCl mixture was reacted with HCl. The reported concentrations in brackets are the final concentrations in the reacting solution.

Plots of Infinite Time (Figure 4.13), Guggenheim (Figure 4.14) and Kezdy-Swinebourne (Figure 4.15) methods for the determination of rate constants applied in this study, were obtained as straight lines which indicate that the reaction systems are first-order reactions with respect to sucrose concentration. As described in Section 2.2, the slope of the graph of ℓn (α_t-α_∞) versus time, and ℓn (α_1-$\alpha_{1'}$) versus time is equal to the negative of the rate constant of Infinite Time method and Guggenheim method, respectively. In Kezdy-Swinebourne, the rate constant is given by k=1/Δt ℓn (slope) from the plot of α' versus α. The values of rate constants obtained graphically from the slopes of the plots are recorded in Tables 4.6-4.7.

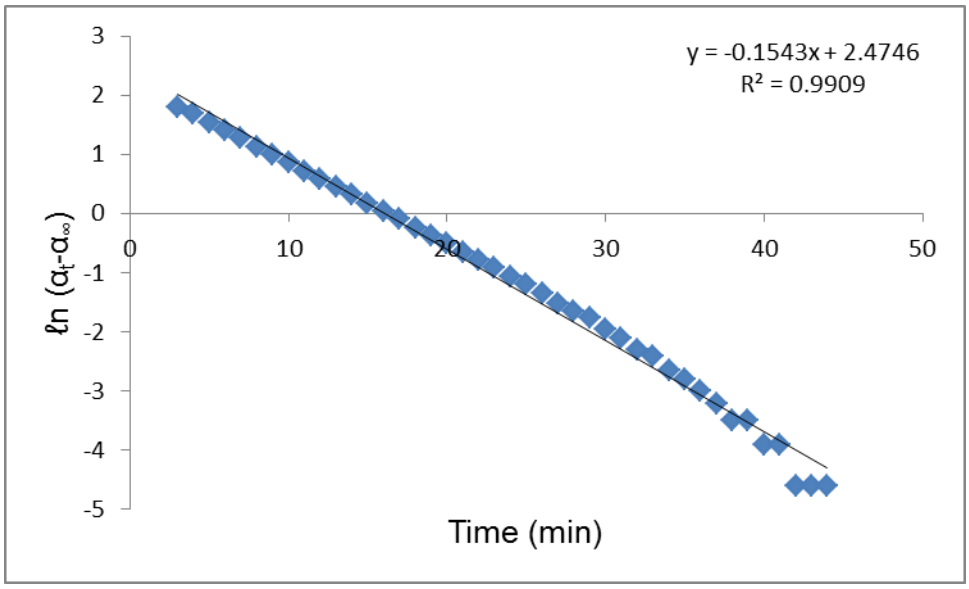

Figure 4.13 A typical graph of ℓn (α_t- α_∞) versus time, obtained for sucrose inversion (NaCl (0.250 M)-sucrose (10 % mass/volume)-HCl (2.507 M mixture)) at 29 °C. Sucrose-NaCl mixture was reacted with HCl. The reported concentrations in brackets are the final concentrations in the reacting solution.

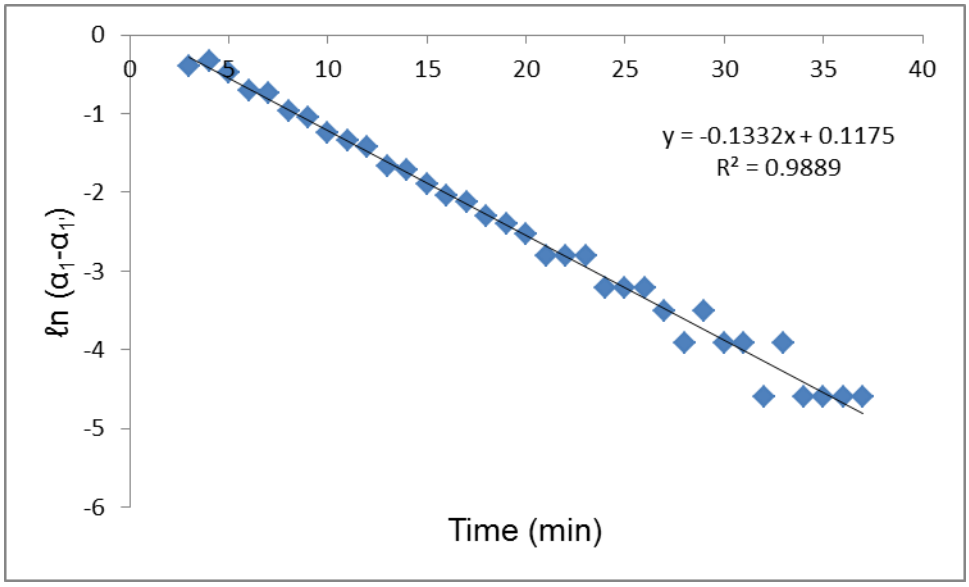

Figure 4.14 A typical graph of ℓn (α_1-$\alpha_{1'}$) versus time, obtained for sucrose inversion (NaCl (0.250 M)-sucrose (10 % mass/volume)-HCl (2.507 M mixture)) at 29 °C. Sucrose-NaCl mixture was reacted with HCl. The reported concentrations in brackets are the final concentrations in the reacting solution.

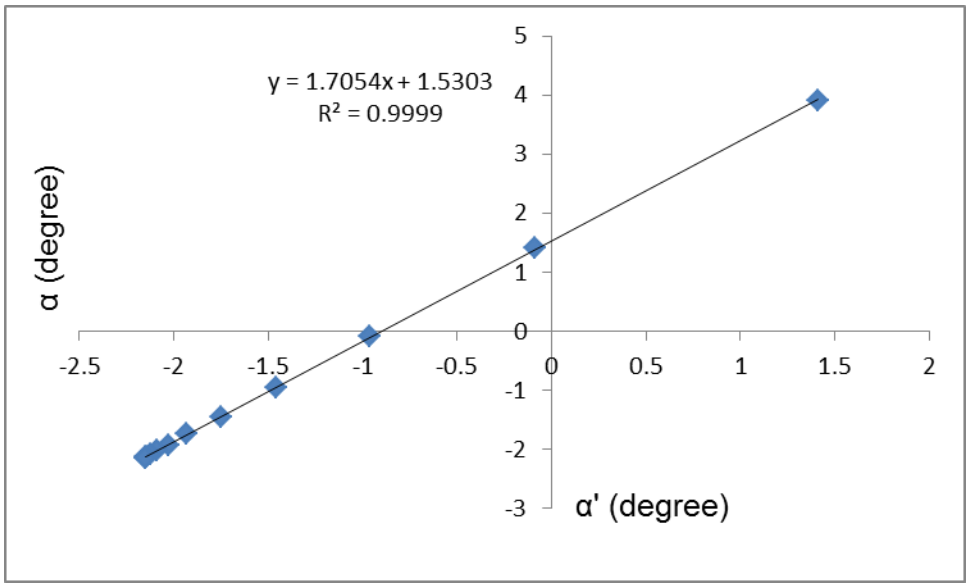

Figure 4.15 A typical graph of α' versus α obtained for sucrose inversion (NaCl (0.250 M)-sucrose (10 % mass/volume)-HCl (2.507 M mixture)) at 29 °C. k = 1/240 ℓn slope. Sucrose-NaCl mixture was reacted with HCl. The reported concentrations in brackets are the final concentrations in the reacting solution.

Table 4.6 Rate constants of Sucrose-NaCl-HCl systems determined by graphical methods.

1st Order rate constant Methods	[NaCl], M (Final concentration)	Sucrose-NaCl mixture, ($k\ s^{-1}$)	HCl-NaCl mixture, (ks^{-1})
Infinite Time			
	0.050	0.002437	0.002405
	0.125	0.002463	0.002440
	0.250	0.002571	0.002503
	0.375	0.002668	0.002596
	0.500	0.002683	0.002620
	0.750	0.002917	Not Determined
Kezdy-Swinbourne			
	0.050	0.002204	0.002090
	0.125	0.002109	0.002111
	0.250	0.002222	0.002205
	0.375	0.002394	0.002272
	0.500	0.002737	0.002404
	0.750	0.002423	Not Determined
Guggenheim			
	0.050	0.002102	0.002085
	0.125	0.002132	0.002085
	0.250	0.002220	0.002137
	0.375	0.002423	0.002192
	0.500	0.002608	0.002335
	0.750	0.002723	Not Determined

The rate constant of sucrose + HCl: IT = 0.002377 s^{-1}, KS = 0.001992 s^{-1}, G = 0.002003 s^{-1}.

Table 4.7 Rate constants of Sucrose-Na_2SO_4-H_2SO_4 systems determined by graphic methods.

Graphic Methods	$[Na_2SO_4]$, M (Final concentration)	Sucrose-Na_2SO_4 mixture (ks^{-1})	H_2SO_4-Na_2SO_4 mixture (ks^{-1})
Infinite Time			
	0.050	0.002658	0.002618
	0.250	0.002532	0.002542
	0.500	0.002520	0.002425
	0.750	0.002487	0.002290
Kezdy-Swinbourne			
	0.050	0.002393	0.002337
	0.250	0.002284	0.002306
	0.500	0.002259	0.002185
	0.750	0.002142	0.001918
Guggenheim			
	0.050	0.002422	0.002343
	0.250	0.002300	0.002287
	0.500	0.002235	0.002143
	0.750	0.002178	0.001927

The rate constant of sucrose + H_2SO_4, IT = 0.002608 s^{-1}, KS = 0.002241 s^{-1}, G = 0.002172 s^{-1}.

..

4.3.1.1 Reactions of sucrose-NaCl mixture with HCl and HCl-NaCl mixture with sucrose solution.

The initial concentrations of sodium chloride before dilution studied in the inversion reaction were 0.100, 0.500, 1.000 and 1.500 M. This was further extended by adding 0.250 and 0.750 M of NaCl to investigate the behavior of the relationship between the concentration added and the rate constants to evaluate the effect on the rate at a wide range of concentrations. A concentration of 1.500 M NaCl could not be dissolved in the solution of HCl, and as such no results were obtained for this reaction. The rate constant values in the two last columns in Table 4.6 show that in all methods of first-order determination the rate of reaction in the presence of HCl increases markedly with increasing the concentration of NaCl, except at 0.125 and 0.500 M for sucrose-salt mixture where the rates are determined by Kezdy-Swinebourne method. Figure 4.16 shows the relationship observed between the concentration of the salts and the rate constants within the range of the concentrations investigated.

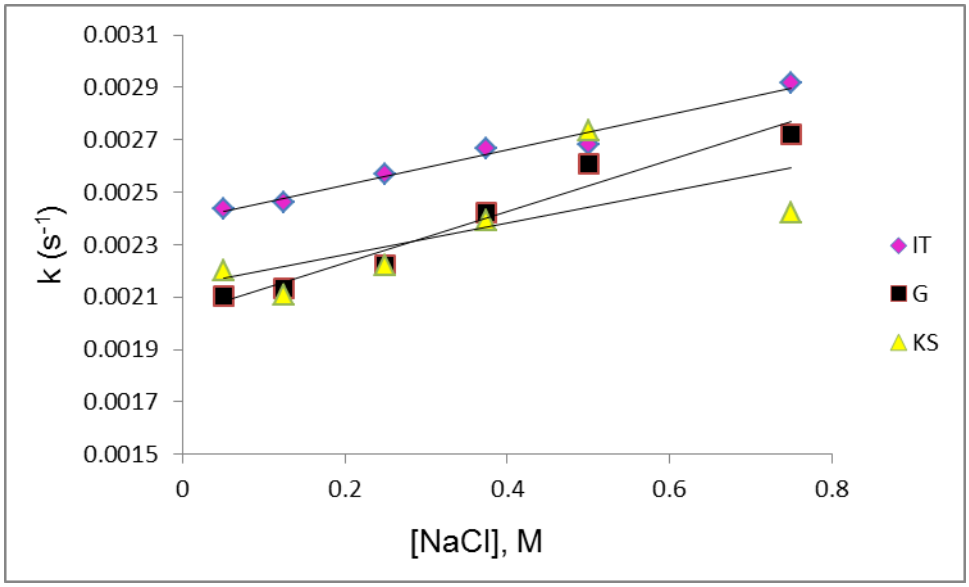

Figure 4.16 A typical graph showing the relationship between [NaCl] (Sucrose-salt mixture) and rate constants (ks^{-1}) at 29 °C.

Despite the discrepancy at 0.125 and 0.500 M, on the whole the results are reasonably consistent with literature [85]. Considering the reproducibility of the results (Section 4.3.3), there is no significant difference between the rates measured for the sucrose-salt and the acid-salt mixtures. With the Guggenheim and Kezdy-Swinbourne methods, the rate constants determined are consistently lower than those for the Infinite Time Method.

4.3.1.2 Reactions of sucrose-Na_2SO_4 mixture with H_2SO_4 and H_2SO_4-Na_2SO_4 mixture reacted with sucrose solution.

The results presented in Table 4.7 clearly show the variations of the rate constants obtained for the inversion of sucrose catalyzed by H_2SO_4 as compared to the data for the inversion catalyzed by HCl. The values of the rate constants decrease with increasing the concentration of sodium sulfate as shown in Figure 4.17.

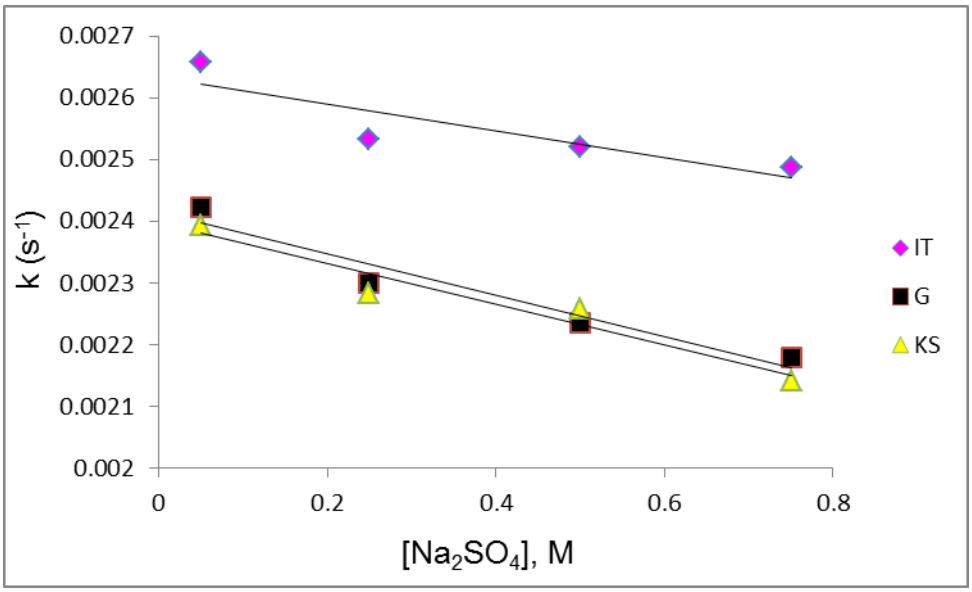

Figure 4.17 A typical graph showing the relationship between [Na_2SO_4] (Sucrose-salt mixture) and rate constants (ks^{-1}) at 29 °C.

The negative slopes in Figure 4.17 as compared to the slopes in Figure 4.16 are in good agreement with known retarding effects of sulfate salts observed in the inversion of sucrose. These effects are attributed to the decreasing concentration of hydronium ions resulting from HSO_4^-/SO_4^{2-} equilibrium [86]. This particular kind of behavior together with the inert property (not chemically reactive) of sulfate salts [87] could possibly bear some relation on the equilibrium stability of Na_2SO_4 with H_2SO_4 in the reacting solution. On the other hand, it was expected that the hydration number of a sodium ion will decrease the rate of a reaction. However, it is difficult to state with certainty if in effect the hydration number of sodium ion caused the decrease in rate considering the fact that the cation was fixed and regarded as a spectator ion while both Cl^- and SO_4^{2-} anions were varied. Thorough studies need to be conducted to clarify this issue by varying cations of added salts in the kinetics of sucrose inversion.

4.3.1.3 Comparison with literature.

There is quite a large number of published papers concerned with the inversion of sucrose. The emphasis, therefore, is not to cover almost every paper regarding the topic, but to focus only on a number of limited studies which were thought to be closely related to the present study. Therefore, the literature based on the inversion of sucrose catalyzed by acids was considered. A comparison of some closely related studies has been made. The summary of rate constants is presented in Table 4.8. Please note that the units of the rate constant values listed in the literature have all been converted to in s^{-1} in the Table.

Table 4.8 Rate constants of the inversion of sucrose from literature.

Method	Temperature	[HCl] (M)	K (s^{-1})	Reference
Polarimetry	25 °C	0.05	0.00000597	Guggenheim &
		0.10	0.00001240	Wisemann
		0.20	0.00002650	(1950)
Polarimetry	28 °C	0.05	0.001878	Ashrafi (2011)
Polarimetry	20 °C	3.28 molality	0.000734	Krieble (1935)
Saccharimeter	25 °C	0.50	0.005897	Clark (1921)
Polarimetry	20 °C	2.00	0.000383	Dawber (1966)
IR measurements	25 °C	2.274	0.000523	Pintar (2000)
Polarimetry	29 °C	2.507	0.002377	This work

Method	Temperature	[H_2SO_4] (M)	K (s^{-1})	Reference
Polarimetry	20 °C	2.230	0.00870	Krieble (1935)
Polarimetry	29 °C	2.506	0.002608	This work

Guggenheim and Wiseman [37] measured the inversion of sucrose kinetics at 25ºC using concentration of HCl which are much lower than the 2.507 M HCl used in the present study. Their values are included in Table 4.8 for comparison. Their measurements of the rate constants were found to increase with increasing the concentration of salt.

Ashrafi *et al*. [88] measured the rate of inversion of 0.050 M sucrose at 28 °C by HCl (0.050 M). The value for the rate constant reported in Table 4.8 has been obtained by plotting the data listed in the publication and fitting an exponential curve. The rate constant of 0.002234 s^{-1} reported by Ashrafi *et al*. is the average of the rate constants at each time interval.

Krieble [35] studied the inversion of sucrose (0.146 M) at 20 °C both by HCl and H_2SO_4. His results show that the rate constant increases with increasing the concentration of acid.

We have re-calculated the rate of sucrose inversion by Clarke [8] to include the factor 2.303 for converting log to ℓn in the first-order rate equation. The value is listed in Table 4.8.

A paper by Dawber *et al.* [20] describes an acid-catalyzed inversion of sucrose in terms of acidity function and acid molarity at 20 ºC on solutions containing 10 g of sucrose per 100.00 mL of acidified solution. From their plot of the rate constant versus molarity of HCl, a rate constant of 0.000383 s^{-1} for 2.00 M NaCl was estimated by extrapolation.

While the inversion of sucrose kinetics is largely studied by polarimetry, Pintar *et al.* [24] were able to apply Infrared Spectroscopy (IR) to monitor this reaction. Their results show that the bimolecular sucrose disappearance rate increases linearly by increasing hydrogen ion concentration in the reaction mixture. Their value of reaction rate constant determined at 25 °C was found to be 0.0005230 s^{-1}.

Considering the wide range of temperatures and concentrations of the HCl catalysts, it is difficult to compare the results obtained for the rate constant of the inversion of sucrose. However, it appears that the measurements which have been reported are of right order of magnitude compared to previous values listed in the literature.

4.3.2 Kinetic salt effect.

The consideration of the kinetic salt effect (Section 2.3) can be of great importance, both in elucidating and providing information about the mechanism of a reaction [65, 89-90]. The kinetic salt effect on the hydrolysis of sucrose has been investigated before [37]; fairly dilute solutions of 0.08764 m sucrose have been studied over several days (48 hr., old or more). The effect on the addition of salts to more concentrated solutions has been mentioned by Dordick and Clarke [86]. The equation for the kinetic salt effect is only valid for dilute solutions, where the activity coefficients can be expressed in terms of ionic strength (Section 2.3, equation 2.13). Unfortunately, the use of dilute salt solutions has small effects on the inversion of concentrated (of the order of 0.3 M, used in the present study) sucrose solutions, and the use of dilute sucrose solutions would require a measurement time which would be impractically long for the present experimental set-up.

If ions of the same charge are involved in the rate determining step of the reaction, an increase in ionic strength increases the rate of reaction, whereas a decrease in the rate is caused if the ions are of different charge [64]. Considering that an activity coefficient does not cause a change in sign of the ionic charge, a qualitative conclusion can be made about the mechanism of the sucrose inversion if the slope of the plot of log k versus $I^{1/2}$ (Section 2.3, equation 2.4) is either positive or negative, even if the activity coefficients are not known.

The ionic strength was calculated from the concentrations of the added salts varied between 0.050-0.750 M present in the final reacting solutions of the inversion reaction by using equation $I = 1/2\sum (M\ z^2)$. Table 4.9 presents the values of the rate constants and the ionic strengths calculated from Infinite Time method.

Table 4.9 The effect of the ionic strength on the kinetics of the sucrose inversion. SSM and ASM denote reactions of sucrose-salt mixture and acid-salt mixture, respectively. ND (the reaction was not determined).

[NaCl]	*k*/s, SSM	ℓog *k*	*I*	$I^{1/2}$	*k*/s, ASM	ℓog *k*	$I^{1/2}$
0.050	0.002437	-2.6131	2.560	1.600	0.002405	-2.6189	1.600
0.125	0.002463	-2.6085	2.635	1.623	0.002440	-2.6126	1.623
0.250	0.002571	-2.5898	2.760	1.661	0.002503	-2.6015	1.661
0.375	0.002668	-2.5738	2.885	1.698	0.002596	-2.5857	1.698
0.500	0.002683	-2.5714	3.010	1.735	0.002620	-2.5817	1.735
0.750	0.002917	-2.5350	3.260	1.805	ND	ND	ND
[Na_2SO_4]	*k*/s, SSM	ℓog *k*	*I*	$I^{1/2}$	*k*/s, ASM	ℓog *k*	$I^{1/2}$
0.050	0.002658	-2.5754	7.670	2.769	0.002618	-2.5820	2.769
0.250	0.002532	-2.5965	8.270	2.876	0.002542	-2.5948	2.876
0.500	0.002520	-2.5986	9.020	3.003	0.002425	-2.6153	3.003
0.750	0.002487	-2.6043	9.770	3.126	0.002290	-2.6402	3.126

The rate constant increases with increasing the ionic strength of a solution containing NaCl while it decreases with increasing the ionic strength of the solution containing H_2SO_4. Figures 4.18 and 4.19 illustrate the linear plots of the log *k* versus $I^{0.5}$ for all the reaction mixtures whose slopes are positive and negative for the addition of NaCl and Na_2SO_4 in the inversion reaction, respectively.

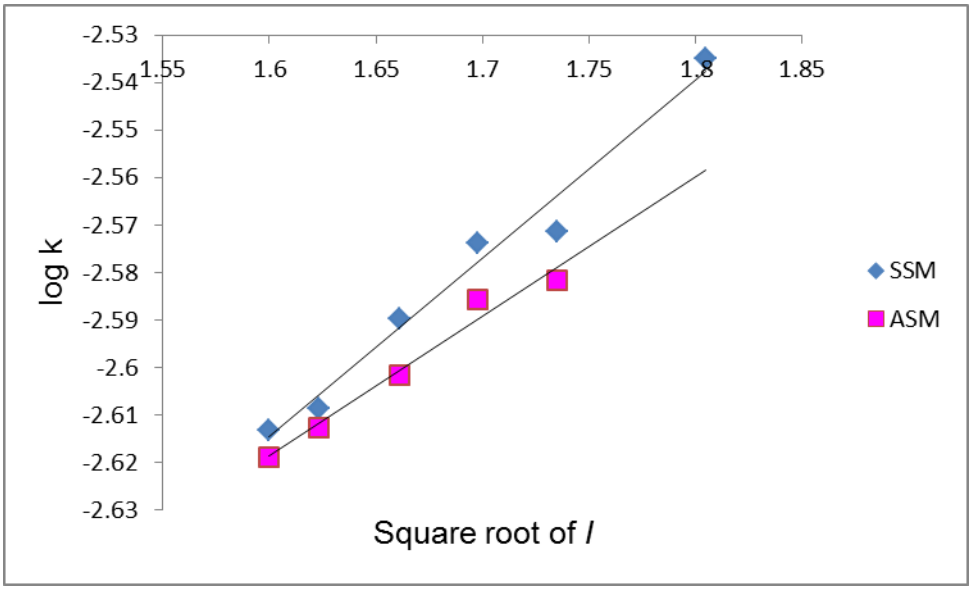

Figure 4.18 The effect of ionic strength (I) on the inversion of sucrose promoted by HCl with increasing concentration of NaCl at 29 °C. SSM and ASM denote reactions of sucrose-salt mixture and acid-salt mixture, respectively.

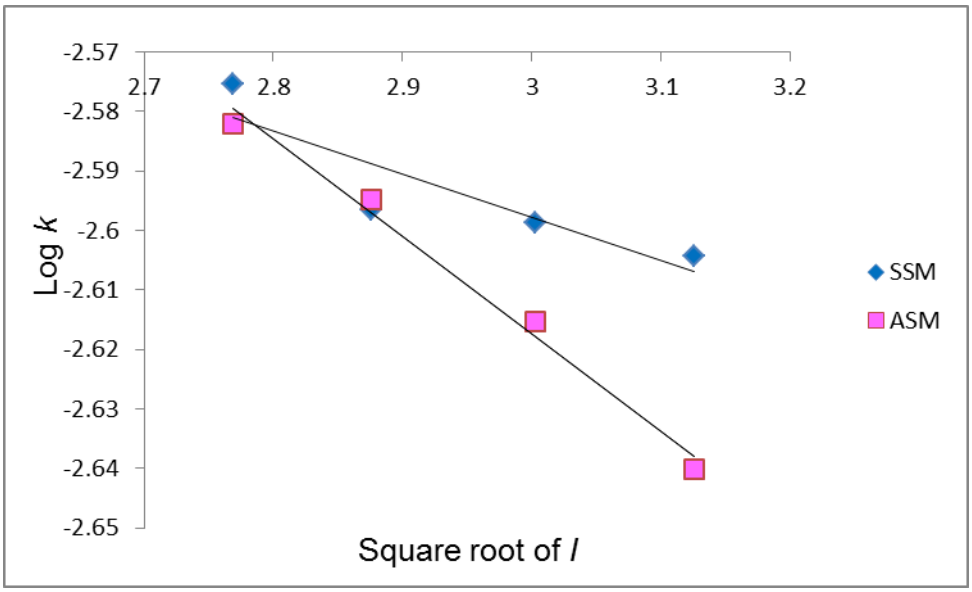

Figure 4.19 The effect of ionic strength (I) on the inversion of sucrose promoted by H_2SO_4 with increasing concentration of Na_2SO_4 at 29 °C. SSM and ASM denote reactions of sucrose-salt mixture and acid-salt mixture, respectively.

Similar observations in terms of the behavior of the slopes in the plots of log k versus $I^{1/2}$ as determined from the rates of Guggenheim and Kezdy-Swinebourne methods were also observed. The values are found in Appendix 15. The difference in the sign of the slopes among the ionic strength graphs of all reaction mixtures indicates the presence of a negative and a positive kinetic salt effect in the inversion reaction. These effects are possibly due to the involvement of opposite charges and like charges in the rate determining step of the reaction. It can be shown that there exists a dependence of the rate on the ionic strength even if a neutral species and an ion are the reacting species [Question 27.16, 91]. The dependence is not zero as would be expected from equation 2.14 in Section 2.3.

When a reaction of a neutral species, such as sucrose, is catalyzed by H^+ (*aq*), then the following dependence can be deduced:

Consider the mechanism, H^+ (*aq*) + B (*aq*) → P (*aq*), where H^+ (*aq*) comes from the dissociation of the weak acid HA (*aq*) with the weak acid having a fixed concentration. It can be shown that the log [H^+ (*aq*)], derived from the ionisation of HA (*aq*), depends on the activity coefficients of ions and thus depending on the ionic strength. The relationship between log (rate) and log (H^+ (*aq*) can be found to show that the rate also depends on the ionic strength:

$$K_a = \frac{[H^+(aq)][A^-(aq)]}{[HA(aq)]} \tag{4.1}$$

By expressing equation (4.1) in terms of the activity coefficients it follows that;

$$K_a = \frac{\gamma_\pm^2[H^+(aq)][Cl^-(aq)]}{[HA\,(aq)]_{\gamma HA(aq)}} \tag{4.2}$$

Assuming that the activity coefficient for neutral HA (*aq*) is 1, equation (4.2) becomes

$$K_a = \frac{\gamma_\pm^2[H^+(aq)][A^-(aq)]}{[HA(aq)]} \tag{4.3}$$

and can be expressed in terms of [H^+ (*aq*)]

$$[H^+(aq)] = \frac{K_a[HA(aq)]}{[A^-(aq)]\gamma_\pm^2} \tag{4.4}$$

Taking the log at both sides of equation (4.4) gives the following equations:

$$\log[H^+(aq)] = logK_a + log[HA(aq)] - (\log[A^-(aq)] - 2log\gamma_{\pm}) \quad (4.5)$$

At low concentration, the activity coefficient can be explained in terms of ionic strength of the solution by using the Debye-Huckel limiting law;

$$log\gamma_{\pm} = -A\left|z(H^+(aq))z(B^-(aq)\right|I^{1/2} \quad (4.6)$$

By substituting equation (4.6) in (4.5) gives

$$logH^+(aq) = logK_a + log\frac{[HA(aq)]}{[A^-(aq)]} + 2AI^{1/2} \quad (4.7)$$

From the above equation, the rate can be written as:

$$rate = k[H^+(aq)][B^-(aq)] \quad (4.8)$$

By taking the log and substituting the expression for log [H^+ (*aq*)], gives

$$\log(rate) = \log rate' + 2AI^{1/2} \quad (4.9)$$

This indeed has been confirmed experimentally in the present study for the inversion of sucrose.

4.3.3 Precision measurements.

4.3.3.1 Repeatability.

The precision of the instrument was assessed for its stability and capability of resulting in the same measurements over time. It was determined by monitoring the rate of the inversion of sucrose (10%, mass/volume) catalyzed by HCl (2.507 M) in the presence of the added 0.250 M NaCl in sucrose or acid solutions. The concentration of NaCl was randomly chosen. The inversion reaction was monitored by running the reaction mixtures of sucrose-NaCl and HCl-NaCl in succession, each prepared in triplicates (n=3) from the same sucrose and acid solutions. The analysis was done on the same day, under the same experimental conditions according to the method procedure as outlined in Section 3.3.2. The rate constants were determined by Infinite Time, Guggenheim, and Kezdy-Swinebourne methods.

The results obtained are reported in Table 4.10 as relative standard deviation (RSD), which is the usual way of quantifying precision as a percentage of the mean (average).

Table 4.10 Repeatability of rate constants in final reacting solution containing sucrose (10% mass/volume)-NaCl (0.250 M)-HCl (2.507 M) determined by curve fitting methods.

1^{st} Order rate constant Methods	Run	Sucrose-NaCl mixture (k, s^{-1})	HCl-NaCl mixture (k, s^{-1})
Infinite Time Method	Repeatability (Polarimeter variation)		
	1^{st}	0.002467	0.002463
	2^{nd}	0.002495	0.002547
	3^{rd}	0.002485	0.002475
	Mean ± RSD	0.002482 ± 0.46%	0.002373 ± 1.82%
Kezdy-Swinbourne	Repeatability (Polarimeter variation)		
	1^{st}	0.002275	0.002243
	2^{nd}	0.002220	0.002221
	3^{rd}	0.002129	0.002209
	Mean ± RSD	0.002208 ± 3.34%	0.002232 ± 0.49%
Guggenheim	Repeatability (Polarimeter variation)		
	1^{st}	0.002240	0.002182
	2^{nd}	0.002240	0.002188
	3^{rd}	0.002182	0.002152
	Mean ± RSD	0.002220 ± 1.51%	0.002174 ± 0.89%

The rate constants for sucrose-NaCl and HCl-NaCl mixtures were found on average deviation from the individual mean to be at most 1.42% for all three evaluation methods. In general, there is a good agreement within the experimental precision of the instrument for the determination of rate constants by various curve fitting methods within analysis on the same day. Any measurements errors occurred were considered to be the results of the known general uncertainties caused by various experimental error sources. These measurement errors may arise from imperfections in the components of the polarimeter which include, among others, the quarter-wave plate, polarizers, detectors, and rotational stages of a polarimeter [92].

4.3.3.2 Reproducibility.

The within-laboratory (same laboratory) reproducibility was assessed to get an estimate of the between-laboratory (different laboratory) reproducibility since it was not possible to involve additional laboratories for the determination of the between-laboratory reproducibility. It was evaluated by monitoring the rate of the inversion of sucrose (10%, mass/volume) catalyzed by HCl (2.507 M) in the presence of the added 0.250 M NaCl in sucrose or acid solutions. The inversion reaction was monitored by running each reaction mixture of sucrose-NaCl and HCl-NaCl on three different days, under the same experimental conditions according to the method procedure as outlined in Section 3.3.2. The rate constants were determined by Infinite Time, Guggenheim, and Kezdy-Swinebourne methods**.** The mean rate constants obtained are presented in Table 4.11.

Table 4.11 Reproducibility of rate constants in final reacting solution containing sucrose (10% mass/volume)-NaCl (0.250 M)-HCl (2.507 M) determined by curve fitting methods.

1st Order rate constant Methods	Run	Sucrose-NaCl mixture (s^{-1})	HCl-NaCl mixture (s^{-1})
Infinite Time Method			
	1st	0.002572	0.002543
	2nd	0.002467	0.002545
	3rd	0.002573	0.002463
	Mean ± RSD	0.002537 ± 2.40%	0.002517 ± 1.86%
Kezdy-Swinbourne			
	1st	0.002224	0.002241
	2nd	0.002275	0.002218
	3rd	0.002299	0.002244
	Mean ± RSD	0.002265 ± 1.73%	0.002234 ± 0.64%
Guggenheim			
	1st	0.002220	0.002230
	2nd	0.002240	0.002192
	3rd	0.002277	0.002182
	Mean ± RSD	0.002246 ± 1.29%	0.002201 ± 1.15%

The obtained results indicate that the variability of rate constants measured as relative standard deviation (RSD) ranges between 0.64 to 2.40% for all rate evaluation methods. These % RSD values are acceptable and show good reproducibility of the method. If the measurements including the one used for reproducibility and repeatability are considered at 95% confidence level (n=16, Appendix 16), the mean rate constant of 0.002518 s^{-1} is obtained and the % RSD is 2.5, which is a realistic reflection of the error involved in the measurements.

4.3.4 Effect of adding more than one salt in the inversion reaction.

In Table 4.12, the experimental results based on the reaction systems involving the addition of more than one salt in single inversion kinetics are presented. The reaction system catalyzed by HCl was chosen to investigate further the effect of these salts on the kinetics of sucrose inversion. The salts were dissolved in sucrose solution prior the reaction with acid solution, but did not dissolve in acid solution. Consequently, only the results for sucrose-salt mixtures are presented. In column 1, the graphical methods for the determination of first order rate constants are shown. In column 2, are the rate constants of the reaction system of acid (HCl) catalyzed inversion of sucrose with added NaCl. In column 2 and 3, are the rate constants of the reaction system of inversion of sucrose kinetics with the addition of NaCl + Na_2SO_4 and NaCl + Na_2SO_4 + NaH_2PO_4, respectively.

Table 4.12 Values of the rate constants k (s^{-1}) of the effect of adding more than one salt in the inversion reaction.

Graphic Methods	Solution		
	Sucrose + NaCl (0.5 M)	Sucrose + NaCl (0.5 M) + Na_2SO_4 (0.5 M)	Sucrose + NaCl (0.5 M) + Na_2SO_4 (0.5 M) + NaH_2PO_4 (0.5 M)
Infinite Time	0.002683	0.002557	0.002267
Kezdy-Swinbourne	0.002737	0.002258	0.001904
Guggenheim	0.002608	0.002265	0.001890

It is clear that for all the reaction systems studied the rate of sucrose inversion decreases with increasing the number of salts added in the reacting system. The values of the rate constants as determined from the graphical methods of first order reaction kinetics show that the inversion process is faster for sucrose + NaCl system than sucrose + NaCl + Na_2SO_4 and NaCl + Na_2SO_4 + NaH_2PO_4 systems. Possibly, the decrease in the rate constants when more than one salt is added in the reacting system could be due to the blocking of the most reactive sites of sucrose by these salts. Sucrose has eight hydroxyl groups, i.e. three primary and five secondary hydroxyl groups as shown in Figure 1a in Chapter 1. Several research groups have indicated that the chemical reactivity of the primary hydroxyl groups at the C-6 and C-6' positions are higher than that at C-1' position, followed by the hydroxyl groups [93]. Possibly, these salts may be in competition for a common interaction site, and thus causing the decrease in rate constants.

CHAPTER 5

5.1 Conclusions.

The study of angles of rotation of saccharide solutions, and of salts added to the saccharides and the kinetics of the sucrose inversion have been evaluated by using polarimetry technique as the main analytical tool employed in this study. The angle of rotation which has been largely referred to as the optical rotation in this work, is affected in the order NaCl < Na_2SO_4 < NaH_2PO_4, particularly, when salts are added to sucrose solutions. On the other hand, it emerged that both glucose and fructose are also affected by the addition of salts; with the salts seem to have the least effect on the optical rotation of glucose. The change in optical rotation depends predominantly on the pH of the solution and the nature of the salt added. It increases with increasing concentration of the added salts. The Hofmeister series for ions in solution was not followed as far as changes in optical rotation were concerned.

From the above, it could be concluded that there is some interaction between the sugars and the salts, and that different salts interact in different ways with the different sugars. Apart from the structure which gives rise to the optical rotation of the molecules of various saccharides, it could also be possible that there is some close association, such as the formation of complexes, between the sugar and salt molecules. However, such formation of complexes would have to be proven experimentally. Some attempts were made to detect any evidence of complex formation using spectroscopic methods such as Raman spectroscopy; however, nothing could be detected.

By assessing the results for the kinetics of the inversion of sucrose catalyzed by an acid against values available from the literature; taking into consideration the large variety of different parameters which were used in the different publications, the present results are in reasonable agreement with what has been determined previously. Three different methods, namely Infinite Time, Guggenheim, and Kezdy-Swinbourne were used to evaluate the kinetic measurements.

Guggenheim and Kezdy-Swinbourne can be regarded as approximate methods. They invariably yielded results which were lower than those obtained from the direct curve fitting Infinite Time method.

The addition of salts to the acid catalyzed inversion reaction of sucrose showed some pronounced effect on the rate of reaction. Having ascertained the reproducibility of the measurements and estimated the error involved, different methods of preparing the reagent solutions were investigated. The salt was either added to the acid solution or to the sucrose solution before mixing the solutions to start the inversion reaction. No significant differences could be detected for the two methods.

The addition of NaCl to the HCl catalyzed sucrose inversion reaction resulted in an increase of reaction rate with the ionic strength of the solution. When Na_2SO_4 was added to the sucrose inversion catalyzed by H_2SO_4, the reaction rate decreased with increasing the ionic strength of the solution. This finding indicates that the reaction rates result from two possible conditions. Firstly, from the reactant ions of the same sign or charge which gave rise to a positive kinetic salt effect and secondly, from the reactant ions of opposite charges which gave rise to a negative kinetic salt effect. The above two conditions suggest that the inversion of sucrose proceeded through two different reaction mechanisms.

Based on the observed kinetic data, the work presented in this dissertation has a scope for improvement. For future studies of the reaction, more parameters can be varied, such as acid strength, temperature, combination of different salts and concentration of H^+ (*aq*). The effect of other neutral salts such as sodium perchlorate on the inversion of sucrose can also be investigated, because perchlorate is known not form complexes during reaction. The experiment can be set up to study more dilute solutions, so that the kinetic salt effect can be evaluated quantitatively.

A variety of techniques such as Polarimetry; Spectroscopy; Chromatography; Colorimetry and Spectrophotometry; Nuclear Magnetic Resonance; Isotope Dilution; Titrimetry; Enzymatic Analysis; and Enzyme Electrodes have already been described as potential instruments that can be used to monitor the inversion of sucrose kinetics [12]. More recently, experiments employing NMR to explore the kinetics of invertase

catalyzed inversion of sucrose have been described by Kehlbeck et al. [94]. NMR was used to visualise the appearance of products and disappearance of reactants to demonstrate reaction monitoring and kinetics. In addition to kinetics, they have also indicated that the study of mutarotation can be easily monitored from individual and mixtures of sucrose and its component saccharides. In contrast to NMR, GC-MS can also be used to monitor the inversion of sucrose kinetics. However, the main disadvantage of GC-MS is the need to prepare appropriate derivatives due to relatively low volatility of saccharides compounds [95]. Fortunately, evaporation based detection systems such as charged aerosol detector (CAD) have been developed in the recent past. CAD detects any nonvolatile or semivolatile analyte with or without a chromophore and can be coupled with chromatographic method such as HPLC [96].

While the inversion of sucrose catalyzed by invertase or H^+ (*aq*) has been extensively studied previously [25, 94], the specific influence of H^+ (*aq*) concentration and the presence of added salts on the inversion reaction as monitored by NMR and HPLC-CAD have not been much explored systematically. Thus, in future the coupling of NMR and HPLC-CAD can be employed to study this inversion reaction catalyzed by H^+ (*aq*) or inorganic promoters with or without the presence of added salts. Their results can be compared with those obtained from traditional polarimetrIc method.

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APPENDICES

Appendix 1 Calculated and weighed mass of sucrose and salt solutes (NaCl, Na_2SO_4, and NaH_2PO_4) for sucrose-salt mixtures.

NaCl + Sucrose + Water system	[NaCl]	Calculated mass of salt	Weighed mass of salt	Weight mass of sucrose
	0.1	0.5844	0.5846	20.0001
	0.5	2.9220	2.9224	20.0003
	1.0	5.844	5.8445	20.0002
	2.0	11.688	11.6883	20.0005
	3.0	17.5320	17.5322	20.0004
	4.0	23.3760	23.3761	20.0002
	5.0	29.220	29.2226	20.0003
Na_2SO_4 + Sucrose + Water system	[Na_2SO_4]	Calculated mass of salt	Weighed mass of salt	Weighed mass of sucrose
	0.1	1.420	1.4204	20.0004
	0.3	4.261	4.2614	20.0001
	0.5	7.102	7.1023	20.0005
	0.75	10.653	10.6532	20.0003
	1.0	14.204	14.2043	20.0004
	1.25	17.755	17.7554	20.0004
	1.5	21.306	21.3063	20.0002
NaH_2PO_4 + sucrose + water system	[NaH_2PO_4]	Calculated mass of salt	Weighed Mass of salt	Weighed mass of sucrose
	0.1	1.199	1.1993	20.0004
	0.3	3.599	3.5995	20.0001
	0.5	5.999	5.9993	20.0003
	0.75	8.998	8.9984	20.0000
	1.0	11.998	11.9984	20.0002

	1.25	14.996	14.9965	20.0003
	1.5	17.997	17.9971	20.0002

Appendix 2 Calculated and weighed mass of sucrose and salt solutes for the inversion reaction (sucrose-salt mixture).

Sucrose-NaCl +HCl	[NaCl]	Calculated mass of salt	Weighed mass of salt	Weighed mass of sucrose
	0.100	0.5844	0.5845	20.0001
	0.250	1.461	1.4611	20.0001
	0.500	2.922	2.9223	20.0002
	0.750	4.383	4.3832	20.0001
	1.000	5.844	5.8442	20.0004
	1.500	8.766	8.7662	20.0003
Sucrose-Na_2SO_4+H_2SO_4	[Na_2SO_4]	Calculated mass of salt	Weighed mass of salt	Weighed mass of sucrose
	0.100	1.420	1.4206	20.0003
	0.500	7.102	7.1021	20.0002
	1.000	14.204	14.2044	20.0002
	1.500	21.306	21.3064	20.0001

Appendix 3 Calculated and weighed mass of sucrose and salt solutes for the inversion reaction (acid-salt mixture).

HCl-NaCl+ sucrose	[NaCl]	Calculated mass of salt	Weighed mass of salt	Weighed mass of sucrose
	0.100	0.5844	0.5845	20.0004
	0.250	1.461	1.4610	20.0004
	0.500	2.922	2.9221	20.0004
	0.750	4.383	4.3830	20.0004
	1.000	5.844	5.8441	20.0004
	1.500	8.766	Not dissolved	20.0004
H_2SO_4-Na_2SO_4+ Sucrose	[Na_2SO_4]	Calculated mass of salt	Weighed mass of salt	Weighed mass of sucrose
	0.100	1.420	1.4202	20.0004
	0.500	7.102	7.1021	20.0004
	1.000	14.204	14.2043	20.0004
	1.500	21.306	21.3063	20.0004

Appendix 4 Optical rotation of HCl (2.507 M) catalyzed inversion of sucrose 0(10%) with the addition of sodium chloride (sucrose-salt mixture) at 29 °C.

Optical Rotation						
Time (min)	0.050 M	0.125 M	0.250 M	0.375 M	0.500 M	0.750 M
3	3.99	3.98	3.91	3.79	3.51	3.67
4	3.21	3.23	3.24	2.99	2.6	2.92
5	2.63	2.61	2.52	2.29	1.88	2.23
6	1.97	2.03	1.9	1.72	1.23	1.67
7	1.45	1.55	1.41	1.18	0.72	1.13
8	1.02	1.11	0.93	0.74	0.22	0.64
9	0.63	0.71	0.55	0.33	-0.12	0.27
10	0.3	0.39	0.2	-0.03	-0.48	-0.22
11	-0.03	0.08	-0.09	-0.31	-0.74	-0.52
12	-0.28	-0.17	-0.35	-0.58	-0.98	-0.81
13	-0.52	-0.41	-0.59	-0.81	-1.17	-1.02
14	-0.71	-0.6	-0.78	-0.99	-1.34	-1.22
15	-0.89	-0.79	-0.96	-1.17	-1.46	-1.38
16	-1.04	-0.95	-1.11	-1.31	-1.6	-1.52
17	-1.18	-1.08	-1.24	-1.43	-1.69	-1.62
18	-1.3	-1.21	-1.36	-1.53	-1.78	-1.71
19	-1.4	-1.32	-1.46	-1.63	-1.85	-1.8
20	-1.5	-1.42	-1.55	-1.71	-1.92	-1.87
21	-1.58	-1.5	-1.63	-1.78	-1.97	-1.92
22	-1.65	-1.58	-1.69	-1.83	-2.02	-1.97
23	-1.71	-1.64	-1.75	-1.89	-2.06	-2.01
24	-1.77	-1.71	-1.81	-1.93	-2.09	-2.04
25	-1.82	-1.76	-1.85	-1.97	-2.12	-2.07
26	-1.86	-1.8	-1.89	-2	-2.15	-2.09
27	-1.9	-1.85	-1.93	-2.03	-2.16	-2.11
28	-1.93	-1.88	-1.96	-2.05	-2.18	-2.13
29	-1.96	-1.92	-1.98	-2.07	-2.2	-2.14
30	-1.99	-1.94	-2.01	-2.09	-2.21	-2.16
31	-2.01	-1.97	-2.03	-2.1	-2.22	-2.17
32	-2.02	-1.99	-2.05	-2.12	-2.23	-2.18

33	-2.05	-2.01	-2.06	-2.13	-2.24	-2.18
34	-2.07	-2.03	-2.08	-2.14	-2.25	-2.19
35	-2.08	-2.04	-2.09	-2.15	-2.26	-2.19
36	-2.09	-2.05	-2.10	-2.16	-2.26	-2.2
37	-2.1	-2.06	-2.11	-2.16	-2.26	-2.2
38	-2.11	-2.07	-2.12	-2.16	-2.27	-2.21
39	-2.12	-2.07	-2.12	-2.17	-2.27	-2.21
40	-2.13	-2.08	-2.13	-2.17	-2.28	-2.21
41	-2.14	-2.08	-2.13	-2.18	-2.28	-2.22
42	-2.14	-2.09	-2.14	-2.18	-2.28	-2.22
43	-2.15	-2.1	-2.14	-2.18	-2.28	-2.22
44	-2.15	-2.1	-2.14	-2.19	-2.29	-2.22
45	-2.16	-2.11	-2.15	-2.19	-2.29	-2.22
46	-2.16	-2.11	-2.15	-2.19	-2.29	-
47	-2.16	-2.11	-2.15	-2.19	-2.29	-
48	-2.16	-2.11	-2.15	-2.19	-2.29	-
49	-2.16	-2.11	-2.15	-	-	-

Appendix 5 Optical rotation of HCl (2.507 M) catalyzed inversion of sucrose (10%) with the addition of sodium chloride (HCl-NaCl mixture) at 29 °C. ND (The reaction was not determined)

Optical Rotation						
Time (min)	0.050 M	0.125 M	0.250 M	0.375 M	0.500 M	0.750 M
3	4	4.03	3.93	3.87	3.88	ND
4	3.31	3.29	3.12	3.06	3.01	ND
5	2.69	2.66	2.49	2.41	2.4	ND
6	2.06	2.06	1.89	1.8	1.76	ND
7	1.58	1.58	1.39	1.3	1.2	ND
8	1.16	1.15	0.97	0.87	0.76	ND
9	0.75	0.71	0.56	0.46	0.35	ND
10	0.43	0.38	0.26	0.13	0.04	ND
11	0.1	0.08	-0.08	-0.12	-0.3	ND

12	-0.15	-0.2	-0.31	-0.43	-0.54	ND
13	-0.38	-0.43	-0.59	-0.65	-0.74	ND
14	-0.6	-0.63	-0.78	-0.86	-0.96	ND
15	-0.77	-0.82	-0.96	-1.02	-1.12	ND
16	-0.94	-0.98	-1.12	-1.18	-1.28	ND
17	-1.08	-1.11	-1.25	-1.3	-1.4	ND
18	-1.2	-1.24	-1.38	-1.42	-1.51	ND
19	-1.32	-1.36	-1.48	-1.52	-1.61	ND
20	-1.41	-1.45	-1.57	-1.61	-1.7	ND
21	-1.5	-1.54	-1.65	-1.68	-1.76	ND
22	-1.58	-1.61	-1.73	-1.75	-1.82	ND
23	-1.64	-1.68	-1.78	-1.8	-1.88	ND
24	-1.7	-1.73	-1.84	-1.85	-1.92	ND
25	-1.76	-1.79	-1.89	-1.9	-1.96	ND
26	-1.8	-1.83	-1.93	-1.94	-2	ND
27	-1.84	-1.87	-1.97	-1.97	-2.03	ND
28	-1.88	-1.91	-2	-2	-2.06	ND
29	-1.91	-1.94	-2.03	-2.03	-2.07	ND
30	-1.94	-1.96	-2.05	-2.05	-2.1	ND
31	-1.96	-1.99	-2.07	-2.07	-2.13	ND
32	-1.98	-2.01	-2.09	-2.08	-2.13	ND
33	-2	-2.03	-2.11	-2.1	-2.14	ND
34	-2.02	-2.04	-2.13	-2.11	-2.16	ND
35	-2.03	-2.06	-2.14	-2.12	-2.16	ND
36	-2.05	-2.07	-2.15	-2.14	-2.17	ND
37	-2.06	-2.08	-2.16	-2.14	-2.18	ND
38	-2.07	-2.09	-2.17	-2.15	-2.18	ND
39	-2.08	-2.1	-2.18	-2.15	-2.18	ND
40	-2.08	-2.11	-2.19	-2.16	-2.19	ND
41	-2.09	-2.12	-2.19	-2.17	-2.2	ND
42	-2.1	-2.12	-2.19	-2.17	-2.2	ND
43	-2.1	-2.12	-2.2	-2.17	-2.21	ND
44	-2.11	-2.13	-2.2	-2.18	-2.21	ND
45	-2.11	-2.13	-2.21	-2.18	-2.21	ND
46	-2.12	-2.14	-2.21	-2.18	-2.21	ND

47	-2.12	-2.14	-2.21	-2.18	-2.21	ND
48	-2.12	-2.14	-2.21	-2.18	-	ND
49	-2.12	-2.14	-2.21	-	-	ND
50	-2.12	-2.14	-2.21		-	ND
51		-	-		-	ND

Appendix 6 Optical rotation of H_2SO_4 (2.506 M) catalyzed inversion of sucrose (10%) with the addition of sodium sulfate (sucrose-Na_2SO_4 mixture) at 29 °C.

Time (min)	0.050 M	0.250 M	0.500 M	0.750 M
3	3.38	3.7	3.91	4.01
4	2.64	3.09	3.09	3.19
5	1.94	2.23	2.48	2.52
6	1.4	1.65	1.83	1.89
7	0.87	1.14	1.26	1.47
8	0.46	0.67	0.78	0.89
9	0.11	0.25	0.39	0.47
10	-0.24	-0.08	0.04	0.12
11	-0.51	-0.36	-0.29	-0.18
12	-0.74	-0.64	-0.55	-0.47
13	-0.95	-0.87	-0.8	-0.69
14	-1.14	-1.06	-1	-0.92
15	-1.3	-1.23	-1.17	-1.1
16	-1.44	-1.38	-1.34	-1.24
17	-1.55	-1.5	-1.48	-1.4
18	-1.66	-1.62	-1.6	-1.52
19	-1.75	-1.72	-1.71	-1.64
20	-1.83	-1.81	-1.8	-1.73
21	-1.9	-1.88	-1.88	-1.81
22	-1.95	-1.94	-1.95	-1.89
23	-2	-2	-2.01	-1.95
24	-2.05	-2.05	-2.06	-2.01
25	-2.09	-2.09	-2.11	-2.06

26	-2.12	-2.13	-2.15	-2.11
27	-2.17	-2.16	-2.18	-2.15
28	-2.18	-2.19	-2.21	-2.18
29	-2.19	-2.22	-2.24	-2.21
30	-2.21	-2.24	-2.26	-2.24
31	-2.23	-2.26	-2.28	-2.26
32	-2.25	-2.27	-2.3	-2.28
33	-2.26	-2.29	-2.32	-2.29
34	-2.27	-2.3	-2.33	-2.31
35	-2.27	-2.31	-2.34	-2.32
36	-2.29	-2.32	-2.35	-2.34
37	-2.29	-2.33	-2.36	-2.35
38	-2.3	-2.33	-2.37	-2.36
39	-2.31	-2.34	-2.38	-2.36
40	-2.29	-2.35	-2.38	-2.37
41	-2.31	-2.35	-2.39	-2.38
42	-2.31	-2.35	-2.39	-2.38
43	-2.32	-2.36	-2.4	-2.39
44	-2.32	-2.36	-2.4	-2.39
45	-2.32	-2.36	-2.4	-2.4
46	-2.34	-2.36	-2.4	-2.4
47	-2.32	-2.37	-2.41	-2.4
48	-2.32	-2.37	-2.41	-2.4
49	-2.32	-2.37	-2.41	-2.4
50	-2.32	-2.37	-2.41	-
51	-2.32	-2.37	-2.41	-
52	-2.32	-	-	-
53	-	-	-	-
54	-	-	-	-
55	-	-	-	-

Appendix 7 Optical rotation of H_2SO_4 (2.506 M) catalyzed inversion of sucrose (10%) with the addition of sodium sulfate (H_2SO_4-Na_2SO_4 mixture) at 29 °C.

Time (min)	0.050 M	0.250 M	0.500 M	0.750 M
3	3.43	3.56	3.64	3.84
4	2.69	2.75	2.98	3.17
5	2.05	2.16	2.23	2.52
6	1.43	1.55	1.67	1.99
7	0.95	1.02	1.15	1.52
8	0.53	0.59	0.72	1.06
9	0.13	0.19	0.31	0.69
10	-0.19	-0.14	-0.01	0.32
11	-0.44	-0.44	-0.33	0.03
12	-0.72	-0.69	-0.58	-0.26
13	-0.94	-0.9	-0.8	-0.5
14	-1.12	-1.1	-1.01	-0.73
15	-1.28	-1.27	-1.18	-0.91
16	-1.42	-1.41	-1.33	-1.08
17	-1.54	-1.54	-1.48	-1.24
18	-1.65	-1.65	-1.59	-1.37
19	-1.74	-1.75	-1.7	-1.49
20	-1.82	-1.84	-1.79	-1.61
21	-1.89	-1.91	-1.87	-1.7
22	-1.95	-1.98	-1.95	-1.79
23	-2	-2.03	-2.01	-1.87
24	-2.04	-2.08	-2.06	-1.93
25	-2.08	-2.13	-2.11	-2
26	-2.11	-2.16	-2.15	-2.05
27	-2.14	-2.19	-2.19	-2.1
28	-2.17	-2.22	-2.23	-2.14
29	-2.19	-2.24	-2.25	-2.18
30	-2.21	-2.27	-2.28	-2.21
31	-2.23	-2.29	-2.3	-2.24
32	-2.24	-2.3	-2.32	-2.27
33	-2.25	-2.31	-2.34	-2.29
34	-2.26	-2.33	-2.35	-2.32

35	-2.27	-2.34	-2.37	-2.33
36	-2.28	-2.35	-2.38	-2.35
37	-2.29	-2.36	-2.39	-2.37
38	-2.29	-2.36	-2.4	-2.38
39	-2.3	-2.37	-2.41	-2.39
40	-2.3	-2.38	-2.41	-2.4
41	-2.31	-2.38	-2.42	-2.41
42	-2.31	-2.39	-2.43	-2.42
43	-2.31	-2.39	-2.43	-2.43
44	-2.32	-2.39	-2.44	-2.43
45	-2.32	-2.4	-2.44	-2.44
46	-2.32	-2.4	-2.44	-2.45
47	-2.32	-2.4	-2.44	-2.45
48	-2.32	-2.4	-2.45	-2.45
49	-	-2.4	-2.45	-2.45
50	-	-	-2.45	-2.45
51	-	-	-2.45	-
52	-	-	-2.45	-
53	-	-	-	-
54	-	-	-	-
55	-	-	-	-

Appendix 8 Optical rotation values for repeatability of HCl (2.507 M) catalyzed inversion of sucrose (10%) with the addition of sodium chloride (0.50 M, n=3, sucrose-NaCl mixture at 29 °C).

Optical Rotation			
Time (min)	0.500 M (n=1)	0.500 M (n=2)	0.500 M (n=3)
3	3.89	3.9	4.05
4	3.13	3.16	3.27
5	2.42	2.47	2.65
6	1.86	1.91	2.04
7	1.35	1.41	1.58
8	0.89	0.93	1.07
9	0.49	0.56	0.69
10	0.13	0.23	0.36
11	-0.15	-0.08	0.09
12	-0.4	-0.33	-0.22
13	-0.67	-0.59	-0.47
14	-0.83	-0.77	-0.67
15	-1.04	-0.97	-0.84
16	-1.15	-1.15	-1.01
17	-1.29	-1.24	-1.15
18	-1.41	-1.33	-1.27
19	-1.5	-1.48	-1.37
20	-1.59	-1.53	-1.47
21	-1.66	-1.62	-1.55
22	-1.73	-1.69	-1.62
23	-1.79	-1.77	-1.69
24	-1.84	-1.77	-1.74
25	-1.88	-1.85	-1.79
26	-1.92	-1.89	-1.84
27	-1.95	-1.92	-1.87

28	-1.98	-1.95	-1.91
29	-2.01	-1.98	-1.93
30	-2.03	-2	-1.96
31	-2.05	-2.02	-1.98
32	-2.07	-2.04	-2
33	-2.08	-2.06	-2.02
34	-2.09	-2.07	-2.03
35	-2.1	-2.08	-2.04
36	-2.11	-2.09	-2.06
37	-2.12	-2.1	-2.07
38	-2.13	-2.11	-2.07
39	-2.14	-2.12	-2.08
40	-2.14	-2.12	-2.09
41	-2.15	-2.13	-2.1
42	-2.15	-2.13	-2.1
43	-2.15	-2.14	-2.11
44	-2.16	-2.14	-2.11
45	-2.16	-2.14	-2.11
46	-2.16	-2.15	-2.12
47	-2.17	-2.15	-2.12
48	-2.17	-2.15	-2.12
49	-2.17	-2.15	-2.12
50	-2.17	-2.15	-2.12
51	-2.17	-	-
52	-	-	-
53	-	-	-

Appendix 9 Optical rotation values for repeatability of HCl (2.507 M) catalyzed inversion of sucrose (10%) with the addition of sodium chloride (0.500 M, n=3, HCl-NaCl mixture at 29 °C).

Optical Rotation			
Time (min)	0.500 M (n=1)	0.500 M (n=2)	0.500 M (n=3)
3	3.81	3.94	3.95
4	3.03	3.14	3.2
5	2.39	2.5	2.48
6	1.83	1.94	1.9
7	1.3	1.4	1.42
8	0.88	0.97	0.97
9	0.51	0.59	0.61
10	0.16	0.23	0.26
11	-0.13	-0.06	-0.02
12	-0.38	-0.35	-0.3
13	-0.62	-0.56	-0.52
14	-0.8	-0.76	-0.73
15	-0.99	-0.95	-0.9
16	-1.13	-1.1	-1.05
17	-1.27	-1.24	-1.19
18	-1.38	-1.33	-1.3
19	-1.49	-1.45	-1.41
20	-1.57	-1.55	-1.5
21	-1.65	-1.62	-1.58
22	-1.72	-1.69	-1.65
23	-1.78	-1.76	-1.71
24	-1.83	-1.81	-1.77
25	-1.87	-1.86	-1.82
26	-1.91	-1.9	-1.86
27	-1.95	-1.94	-1.89

28	-1.98	-1.97	-1.93
29	-2	-2	-1.96
30	-2.03	-2.02	-1.98
31	-2.05	-2.04	-2
32	-2.06	-2.06	-2.02
33	-2.08	-2.08	-2.04
34	-2.1	-2.09	-2.05
35	-2.11	-2.1	-2.07
36	-2.12	-2.12	-2.08
37	-2.13	-2.12	-2.09
38	-2.14	-2.13	-2.1
39	-2.15	-2.14	-2.11
40	-2.15	-2.15	-2.11
41	-2.16	-2.15	-2.12
42	-2.16	-2.16	-2.12
43	-2.16	-2.16	-2.13
44	-2.17	-2.16	-2.13
45	-2.17	-2.17	-2.14
46	-2.17	-2.17	-2.14
47	-2.18	-2.17	-2.14
48	-2.18	-2.17	-2.14
49	-2.18	-2.17	-2.14
50	-2.18	-	-
51	-2.18	-	-
52	-	-	-
53	-	-	-

Appendix 10 Optical rotation values for reproducibility of HCl (2.507 M) catalyzed inversion of sucrose (10%) with the addition of sodium chloride (0.500 M, n=3, sucrose-NaCl mixture at 29 °C).

Optical Rotation			
Time (min)	0.500 M (n=1)	0.500 M (n=2)	0.500 M (n=3)
3	3.91	3.89	3.92
4	3.24	3.13	3.12
5	2.52	2.42	2.5
6	1.9	1.86	1.84
7	1.41	1.35	1.34
8	0.93	0.89	0.87
9	0.55	0.49	0.48
10	0.2	0.13	0.15
11	-0.09	-0.15	-0.16
12	-0.35	-0.4	-0.41
13	-0.59	-0.67	-0.65
14	-0.78	-0.83	-0.84
15	-0.96	-1.04	-1.01
16	-1.11	-1.15	-1.16
17	-1.24	-1.29	-1.29
18	-1.36	-1.41	-1.41
19	-1.46	-1.5	-1.5
20	-1.55	-1.59	-1.59
21	-1.63	-1.66	-1.67
22	-1.69	-1.73	-1.73
23	-1.75	-1.79	-1.79
24	-1.81	-1.84	-1.84
25	-1.85	-1.88	-1.88
26	-1.89	-1.92	-1.92
27	-1.93	-1.95	-1.95

28	-1.96	-1.98	-1.98
29	-1.98	-2.01	-2.01
30	-2.01	-2.03	-2.03
31	-2.03	-2.05	-2.05
32	-2.05	-2.07	-2.06
33	-2.06	-2.08	-2.08
34	-2.08	-2.09	-2.09
35	-2.09	-2.1	-2.1
36	-2.1	-2.11	-2.11
37	-2.11	-2.12	-2.12
38	-2.12	-2.13	-2.13
39	-2.12	-2.14	-2.13
40	-2.13	-2.14	-2.14
41	-2.13	-2.15	-2.14
42	-2.14	-2.15	-2.15
43	-2.14	-2.15	-2.15
44	-2.14	-2.16	-2.15
45	-2.15	-2.16	-2.15
46	-2.15	-2.16	-2.16
47	-2.15	-2.17	-2.16
48	-2.15	-2.17	-2.16
49	-2.15	-2.17	-2.16
50	-	-2.17	-2.16
51	-	-2.17	-

Appendix 11 Optical rotation values for reproducibility of HCl (2.507 M) catalyzed inversion of sucrose (10%) with the addition of sodium chloride (0.500 M, n=3, HCl-NaCl mixture at 29 °C).

Optical Rotation			
Time (min)	0.500 M (n=1)	0.500 M (n=2)	0.500 M (n=3)
3	4.02	3.88	3.81
4	3.17	3.15	3.03
5	2.5	2.46	2.39
6	1.93	1.89	1.83
7	1.44	1.37	1.3
8	0.96	0.93	0.88
9	0.58	0.56	0.51
10	0.23	0.2	0.16
11	-0.07	-0.09	-0.13
12	-0.32	-0.36	-0.38
13	-0.57	-0.58	-0.62
14	-0.74	-0.76	-0.8
15	-0.93	-0.95	-0.99
16	-1.1	-1.11	-1.13
17	-1.23	-1.24	-1.27
18	-1.35	-1.37	-1.38
19	-1.45	-1.47	-1.49
20	-1.54	-1.55	-1.57
21	-1.62	-1.64	-1.65
22	-1.69	-1.7	-1.72
23	-1.75	-1.76	-1.78
24	-1.81	-1.82	-1.83
25	-1.86	-1.86	-1.87
26	-1.89	-1.9	-1.91
27	-1.93	-1.94	-1.95

28	-1.96	-1.97	-1.98
29	-1.99	-2	-2
30	-2.01	-2.02	-2.03
31	-2.04	-2.04	-2.05
32	-2.05	-2.06	-2.06
33	-2.07	-2.08	-2.08
34	-2.08	-2.09	-2.1
35	-2.1	-2.11	-2.11
36	-2.11	-2.12	-2.12
37	-2.11	-2.12	-2.13
38	-2.13	-2.13	-2.14
39	-2.13	-2.14	-2.15
40	-2.14	-2.15	-2.15
41	-2.14	-2.15	-2.16
42	-2.15	-2.16	-2.16
43	-2.15	-2.16	-2.16
44	-2.16	-2.16	-2.17
45	-2.16	-2.17	-2.17
46	-2.16	-2.17	-2.17
47	-2.16	-2.17	-2.18
48	-2.16	-2.17	-2.18
49	-	-2.17	-2.18
50	-	-2.17	-2.18
51	-	-	-
52	-	-	-
53	-	-	-

Appendix 12 Optical rotation of HCl (2.507 M) catalyzed inversion of sucrose (10%) with the addition of more than one salt (each at 0.500 M from the preparation of sucrose-salt mixture) at 29 °C.

Optical Rotation		
Time (min)	NaCl-Na_2SO_4-sucrose + HCl	NaCl-Na_2SO_4-NaH_2PO_4-sucrose + HCl
3	3.88	4.11
4	3.1	3.43
5	2.52	2.84
6	1.88	2.32
7	1.34	1.8
8	0.9	1.38
9	0.5	0.97
10	0.16	0.63
11	-0.16	0.33
12	-0.41	0.04
13	-0.66	-0.19
14	-0.85	-0.42
15	-1.06	-0.61
16	-1.19	-0.79
17	-1.3	-0.94
18	-1.43	-1.08
19	-1.52	-1.2
20	-1.61	-1.31
21	-1.69	-1.41
22	-1.79	-1.49
23	-1.82	-1.57
24	-1.87	-1.64
25	-1.91	-1.7
26	-1.95	-1.76
27	-1.99	-1.8

28	-2.02	-1.85
29	-2.04	-1.89
30	-2.07	-1.92
31	-2.08	-1.95
32	-2.1	-1.98
33	-2.12	-2
34	-2.13	-2.02
35	-2.14	-2.04
36	-2.15	-2.06
37	-2.16	-2.08
38	-2.17	-2.09
39	-2.17	-2.1
40	-2.18	-2.11
41	-2.18	-2.12
42	-2.19	-2.13
43	-2.19	-2.14
44	-2.2	-2.15
45	-2.2	-2.15
46	-2.2	-2.16
47	-2.2	-2.16
48	-2.2	-2.17
49	-	-2.17
50	-	-2.17
51	-	-2.17
52	-	-2.17
53	-	-

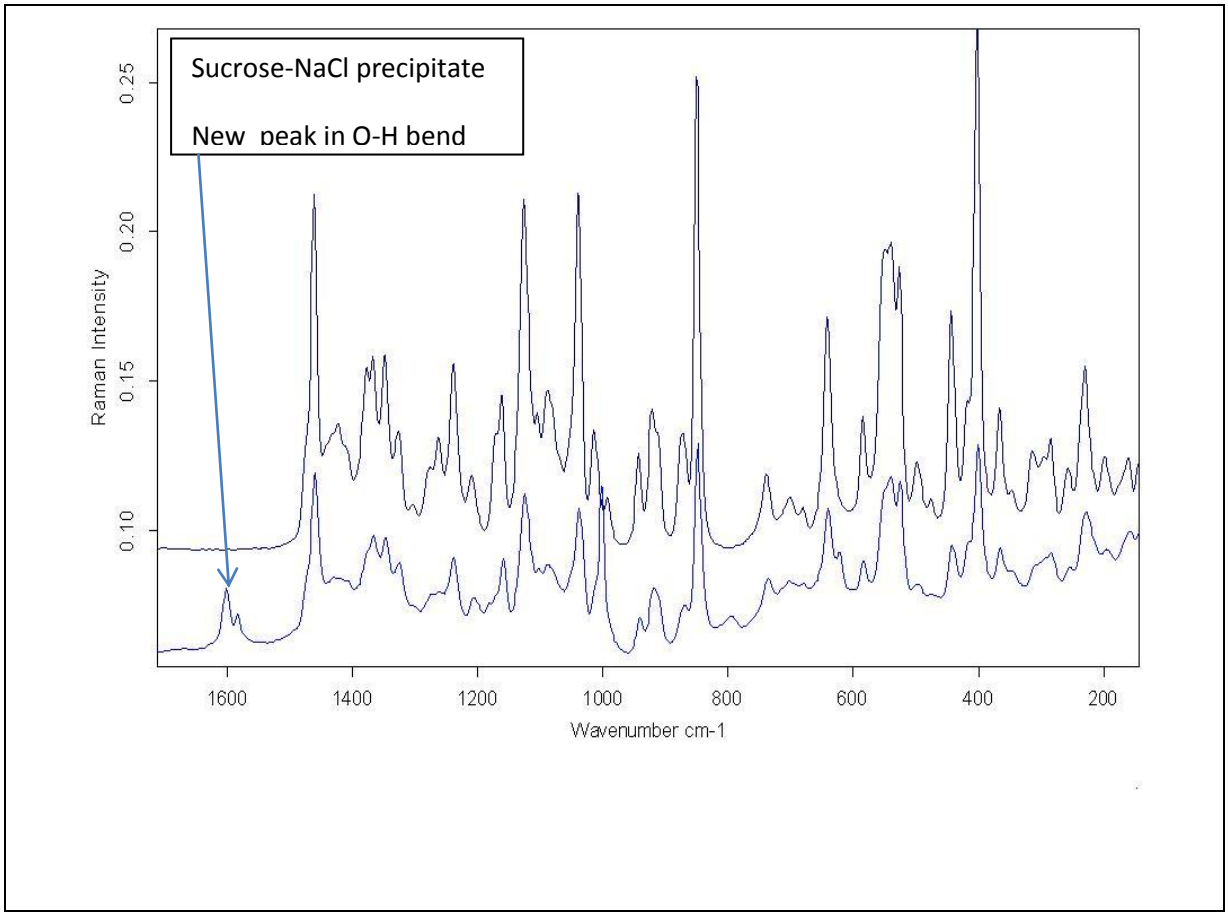
Appendix 13 Optical rotation values of the inversion of sucrose (10%) catalyzed by HCl (2.507 M) or H_2SO_4 (2.506 M) at 29 °C.

Time (min)	Sucrose + HCl	Sucrose + H_2SO_4
3	4.36	3.7
4	3.77	2.95
5	3	2.28
6	2.45	1.74
7	1.89	1.23
8	1.44	0.85
9	1.01	0.45
10	0.64	0.1
11	0.3	-0.19
12	0.01	-0.44
13	-0.23	-0.65
14	-0.46	-0.86
15	-0.61	-1.02
16	-0.82	-1.17
17	-0.97	-1.31
18	-1.11	-1.42
19	-1.23	-1.52
20	-1.33	-1.61
21	-1.42	-1.68
22	-1.51	-1.76
23	-1.59	-1.81
24	-1.65	-1.87
25	-1.71	-1.91
26	-1.76	-1.95
27	-1.81	-1.99
28	-1.85	-2.02
29	-1.88	-2.04
30	-1.92	-2.07
31	-1.94	-2.08
32	-1.97	-2.1
33	-1.99	-2.12
34	-2.01	-2.13

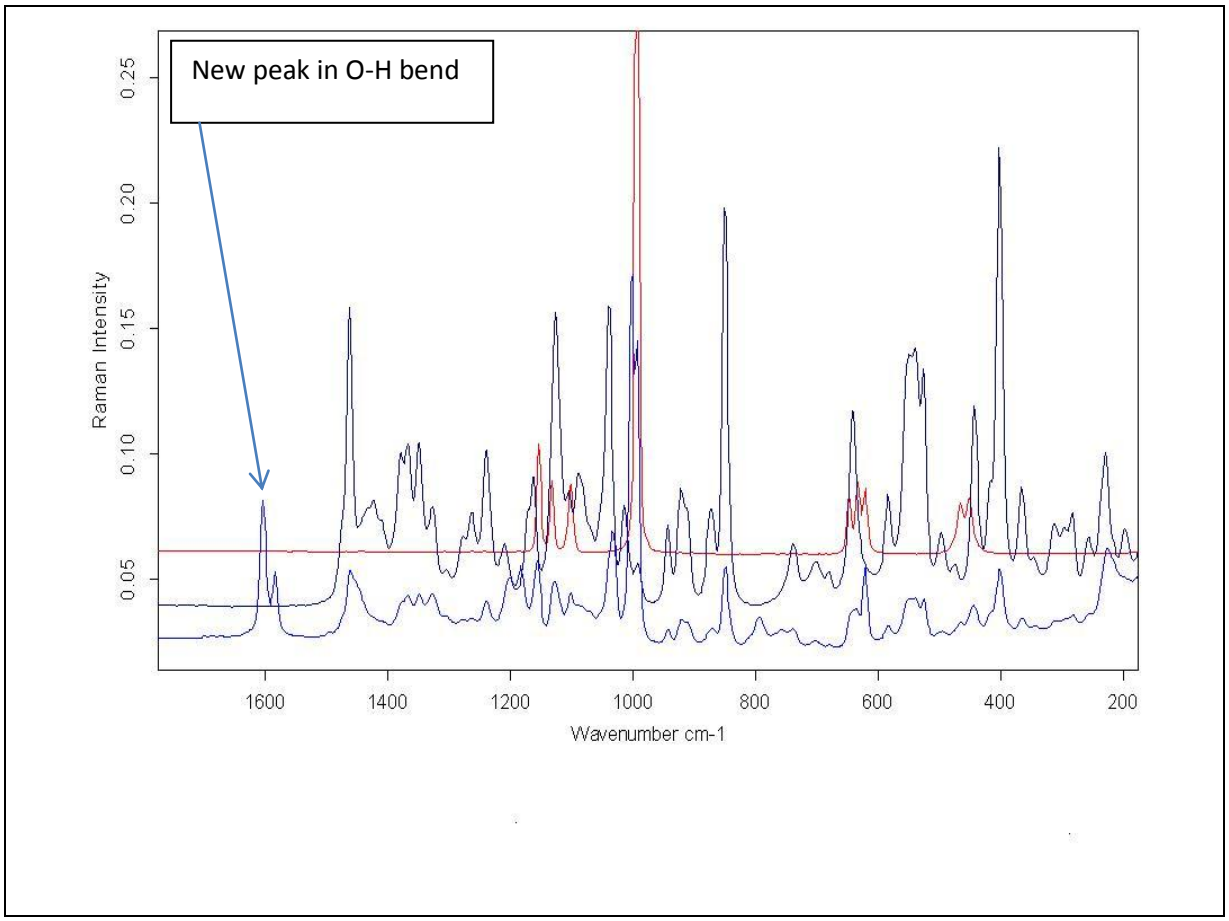
35	-2.02	-2.14
36	-2.04	-2.15
37	-2.05	-2.16
38	-2.07	-2.17
39	-2.08	-2.18
40	-2.09	-2.19
41	-2.1	-2.19
42	-2.1	-2.19
43	-2.11	-2.19
44	-2.12	-2.19
45	-2.12	-
46	-2.13	-
47	-2.13	-
48	-2.13	-
49	-2.13	-
50	-2.13	-
51	-	-

Appendix 14 The spectra showing the O-H bending vibrational region of sucrose-salt precipitates.

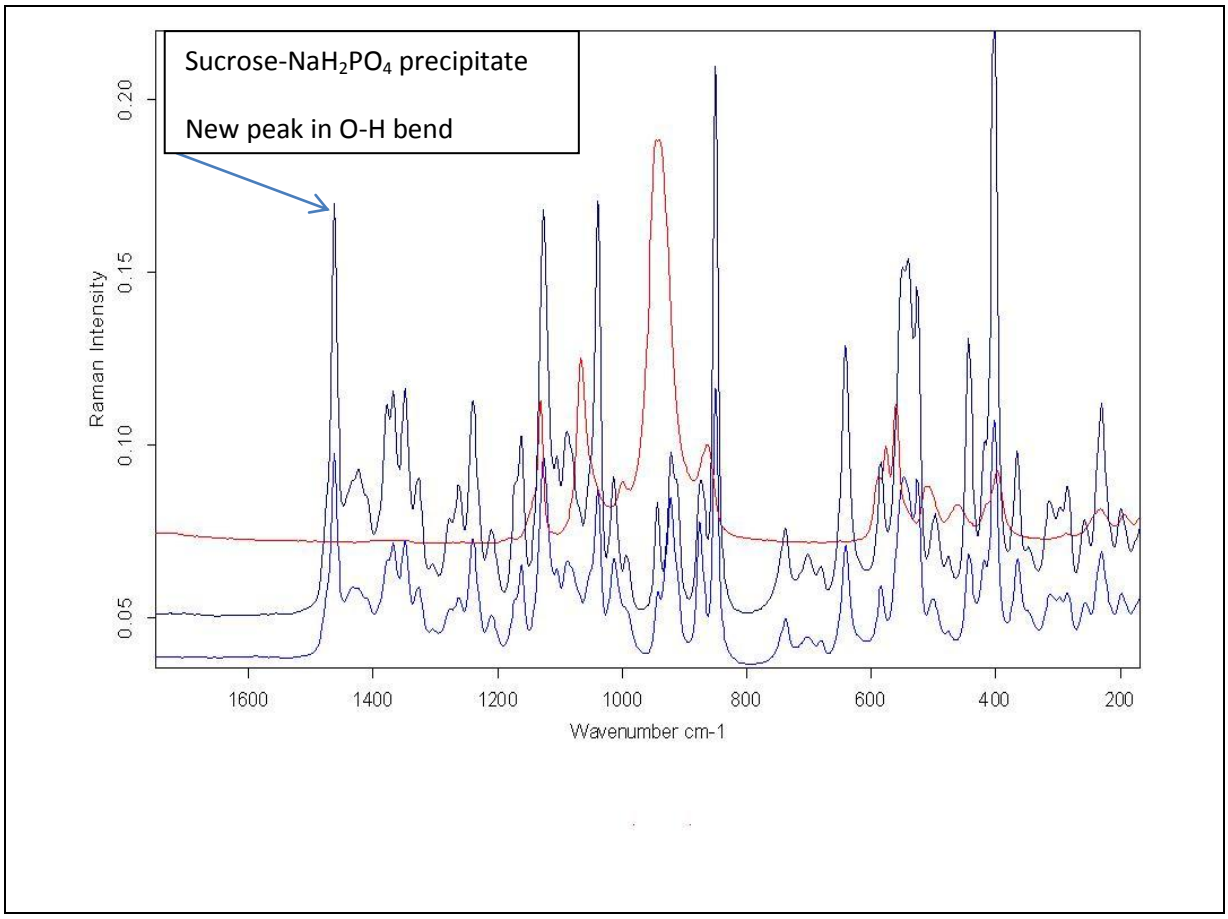
Sucrose-NaCl precipitate O-H bend



Sucrose-Na_2SO_4 precipitate O-H bend



Sucrose-NaH_2PO_4 precipitate O-H bend



Appendix 15 The values of log k versus $I^{1/2}$ for Guggenheim and Kezdy-Swinebourne methods.

Guggenheim Method

[NaCl]	*k*/s, SSM	ℓog *k*	*I*	$I^{1/2}$	*k*/s, ASM	ℓog *k*	$I^{1/2}$
0.050	0.002102	-2.6773	2.560	1.600	0.002085	-2.6808	1.600
0.125	0.002132	-2.6712	2.635	1.623	0.002085	-2.6808	1.623
0.250	0.002220	-2.6575	2.760	1.661	0.002137	-2.6701	1.661
0.375	0.002423	-2.6156	2.885	1.698	0.002192	-2.6591	1.698
0.500	0.002608	-2.5836	3.010	1.735	0.002335	-2.6317	1.735
0.750	0.002723	-2.5649	3.260	1.805	ND	ND	ND
$[Na_2SO_4]$	*k*/s, SSM	ℓog *k*	*I*	$I^{1/2}$	*k*/s, ASM	ℓog *k*	$I^{1/2}$
0.050	0.002422	-2.6158	7.670	2.769	0.002343	-2.6302	2.769
0.250	0.002300	-2.6382	8.270	2.876	0.002287	-2.6407	2.876
0.500	0.002235	-2.6507	9.020	3.003	0.002143	-2.6689	3.003
0.750	0.002178	-2.6619	9.770	3.126	0.001927	-2.7151	3.126

Kezdy-Swinebourne Method

[NaCl]	*k*/s, SSM	ℓog *k*	*I*	$I^{1/2}$	*k*/s, ASM	ℓog *k*	$I^{1/2}$
0.050	0.002204	-2.6567	2.560	1.600	0.002090	-2.6798	1.600
0.125	0.002109	-2.6759	2.635	1.623	0.002111	-2.6755	1.623
0.250	0.002222	-2.6532	2.760	1.661	0.002205	-2.6565	1.661
0.375	0.002394	-2.6208	2.885	1.698	0.002272	-2.6435	1.698
0.500	0.002737	-2.5635	3.010	1.735	0.002404	-2.6190	1.735
0.750	0.002423	-2.6156	3.260	1.805	ND	ND	ND
$[Na_2SO_4]$	*k*/s, SSM	ℓog *k*	*I*	$I^{1/2}$	*k*/s, ASM	ℓog *k*	$I^{1/2}$
0.050	0.002658	-2.5754	7.670	2.769	0.002618	-2.5820	2.769
0.250	0.002532	-2.5965	8.270	2.876	0.002542	-2.5948	2.876
0.500	0.002520	-2.5986	9.020	3.003	0.002425	-2.6153	3.003
0.750	0.002487	-2.6043	9.770	3.126	0.002290	-2.6402	3.126

Appendix 16 Precision measurements (n=16) of the rate of sucrose inversion (10%, mass/volume) catalyzed by HCl (2.507 M) in the presence of the added 0.250 M NaCl in sucrose or acid solutions.

Rate constants obtained by Infinite Time method	Statistics evaluation	
0.002693	Mean	0.002518
0.002466	Standard Error	1.56E-05
0.002494	Median	0.002466
0.002485	Mode	0.002466
0.002545	Standard Deviation	6.24E-05
0.002474	Sample Variance	3.89E-09
0.002548	Kurtosis	2.920534
0.002468	Skewness	1.497763
0.002458	Range	0.000235
0.002571	Minimum	0.002458
0.002573	Maximum	0.002693
0.002466	Sum	0.04029
0.002503	Count	16
0.002543	Largest	0.002693
0.002463	Smallest	0.002458
0.002545	Confidence Level (95%)	3.32E-05