

Specific Rotation of Aqueous Dextrose and Sucrose Under the Influence of Amino Acids Glycine and L-alanine Using LED Polarimeter

by

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Dedication

This work is dedicated to those who guided me throughout my life.

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List of Abbreviations

BOD – Biological Oxygen Demand

C – Concentration

CE – Capillary Electrophoresis

cm – centimeter

COD – Chemical Oxygen Demand

d – Dark band

D – Dextrorotatory

dm – decimeter

ee – Enantiomeric Excess

EPA – Environmental Protection Agency

g – gram

GC – Gas Chromatography

HPLC – High Performance Liquid Chromatography

l – Length

L- Levorotatory

LA-MB-FTMW – Laser Ablation-Molecular Beam-Fourier Transform Microwave

LC – Liquid Chromatography

M – molarity

mL – milliliter

mm/ha – millimeter/hectare

MS – Mass Spectrometry

Mt - Megatonne

OECD – Organization for Economic Co-operation and Development

T – Temperature

TDS -Total Dissolved Solids

Abstract

The sugar industry produces wastewater high in organic content that typically must be treated before it is discharged into streams or municipal sewer systems. Sugars and amino acids play an important role in cellular metabolism and thus flooding an environment with excess cellular fuel can result in increased bacteria in rivers and streams as well as oxygen depleted dead zones in marine environments. A quick and inexpensive approach to the detection of simple sugars in water would be helpful in localizing and identifying sources of excess sugars in the environment.

Polarimetry is potentially one option to detect sugars since most sugars are optically active. Unfortunately, organic compounds can interact with the sugar and greatly alter the polarimetry. This work studies the effect of amino acids such as glycine and L-alanine on the optical rotation of aqueous dextrose and sucrose. The optical activity of dextrose and sucrose solutions were measured in the presence of the amino acids glycine, and L-alanine, by a single wavelength LED polarimeter. The optical rotation was recorded in terms of absolute rotation and thereafter converted to specific rotation taking the solution concentration into consideration. It also has been observed that the pH of the solution mixtures was very minimally influenced by amino acid interaction with sugar solution demonstrating that there has been no major reaction between molecules within the solution. Glycine and L-alanine are optically inactive and optically active amino acids respectively. Experimental results showed that they decrease the specific rotation of sugar solutions. This research indicates that the interactions of amino acids with sugars in solution significantly impacts the specific rotation of the optically active sugars.

Therefore, to use polarimetry to detect concentrations of sugar in water, it is important to consider the interaction of other organic compounds such as amino acids that may also be present in the streams.

Chapter 1: Introduction

It has been estimated that a teaspoon contains 4.2 g of table sugar (*Sugary Drinks*, n.d.). A 12-ounce soda contains 8 to 10 teaspoons of sugar which means that a 12-ounce soda can contain 40 g of sugar (*Sugary Drinks*, n.d.). The amount of process water required to refine this sugar is very large: 38 liters of water is required to produce 454 g (*Sugarcane Farming's Toll on the Environment | Magazine Articles | WWF*, 2015). In the process, 20000 liters of wastewater is generated for 1000 kg of sugarcane processing (Macarie & Le Mer, 2006). Processed sugar, a part of many ingredients in prepared food, has been around since ancient times and has always been part of the dietary needs for energy as well as taste. Raw sugar is first processed from sugarcane and usually consists of yellow or golden-brown crystals. This raw sugar contains 98% sucrose, 1% moisture, and 0.5% of inorganic substances (Clarke et al., 1997). The raw sugar is then transported to refineries for further processing. In order to cater to the needs of the growing population, as per OECD (Organization for Economic Co-operation and Development) report, for over the course of the next decade, it is projected that 2000-2500 Mt of sugar will be produced from sugarcane (*saccharum officinarum*), and 100-450 Mt of sugar from sugarbeet (*Beta vulgaris subsp. vulgaris*) will be produced (OECD et al., 2020). It is reported that sugarcane crop is considered as one of the most water thirsty crops requiring 1500-2000 mm/ha/year water for different growth stages (*Sugarcane | Industries | WWF*, n.d.) whereas for sugarbeet crop it requires 550-750 mm/growing period, for the same harvest area as that of sugarcane, water is required (*Sugarbeet | Land & Water | Food and Agriculture Organization of the United Nations | Land & Water | Food and Agriculture Organization of the United Nations*,

2022). Ethanol, historically created as a petrochemical byproduct, is now considered a biofuel as it is primarily produced in ethanol distilleries. As a biofuel, ethanol is normally distilled within sugarcane refineries as sugarcane is one of the main raw materials in high demand (Association, n.d.). It has been estimated that for every liter of ethanol produced, 10-15 liters of spent wash is generated (Christofolletti et al., 2013). Waste is generated at various levels in sugar refineries and ethanol distilleries. According to EPA reports, pollutants released by the industry are both organic as well as inorganic compounds (EPA, 1997a) (EPA, 1997b). Discharge of these sugar loaded effluents, without treatment, into waterbodies can become toxic to the environment and can result in severe stress to aquatic as well as marine species.

Decomposition and Degradation of Sugars

Sucrose, also known as table sugar, produced in sugar refineries, degrades at pH 8.3 into D-glucose and D-fructose via acid hydrolysis mechanism (Clarke et al., 1997). Addition of lime juice in the sugar refinery process enables D-glucose and D-fructose to decompose further.

Under alkali medium or acid medium it can disintegrate and at pH of 5.0-6.0 starts to ionize and mutarotates (Clarke et al., 1997). At higher temperatures and lower pH of 3.0 or 4.0, the D-glucose undergoes enolization, a process in which a carbonyl compound is converted into an enol, and isomerization a process in which isomeric forms of compounds are produced. Further degradation of enediol ions leads to carboxylic acids (Clarke et al., 1997).

D-fructose in water under high temperatures degrades to produce some products such as 5-(Hydroxymethyl)-2-furaldehyde that condense into humin or D-glucose and can fragment into different types of acids such as formic acid, lactic acid, and 2-furaldehyde (Clarke et al., 1997). During sugar production in refineries at high temperature and in the presence of amino acids in

concentrations between 0.002 M to 0.008 M, the sucrose undergoes a Maillard reaction forming undesirable color in the product produced (Clarke et al., 1997).

Sucrose under acid conditions more readily converts to D-glucose and D-fructose than in alkali conditions. Under alkali conditions degradation of D-glucose and D-fructose is complex. The above-mentioned degradation processes should not be taken lightly nor are they limited. Sugar refinery processes and ethanol distillation processes involve several steps and use of chemical compounds. At different levels of refining steps, fresh water is regularly used for the process. The released water from the refineries can be of temperatures higher than normal, having high/low pH value, and contains several toxic chemicals.

Impact of Sugar Effluents

Effluents are discharged from processes such as milling, evaporators, clarification, and cleaning process. Effluents from sugar refineries needs to be treated before being discharged into the waterbodies. Sugar effluents contain Total solids, Total dissolved solids, suspended solids, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD), alkalinity, color, and odor. One of the several processes in the sugar refinery is the degradation of D-glucose into carboxylic acid (Clarke et al., 1997). Untreated water containing carboxylic acid can be very dangerous to the water bodies as they form acidic solutions that has lower pH and cause great harm to aquatic and marine species. Not thermally treating sugar effluents can lead to depleted dissolved oxygen levels. Toxic chemicals such as hydrogen sulfide are formed when sugar effluents are directly released into the waterbodies (A. Yadav et al., 2014).

It has been estimated that for every one tonne of sugar produced, an average of 900-2000 thousand liters of sugar effluents is generated by sugar refinery even with the presence of wastewater recycling facility within the establishment. (Macarie & Le Mer, 2006). Sugar

effluents products such as levoglucosan, produced during the burning of cellulose, and hemicellulose biomass are found in aerosols over ocean bodies rising concerns of impacts of biomass burning of sugarcane, and sugarbeet crops or wildfires (Medeiros et al., 2006).

Sugar Effluents Treatment and Analysis Method

Sugar refineries typically include onsite wastewater treatment facilities to treat effluents and recycle wastewater for reuse or to eliminate environmental contamination. Treatment process techniques include coagulation, flocculation, ultrafiltration, reverse osmosis, biological treatment of activated sludge, membrane bioreactor, and trickling filter (Singh et al., 2019). These processes reduce organic waste substantially and what is discharged is oxidized by natural processes occurring downstream.

However, many refineries suffer from aging infrastructure and quite often the influx of sugar effluents surpasses the capacity of treatment process. Untreated water discharged onto open land can have serious impacts on soil quality. Treated water should be released with a pH of 6.5 (Sahu, 2017) however effluents from sugar refineries have had pH as low as 4.0 during the production stage (Sultana et al., 2017). Low pH is a matter of serious concern as the hydrogen ion (H^+) or hydroxide ion (OH^-) can alter the biochemical reactions impacting aquatic or marine species (M. Yadav et al., 2021). Lower pH indicates the presence of carbon dioxide in high concentrations. Carbon dioxide upon its dissolution in water produces carbonic acid. Although carbonic acid (H_2CO_3) being a weak acid can dissociate into hydrogen (H^+) and bicarbonate ions (HCO_3^-). Imbalance of carbon dioxide concentration in waterbodies can kill aquatic or marine species (Boyd, 2020).

Some of the commonly used analytical methods to determine for sugar concentrations is effluent include Gas Chromatography (GC), High-Performance Liquid Chromatography

(HPLC), Capillary Electrophoresis (CE), Mass Spectrometry (MS), and Liquid Chromatography (LC) (Medeiros & Simoneit, 2007). These state-of-the-art techniques are commonly used in laboratory conditions to evaluate the quantity and type of organic compounds present in dry or wet samples. However, like any other technologies, these methods have their own disadvantages: high costs, high maintenance, skilled manpower, high power, and are not portable.

In the previous section on decomposition of sugars, we have seen how sugars can degrade into other compounds forming toxic products that are dangerous to ecosystem. It would be useful to measure for aqueous sugar compositions at the point source itself and make quick determination in the field. This research suggests the use of polarimetry, using the optical rotation of the solution, as one of the possible detection tools to evaluate sugar compounds in waterbodies.

Polarized Light

The polarization of light was first discovered by Christian Huyghens in the year 1690 (Shurcliff, 1962). In the year 1812 the optical rotation, wherein the polarized light is rotated by substances that possess optical active property, was discovered by D.F.J. Arago, a French scientist (Shurcliff, 1962). In 1815 another French scientist Jean Baptiste Biot discovered that certain chemical compounds possessed the ability rotate plane polarized light (Klein, 2021).

Light consists of propagating waves of electric and magnetic fields. This electromagnetic radiation is typically classified by its wavelength and frequency (M. Alonso 1921 & Finn, 1980). Visible light consists of electromagnetic wavelengths of 400 to 700 nm. Light, technically, is electromagnetic radiation in visible range in that wavelength or frequency is visible to human eye. Electromagnetic radiation of longer wavelength is lower in energy and results from thermal, microwave and radio emissions whereas shorter wavelength radiation result from higher energy electronic emission and includes UV, gamma, x-ray radiation. When the emissions consist of

waves of electric and magnetic fields with the orientation of the fields in multiple directions, it is called unpolarized electromagnetic radiation (Shurcliff, 1962).

The study of behavior of polarized light helps to understand the property of matter which inadvertently finds its uses in various applications (Shurcliff, 1962). A semi-transparent glass that polarizes light does so by filtering all except light that is polarized in one direction. There are numerous applications of light polarizers including sunglasses (Beeson & Mayer, 2008), rear view mirrors in automobiles (*Polarization in Imaging: Things You Did and Didn't Know It Could Do | Vision Systems Design*, n.d.), space applications (Cowen, 2006) and the quantification of chemicals in water (Landolt & Long, 1902).

An optically active molecule is one which contains a tetravalent atom such as carbon surrounded by four different functional groups. Such a molecule is referred as being chiral (Shallenberger & Birch, 1975); A property of molecular symmetry that requires four functional groups to form a nonsuperimposable mirror image. The nonsuperimposable mirror image chemical compound is called Enantiomer (Klein, 2021). Enantiomers tend to have the same physical properties, however, when they are subjected to plane polarized light one enantiomer will rotate light left and the other will rotate light to the right (Klein, 2021).

A polarimeter is an instrument that is used to obtain the specific rotation of an optically active solution (Landolt & Long, 1902). Specific rotation (Klein, 2021) is given by equation [1-1] as follows

$$[\alpha]_d^T = \frac{\alpha_o}{C * l} \quad [1-1]$$

Where, $[\alpha]_d^T$ is specific rotation at temperature T, and wavelength d-line, C is the concentration of solution in (g/mL) and l is the length of the polarimeter sample holder also called pathlength in unit decimeters (1.0 dm = 10.0 cm).

In the event of $T \neq 20^\circ\text{C}$, the following equation [1-2] (Bates & Jackson, 1915) can be applied

$$[\alpha]_d^{20} = [\alpha]_d^T + [\alpha]_d^T * 0.000461 (t - 20) \quad [1-2]$$

The specific rotation formula can be applied to optically active compounds to obtain the specific rotation. The observed rotation α_0 is directly proportional to concentration C and path length l . However, it varies at different conditions based on change in temperature (Kholmanskii & Strebkov, 2007). The observed rotation can also be different at different wavelengths of light. When a solution contains the same concentration of two enantiomeric compounds, it will become optically inactive, and the observed angle will be zero in this case. The mixture of enantiomeric compounds at same concentration is termed as racemic mixture (Klein, 2021). But in the case of racemic mixture of different proportions, the solution will be still optically active. The expression for Enantiomeric Excess (ee) (Klein, 2021), is given below in equation [1-3]

$$\% ee = \frac{[\alpha] \text{ of compound mixture}}{[\alpha] \text{ of pure enantiomer compound}} * 100\% \quad [1-3]$$

In order to perform all functions pertaining to the measurement of specific rotation, an important component of a polarimeter is the polarizer. This material performs the action of polarizing the light by filtering out all incident radiation that is not at a particular angle. Different polarizing material can be synthesized to suit the needs of a specific functional requirement. Polarizers can be built in layers and can filter color, wavelength, and field orientations etc. A schematic of a polarimeter is provided in Figure 1-1. The output of the polarimeter is recorded via PASCO SPARKvue software (*SPARKvue*® Software, n.d.).

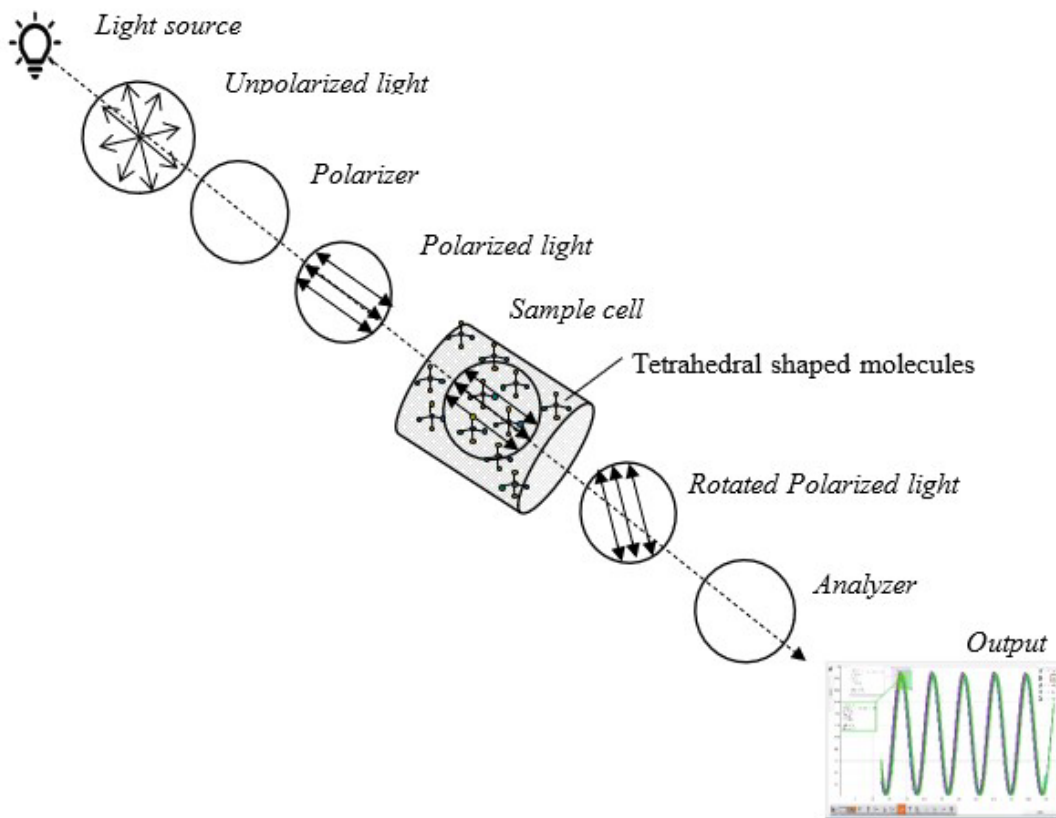


Figure 1-1: Schematic sketch of polarized light through sample cell.

Orientations of Electric Field of Light

There are three different types of polarization in which an electric field of polarized light is oriented; linear, circular, and elliptical (Kliger et al., 1990). The polarized light propagating in one direction is called linear polarization. When there are two linearly produced polarized beams of light, however they are perpendicular to each other with a phase difference of 90° . This electric field rotates circularly along the direction of propagation that is termed as circular polarization. The rotation of polarized light can be left-handed orientation or right-handed orientation. An elliptical polarization is the one in which the amplitudes of two linear polarized lights are not equal and they do not have a phase difference of 90° .

Mechanisms to Generate Different Types of Polarized Light

There are four different ways in which polarization mechanisms work (Kliger et al., 1990) namely dichroic, birefringence, reflection, and scattering. Each of these mechanisms has a specific function in the requirements for application of polarized light. When only orthogonally polarized light is needed then dichroic polarizer is used. Retarders (Kliger et al., 1990) such as quarter-wave plate or half-wave plate are used in case of two different mediums having different refractive indices. Reflection polarizers (Kliger et al., 1990) reflect orthogonal polarization. In scattered polarization (Kliger et al., 1990) unpolarized light is passed through slit where the light emerges partially polarized. Wave plates are features that are added to convert linearly polarized light into circularly polarized light.

Intensity

In an unpolarized light, the light waves are oriented in all directions. If the light along the x-axis is absorbed the intensity of the original light is reduced or lost. The intensity of the emerging polarized light I is given by [1-4] (Chaddha, 2014)

$$I = \frac{1}{2} I_o \quad [1-4]$$

Where I_o is initial light or unpolarized light. When unpolarized light passes through the polarizer the electric field parallel to the polarizer is transmitted and horizontal electric field is absorbed. This electric field parallel to the polarizer is vertically polarized and when passed through a second polarizer it is transmitted. However, when a horizontal unpolarized light is passed through polarizer it will not be transmitted by the second polarizer. In case when the polarized light falls on the polarizer, then the electric field vector is divided into two parts. The parallel electric field E_y is given by the formula [1-5] (Chaddha, 2014)

$$E_y = E \cos \theta \quad [1-5]$$

Horizontal electric field E_x is given by the formula [1-6] (Chaddha, 2014)

$$E_x = E \sin \theta \quad [1-6]$$

Where θ is the angle between the electric field and the direction of the polarized light from the polarizer. Since vertical polarized light is transmitted and horizontal polarized light is absorbed, the intensity of this transmitted polarized light is given by [1-7] (Chaddha, 2014)

$$I = E_y^2 \quad [1-7]$$

As Intensity is square of the amplitude.

Substituting equation [1-5] in [1-7], we obtain [1-8] (Chaddha, 2014)

$$I = E^2 \cos^2 \theta \quad [1-8]$$

This equation is known as Malus' law (Chaddha, 2014) and indicates that the intensity of the light is a function of the angle of the polarizer.

Polarimeter

The device to determine the optical rotation of the solution is called polarimeter. In a modern device component such as LED light source, polarizers, sample cell, a body unit attached to the encoder wheel and a USB connection are incorporated within the body of the polarimeter instrument's enclosure. One of the earliest built and simplest polarimeters is Biot (Mitscherlich) polariscope (Landolt & Heinrich, 1882). Currently there are simple as well as sophisticated polarimeters available on the market that serves purpose of identification of isomers in stereoisomer sample and enantiomer concentration in the samples. If a specific rotation of the sample is known, then its concentration can be determined via this device. A common

polarimetry application in the pharmaceutical industry is to determine the purity of enantiomeric solutions. Polarimeters can be modified to suit other purposes also.

Specific Rotation

Specific rotation for aqueous sucrose at 26.016 g /100 mL at 20°C, and wavelength (λ) of 589 nm is found to be 66.529° (Bates & Jackson, 1915). Landolt's interpolation formula for specific rotation α_d^{20} of sucrose (Bates et al., n.d.) is provided below in [1-9]

$$\alpha_d^{20} = 66.435^\circ + 0.00870C - 0.00023C^2 \quad [1-9]$$

For C between 0 to 65 g/mL. Specific rotation α_d^{20} for dextrose (Bates et al., n.d.) in water is given by [1-10]

$$\alpha_d^{20} = 52.50^\circ + 0.0188p + 0.00517p^2 \quad [1-10]$$

For p between 0 to 35 percent weight.

Since dextrose undergoes mutarotation, the specific rotation achieved at 9.1 g/mL solution and value is found to be +52.2° (Bates et al., n.d.).

Under the effect of acids such as HCl or H₂SO₄ at dextrose concentration of 5 g/mL, for concentrations of HCl at 3.65%, 30.4%, and 44.5%, the specific rotation values at between 16-17°C were +54.5°, +61.0°, and +97.5° respectively (Bates et al., n.d.). Interestingly, under the influence of H₂SO₄ at 22 N concentration, mutarotation is observed and found to have specific rotation at +72.5° for 50 g/mL dextrose solution. Effect on specific rotation of dextrose due to salts such as 4N NH₄Cl, or 2N CaCl₂ or 4N MgCl₂ were +51.2°, +56.0°, and +52.8° respectively. According to Levene and Rothen (Levene & Rothen, 1930), cations and anions in a salt solution has opposing optical rotation caused by deformation of optically active salts in opposite directions.

Sugars and Amino Acids

Dextrose and Sucrose

Sugars are a group of biomolecules also known as carbohydrates encompassing other nutrients such as vitamins, lipids, minerals, and fiber. These molecules are essential for sustaining life. Carbohydrates make up the structure of cells whereas sugars are the energy source for most living organisms on this planet (Shallenberger & Birch, 1975). Carbohydrates contain more than one hydroxyl group or carbonyl group at the end of a carbon (aldehydes) atom or at a second carbon atom (ketones) (Klein, 2021). The natural production of all carbohydrates occurs under photosynthesis action in plants that converts Carbon dioxide and water into carbohydrates as a means of cell construction and energy storage for the plant.

Carbohydrates that are sugars or saccharides are divided into four chemical groups classified as either mono, oligo-, di-, tri-, tetra-, penta- or poly saccharides (Klein, 2021). Monosaccharides are further classified as either aldoses or ketoses. Monosaccharides having carbonyl an aldehyde is an aldose and if the carbonyl is a ketone, then it is a ketose. Monosaccharides are also classified based on the number of carbon atoms present. Three carbon atoms are called trioses which they are the smallest monosaccharides (Klein, 2021). This smallest monosaccharide cannot be hydrolyzed. Four and five carbon atoms carbohydrates are termed as tetroses and pentoses, respectively. Examples of monosaccharides are arabinose, fructose, galactose, glucose, hexoses, mannose, and xyloses. Glucose is a hexose sugar, meaning that it has six carbon atoms in it. Di-saccharides are the composites of two monosaccharides attached by a glycosidic bond. Sucrose, and maltose are examples of disaccharides (Klein, 2021). For this experiment, the focus is limited to sugars sucrose and dextrose (D-glucose).

Glucose is found abundant in all living organisms. It is found in glucosides of plants species (Browne, 1912) through chemical reaction process such as hydrolysis and sometimes hydrolysis with acid or enzymes is converted to glucose. Glucose is also produced through di- and tri- saccharides hydrolyzed with acid (Browne, 1912). Alternatively, glucose can be produced by polysaccharides such as cellulose through the sulfuric acid hydrolysis reaction. Dextrin (Browne, 1912) found in starchy seeds upon reaction with dilute hydrochloric or sulfuric acid can be converted to produce glucose. Glycogen found in the liver, muscles, and other organs of animals, upon hydrolysis by acids can produce glucose. Glucose also goes by other names such as dextrose or diabetes sugar or D-glucose. The 'D' in D-glucose denotes dextrorotatory, its plane of rotation of polarized light is rotated to the right. Dextrose is the term that will be used throughout this paper.

In 1923, after successfully separating the dextrose mixture from a liquid syrup created by hydrolysis with corn or potato starch, dextrose was produced commercially on a large scale (Fellers et al., 1937). By the reaction of honey with 50% ethanol, glucose can also be manufactured. The easiest and most cost-effective approach is the hydrolysis of cane sugar with acid. Under fermentation process (Browne, 1912), glucose can yield some acids such as acetic, formic, and other acids. Decomposition of glucose with alkali yields d,l-lactic acid. Another important product formed by the reaction of glucose with alkali such as lime water can produce saccharin, an artificial sweetener (Browne, 1912).

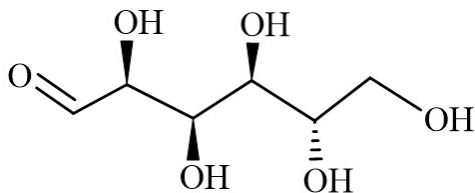


Figure 1-2: Structure of dextrose (D-glucose).

Sucrose, also known as table sugar, is not a hemiacetal ring nor an open chain compound (Ouellette & Rawn, 2018). It is joined by two sugar compounds: glucose and fructose. The IUPAC name for sucrose is β -D-fructofuranosyl- α -D-glucopyranoside (PubChem, n.d.). It is classified as a disaccharide under carbohydrates as it is linked by two monosaccharides units attached by a glycosidic bond (Ouellette & Rawn, 2018). The position of anomers of glucose and fructose at carbon-1 and carbon-2 results in the formation of hemiacetal ring. Therefore, sucrose does not undergo mutarotation phenomenon like glucose (Klein, 2021). Similar to glucose, sucrose (Browne, 1912) also undergoes hydrolysis by acid resulting in invert sugar. Fermentation process also enhances the inversion of sucrose. Certain enzymes such as invertase also results in the change of sucrose composition.

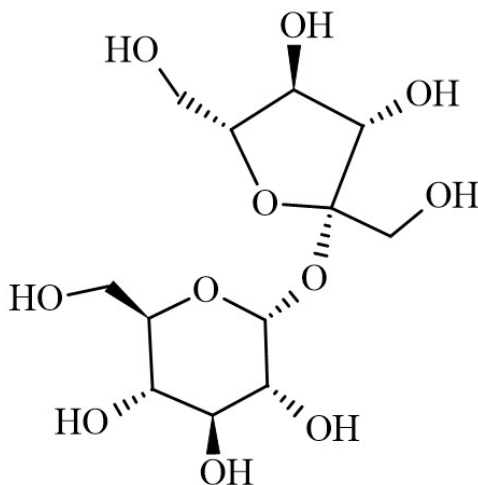


Figure 1-3: Structure of sucrose.

Due to the absence of anomers sucrose does not react with amino acids and proteins without enzymes and does not undergo inversion during the hydrolysis of enzymes such as amylases (Browne, 1912). While glucose, a monosaccharide, is widely found in abundance in nature under disaccharide sucrose is most abundant. Sucrose in the cytosol region of sugar cane

or sugar-beet leaf after the action of few intermediate enzymes such as aldolase, hydrolysis by fructose-6-phosphate and action of phosphatase is released to be transported to other parts of the plant's tissue (Browne, 1912). Sugar is metabolized in the sugar cane through sucrose synthase (Stein & Granot, 2019).

Mutarotation

When an optically active compound is dissolved in a solvent there is a period required for the specific rotation to attain equilibrium. The term for this intermolecular readjustment to the solvent is Mutarotation (Pigman & Isbell, 1968). The phenomena are attributed to the exchange of cyclic hemiacetals with the open chain form in a solution. Therefore, dextrose undergoes mutarotation whereas sucrose does not undergo mutarotation due to absence of cyclic hemiacetals in its ring structure (Ouellette & Rawn, 2018). Mutarotation is observed commonly in reaction such as isomerization modification into another isomer, hydrolysis, acid hydrolysis, inversion, and migration of acyl group such as esters or amides. The kinetics of (Pigman & Isbell, 1968) mutarotation process is readily observed through polarimetry although several other methods exist such as infrared absorption, calorimetric methods, gas chromatography, and solubility. Under the latter one isomer is in excess during the solubility of sugars. The equilibrium constants and optical rotation of sugar compounds in unknown crystalline phase can also be evaluated through mutarotation formula (Pigman & Isbell, 1968) provided below in [1-11]

$$[\alpha] = A * 10^{-m_1 t} + b \quad [1-11]$$

Where b is rotation observed during the equilibrium phase, m is 0.43429, A is the observed rotation obtained based on the difference between initial and final rotation.

Maillard Reaction

A browning color in sugar was first observed by French scientist Louis-Camille Maillard (Amaya-Farfan & Rodriguez-Amaya, 2021). At ambient temperatures, Maillard reactions are more pronounced in stored semi-hydrated or dehydrated protein foods. Glycine and dextrose reaction is one of the simplest Maillard reaction involving a reducing sugar and amino group (Davies et al., 1997). Compared to the mixture of reactants involving glycine, dextrose, and sucrose, it has been observed (Amaya-Farfan & Rodriguez-Amaya, 2021) that sucrose is not a reactant as it is not a reducing sugar like dextrose. Under acid catalyst, it can breakdown forming dextrose and fructose and thereby becoming involved in the Maillard reaction. In aqueous solution, glucose at pH of 6.0 remains a clear transparent solution. As the pH is increased to 8.0 and following a time lapse of 20 minutes, the solution turns brown in color.

Glycine and L-alanine

Of all the properties of twenty-five amino acids, except for glycine and amino malonic acid, can be distinguished by their specific rotation (J. P. 1902-1959 Greenstein & Winitz, 1961). Optically active amino acids have at least two enantiomeric isomers. These enantiomers have the ability to rotate the plane polarized light right or left and have a constant specific rotation at a given concentration. It was proposed that (+) or (-) signs to be used to indicate individual isomer direction of rotation of polarized light. Based on optical rotatory values, 'D' and 'L' were designated to be used as configuration identity of amino acid. Racemic mixtures, equal amount of (+) and (-) enantiomers, is known to make the compound optically inactive. Compounds in organic matter consisting of one or more amino constituents are termed as amino acids. Amino acids are classified into three types: aliphatic, aromatic, and heterocyclic (J. P. 1902-1959 Greenstein & Winitz, 1961). In this experiment the specific rotation resulting from the mixture

of a non-enantiomeric amino acid and an optically active amino acid and optically inactive amino acid with the optically active dextrose and sucrose is studied.

Glycine, one of the twenty-five amino acids that does not rotate plane polarized light, was first isolated from gelatin derived from animals. It is an amphoteric substance that can act as an acid in a basic solution and act as a base in the presence of acidic solution. It is widely distributed throughout both plant and animal tissue. It is estimated that among animal protein, glycine accounts for 10% by total mass of the protein (J. P. Greenstein & Winitz, 1961). Several procedures are adopted to obtain glycine. One of the earliest methods involved isolating glycine from hydrolyzing protein with hydrochloric acid (HCl). In some procedures, sulphuric acid (H₂SO₄) was used for isolating glycine from protein. Glycine has also been isolated from silk fibroin; a protein found in several insects. Treatment of silk fibroin with 5-nitronaphthalene-1-sulfonic acid as also yielded glycine (J. P. Greenstein & Winitz, 1961).

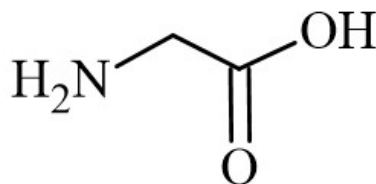


Figure 1-4: Structure of glycine.

L-alanine, an optically active amino acid, rotates the plane polarized light to the left as designated by the symbol provided beside the name. Alanine was first hydrolyzed by Weyl in the year 1888 from silk fibroin, and consequently became a part of protein family (J. P. Greenstein & Winitz, 1961). Just like any other optically active compound, L-alanine has four functional groups, and the configuration of L-alanine is asymmetric. The enantiomer D-alanine is produced naturally by Escherichia Coli. Through the ester distillation process by Emil Fisher, alanine presence in other proteins were shown through the esterification of products obtained through protein hydrolysis in acid and subjecting it to fractional distillation (J. P. Greenstein & Winitz,

1961). Hydrolysis of the ester at low boiling process produced a pure optically active amino acid. The application of this procedure on silk in the year 1901 gave rise to L-alanine amino acid (J. P. Greenstein & Winitz, 1961).

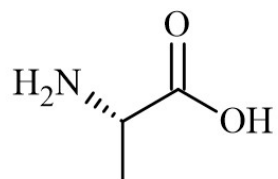


Figure 1-5: Structure of L-alanine.

Conformations

It is essential to understand the conformation of chemical compounds of interest in this research. The conformation of molecules can reveal out certain interesting information. An effort is made to introduce a brief outline of conformations of dextrose, sucrose, glycine, and L-alanine.

Dextrose Conformation

Alonso et al. (J. L. Alonso et al., 2014) have been able to explain the conformation of gas phase structure of D-glucose through LA-MB-FTMW spectrometer (Laser Ablation-Molecular Beam-Fourier Transform Microwave). The OH groups attached to the carbon atoms in the rings of both anomers adopts gauche conformations. The α anomer of D-glucose have one of the carbon atom ring configurations with anomeric of OH group in axial position. Branched chain of hydrogen bonds have hydroxyl groups in equatorial position. From their study, it has been thought that conformation at dihedral angle position has shown that it has a different orientation and have preferences for gauche configurations. In their work (J. L. Alonso et al., 2014), under gas-phase experiments, it was observed that trans configurations had more energy than gauche

configuration. They have been able to explain the difference in structure and stability of D-glucose anomers under different phases.

Sucrose Conformation

Researchers Brown et al. (Brown & Levy, 1963) adopted neutron-diffraction analysis in order to determine the conformation of sucrose crystals. The molecules packed in sucrose is determined by the hydrogen bonds such as OH, of which some of them are asymmetric, some of the hydrogen bonds are placed in position due to contacts with other molecule's oxygen atoms. From the figure [1-3] sucrose is comprised of furanose, and pyranose rings linked by glycosidic bond. The bonds in the furanose rings are explained by the angle of twist. The conformation angle in furanose rings for some of the C-C bonds are $+34.9^\circ$, -27.4° , -31.0° . For the O-C bond is $+8.3^\circ$, and C-O bond is $+14.5^\circ$. The pyranose ring of sucrose adopts chair conformation with OH and CH₂OH functional groups occupying an equatorial position. One of the oxygen atoms, and 5 hydrogen atoms occupy axial position in the pyranose ring. Conformation angle in this ring ranges between $+54.5^\circ$ to 55.5° . The C-O bond length across the ring is larger than the C-O bond adjacent to the glycosidic link. They have reported bond length of C-H to be between 1.08 – 1.11 Å. No value is provided for the O-H bond length although they have, with slight uncertainty, determined that this length is largest compared to others.

Glycine Conformation

Hu et al. (Hu et al., 1993) determined the glycine conformation through self-consistent field (SCF) method, a Hamiltonian iterative process that solves Schrodinger equation to gather different glycine conformers relative stabilities, rotational constants, and vibrational frequencies. Glycine conformers are explained through COOH group, lone pairs of nitrogen bonded to hydrogen and as well as attached to the COOH hydrogen bond forming a ring, hydrogen bond

with a lone pair of nitrogen atom attached to two hydrogen atom, and hydrogen bond with the oxygen atom between on the carbonyl and OH group. They have concluded that COOH functional group, and lone pair of nitrogen bonded to hydrogen and carboxyl hydrogen bond to be important than the other conformations in glycine. In the same breadth they have also been able to determine that the lone pair of nitrogen bonded to hydrogen and also to the carboxyl hydrogen atom, also found in alanine, another amino acid of interest in this study, to be more stable than just COOH group conformation. They have also showcased that NH₃ functional group to be a good hydrogen acceptor and that carbonyl oxygen group to be better hydrogen acceptor than carboxyl oxygen group.

L-alanine Conformation

Kikuchi et.al (Kikuchi et al., 1997) used AB initio method, a method to solve Schrödinger equation, to study the conformation of L-alanine zwitter ions in aqueous solution. This group through their study have been able to determine the stable conformation for L-alanine zwitter ion in aqueous solution. They attributed the difference in energy due to differential solvation, stabilization effect on equilibrium caused by either products or reactants, at different torsional angles. The interactions of oxygen atom in carbon dioxide and hydrogen atom in methyl bond is due to the rotation ability of carbon dioxide at one of the torsional angles. Bond lengths such as C-H, N-H, C-C, C-N, C-O across the L-alanine zwitter ion ranged from 1.084 to 1.531 Å. The bond angles for CCH, CNH, CCO, NCC, and CCC ranged from 110.7° to 116.2°.

Chapter 2: Objective

In order to use polarimetry to detect sugar compounds in wastewater it is important to understand the interactions of certain chemical compounds that can be expected in treated or untreated wastewater systems. For this reason, the role of polar organic molecules and their impact on the specific rotation of the sugar solutions under the influence of amino acids in varying concentrations are studied. Due to the sensitivity of polarimetry, eliminating external factors such as temperature, the influences of pH along with impurities that can influence the specific rotation is crucial. Four interaction systems were studied in this work:

1. To observe the specific rotation behavior of D-glucose, a simple dextrorotatory compound, in the presence of the achiral molecule compound glycine.
2. To observe the specific rotation of D-glucose with L-alanine, a levorotatory as well as an optically active compound.
3. To observe the specific rotation of disaccharide sugar such as sucrose which upon hydrolysis yields D-glucose and D-fructose in its interactions with glycine.
4. Observe the specific rotation of sucrose in its interaction with L-alanine. Sucrose and L-alanine are optically active compounds which rotate the polarized light to the right and left respectively.

Hypothesis

In order to achieve our objectives, it is necessary to state the possibility of select ambiguities to be expected. Some of the questions to be addressed include:

1. How does the interaction of dextrose with glycine affect the specific rotation? How does increasing the concentration of glycine alter the specific rotation of dextrose?
2. Does the interaction of dextrose with the amino acid L-alanine cancel the specific rotation?
3. What is the impact of the polarity of the molecules on the specific rotation?
4. Does the specific rotation in the interaction of sucrose-L-alanine show any variations in specific rotation due to the fact that L-alanine, unlike glycine, is optically active and does it cancel out the optical rotation or any trend is observed?

Chapter 3: Literature Review

Although a natural product of plants and a common metabolic substance for all living things, excess sugar in the environment can be quite detrimental. One of the earlier studies such as the one conducted by Wolff et al. (Wolff & Price, 1960) in their experimental trials, the addition of sugars such as glucose and sucrose on leaves with water supply but no sunlight has shown that these sugars support in the synthesis of plant's chlorophyll. This study showed that plants by oxidizing these sugars undergo endogenous respiration without sunlight. A recent study by Qingxu et al. (Ma, Ma, et al., 2017) (Ma, Cao, et al., 2017) has shown that for plants such as pakchoi (*Brassica Chinensis L*) externally supplied sugars such as glucose and sucrose devoid of microorganisms increase the intake of nitrogen from the rhizosphere. Therefore, showing that these sugars, at optimal concentrations, are indeed necessary for uptake of amino acids present in the soil. However, excessive accumulation of sugars such as sucrose can have a negative impact on growth and flowering time in plant species such as Arabidopsis. The reason for the delayed flowering is explained by Masa-aki Ohto et al (Ohto et al., 2001). The effect of glucose accumulation and the effect on the Arabidopsis growth rate has been well explained by L. Zhou et al. (Zhou et al., 1998) as well. A recent study by Reischke et al. (Reischke et al., 2015) on the bacterial growth in soil has shown that accumulation of glucose led to growth of bacterial activity in the soil. It is not unnatural to find sugars in soils. In fact, some of the types of sugars found in soil (Gunina & Kuzyakov, 2015) are plant sugars, root exudates, sugars from soil organic matter, and dissolved organic matter. The primary source of sugars such as cellulose consisting of mostly glucose to the soil are from the decomposition of plants. Plants not only

release carbon to the soil through rhizodeposition but also sugars. In roots of the plants such as wheat, alfalfa, and peas release 46-52% of sugars. Glucose being the dominant sugar released by the root of these plants followed by fructose and ribose. Microorganisms also produce sugar obtained by carbon from plant litter on the ground. Bacteria, and fungi consisted mainly of ribose sugars. Insect's excreta also act as source of sugars but compared to other sources, they are significantly low.

In one study of pathogens by McKeen (McKeen, 1956), interaction of glucose with glycine was responsible for the growth of fungi such as *Phytophthora fragariae*, a leading cause for disease in strawberry and raspberry fruits. In addition to naturally occurring, sugars are also manufactured by processing sugarcane, sugarbeet, potato, and corn extracts. Given both the global population increase and the expanse of industrial production, it has been estimated that by the end of the year 2030, as per OECD report, 2500 Mt of sugars will be produced around the world (OECD et al., 2020). Processing of sugars from the sugarcane or sugar beets includes several stages requiring large amounts of fresh water through various processing stages. These fresh water after usage becomes very polluted and is not fit to be discharged into the environment without treating it for the effluents. Studies by Hashem et al. (Hashem et al., 2015), Sultana et al (Sultana et al., 2017), Vaithyanathan et al. (Vaithyanathan & Sundaramoorthy, 2017), Fito et al. (Fito et al., 2019), Chavan et al. (Chavan et al., 2016) , and Omwoma et.al (Omwoma et al., 2014) , conducted across the world, have detailed the consequences of the sugar effluents into the environment. The common takeaway from their studies is that the surrounding bodies of water are most vulnerable from this pollution not only rendering them non-potable but also endangering all aquatic species. Therefore, it would be quite practical to have a portable, cost effective, and robust device that can detect sugar compounds in the field itself rather than

having to test in the laboratories. Since sugars have a fairly unique property of rotating plane polarized light, polarimetry offers a simple and plausible option. Given that optical polarimetry has many applications in the field of medicine especially in the development of non-invasive glucose monitoring devices on real-time, the same principle can also be deployed in the study for detection of sugars in treated or untreated water. In the field of medicine, polarimetry was tried on aqueous humor of the rabbit's eye. In this experiment by Rabinovitch et al. (Rabinovitch et al., 1982), through a custom built polarimeter, aqueous humor from the rabbit's eye was withdrawn and placed in the sample cell to determine the blood glucose concentration and were successfully able to obtain optical rotation of glucose concentration at hyper- and hypo-glycemic range. Over the next two decades, several studies such as determination of glucose in cell culture media by Cote et al. (Cote et al., 1992), Lin et al. experiments (Lin et al., 2015) on detection of glucose with scattering samples, detection of glucose in turbidity and human tissues were also conducted through optical polarimetry. Pravdin and company (Pravdin et al., 2016) in their experiment tried to study glucose concentration on human skin. However, they concluded that optical activity of glucose on skin cannot be determined at the required range. Limitations cited in their study can be of importance in future works as it the ability of polarimetry to obtain optical rotation of very low sugar concentration levels mixed with other optically active compounds at higher concentration levels.

It has been acknowledged that the study of optical rotation on sucrose was studied in detail by Bates et al. (Bates & Jackson, 1915). They were able to show that the specific rotation of sucrose is $+66.5^\circ$. This value is widely acknowledged as the accepted experimental value. According to the report from Saccharimetry by Bates et al. (Bates et al., n.d.), specific rotation of dextrose has been determined to be $+52.48^\circ$. Even though these specific rotations were

determined, some of the early reports predates these values are very enlightening to study. Many studies have been conducted to understand the specific rotation of aqueous solutions under various conditions such as Silverman et al. (Silverman et al., 1996) study on influence of certain substances having varying scattering properties in aqueous glucose. Through the study of molecular rotations by Gorin et al. (Gorin et al., 1939), Greenstein et al. (J. P. Greenstein et al., 1953), and Isbell et al. (Isbell, 1929), optical rotation of sugars and amino acids under the influence of different compounds were studied theoretically. One of the most complex studies that involve in the interaction of chiral molecules under different set of conditions have been shown by Craig et al. (Craig et al., 1971) This interaction of chiral molecules and their specific rotation characteristics is of interest in this research.

Specific rotation of sugars such as dextrose, and sucrose in its interactions with amino acids such as glycine, and L-alanine is important. There has been no literature work wherein the specific rotation of aqueous sugar solutions with amino acids such as glycine, and L-alanine is studied or reported. Li et al. (Li et al., 1999) have studied the enthalpy property of glycine in aqueous dextrose and sucrose. In this study, enthalpy coefficients associated with the interactions between hydrophilic-ion group between OH group of sucrose and glycine had negative value whereas interactions between hydrophilic-hydrophobic group between OH group of sucrose and glycine had positive enthalpy coefficient. Interactions of polar amino acid L-alanine in aqueous sucrose, and aqueous glucose was studied by Riyazzudeen and company (Riyazuddeen & Usmani, 2012) through measurement of density and speed of sound of amino acid L-alanine in aqueous sucrose and dextrose under different concentrations of L-alanine. Sand et al. (Li et al., 2002) have studied the partial molar volumes of glycine and L-alanine in aqueous glucose. They have explained some of the interactions between glucose and amino acid molecules: hydrophilic-

ion group, hydrophilic-hydrophobic group, and hydrophilic-hydrophobic group interactions between OH group of dextrose and zwitter ions center, OH group of amino acid, and non-polar group of amino acid respectively. Volumetric properties of L-alanine in aqueous sucrose were studied by Pal et al (Pal & Kumar, 2005).

Chapter 4: Research Methods

Solutions of a dextrose and sucrose from 0.1 M – 0.9 M are prepared, and aliquots of 25.00 mL are drawn. After preparation, dextrose solution was left undisturbed for 10 hours or more. All solutions were prepared in volumetric flasks and were stoppered to limit atmospheric carbon dioxide from absorbing into the solution. Following addition of an amino acid, a period of 10 minutes rest time is given between each solution to allow for complete mixing and any mutarotation of sugar solution with amino acid. Glycine, and L-alanine are the two amino acids used in this study. Temperature and pH were measured for each solution mixture following the 10-minute rest period. Polarimeter readings were taken within 2-3 hours of each sample preparation. Temperature of all solutions were measured before readings and maintained between 22.0- 24.0°C.

Polarimeter

Polarimetry measurements were performed using a PASCO PS-2235 scientific polarimeter. The sample cell length is 1 dm and the output LED light source of wavelength is 589 nm. Two lenses are provided: one attached to LED enables the light to travel along the length of the sample cell axis, and another lens placed in front of LED amplifies the light in the sample cell. The sample cell is filled with the solution and covered by a polarizer plate, washer, and steel ring. Polarizers provided in the polarimeter is made of quartz material. The polarimeter generates pulse of incoming polarized light in such a way that the pulse detected by the encoder is magnified to 4000 cycles per revolution. Polarized glass between the sample and detector is manually rotated allowing the intensity of the light to vary with angle of rotation.

The polarimeter software, SPARKvue, allows for easy determination of the angle of polarization of the solution.

Materials

Chemicals

β -D-Fructofuranosyl- α -D-glucopyranoside ($C_{12}H_{22}O_{11}$ Sucrose) (99.9%), white solid crystals, was purchased from Fisher Scientific. Dextrose ($C_6H_{12}O_6$ glucose), anhydrous reagent grade of high purity, was purchased from Carolina Biological Supply company. 2-Aminoacetic acid ($C_2H_5NO_2$, Glycine), white powder, from Sigma-Aldrich was used. (2S)-2-aminopropanoic acid ($C_3H_7NO_2$, L-alanine) white crystals was also purchased from Sigma-Aldrich.

Measurements

The observed rotation is calculated as an average of several light intensity plots obtained from SPARKvue software. The observed rotation (*Polarimeter • PS-2235*, 2019) is represented by the difference between the reference angle measured only with distilled water and the angle measured with the sugar solution is given by [4-1].

$$\alpha_o = \text{Solution angle} - \text{reference angle} \quad [4-1]$$

Specific rotation (Klein, 2021) is calculated based on the formula provided below in [4-2]

$$[\alpha_{SR}] = \frac{\alpha_o}{C * l} \quad [4-2]$$

Where C is concentration in Molarity (M) and l is length of the sample cell (dm).

$[\alpha_{SR}]$ is the specific rotation. pH of the solutions and weight of the sample was measured using pH/Ion meter S220, and analytical balance from of Mettler Toledo AL 204 model brand was used.

Chapter 5: Results

The optical rotation of dextrose and sucrose as a function of concentration in molarity are shown in Figure 5-1 and Figure 5-2 respectively. The experimental data indicates a linear relation between optical rotation and concentration and this result is consistent with available literature (Bates et al., n.d.). Specific rotation of the sugar solutions at fixed concentrations with varying concentrations of the amino acids: glycine and L-alanine are provided in Figure 5-3, Figure 5-4, Figure 5-5, Figure 5-6, Figure 5-7 and Figure 5-8.

The published value of specific rotation of 1.0 M aqueous dextrose at 20°C is +52.7° (Bates et al., n.d.). Under constant room temperature and sample cell length, during experiment conducted, the specific rotation of aqueous dextrose at 1.0 M was +52.1°, its observed rotation is shown in Figure 5-1. A difference of 0.6° even after adjusting the temperature by applying temperature coefficient formula [1-2] did not show significant variation in specific rotation. Another variable, the concentration difference between the specified and experimental values, might have caused the slight difference. However, in the case of specific rotation of aqueous sucrose, experimentally obtained value had a difference of +7°. The specific rotation value obtained was +59° and its observed rotation shown in Figure 5-2, whereas the literature value is +66.6° at concentration of 0.260 g/mL (Bates & Jackson, 1915) at 25°C.

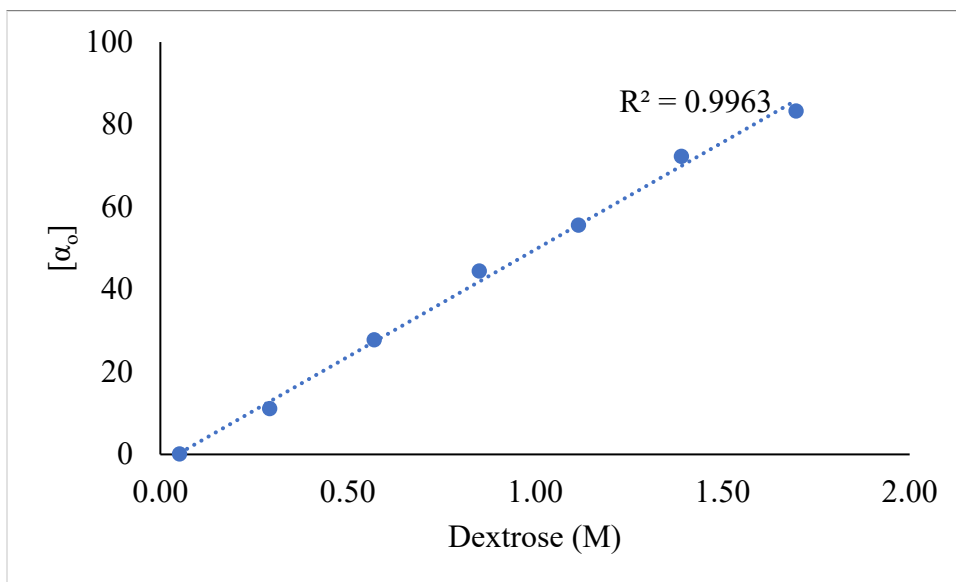


Figure 5-1: Optical rotation of aqueous dextrose for concentrations between 0.10 M - 2.00 M.

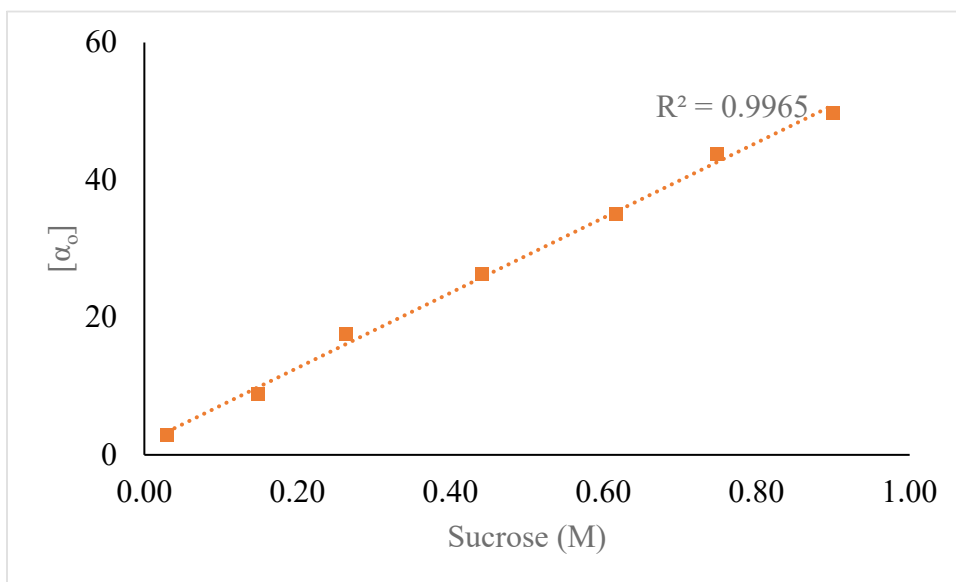


Figure 5-2: Optical rotation of aqueous sucrose for concentrations between 0.03 M - 0.90 M.

Table 5-1: Optical rotation values obtained under different dextrose and sucrose concentrations.

| Dextrose Concentration (M) | Optical rotation (°) | Sucrose Concentration (M) | Optical rotation (°) |
|-----------------------------------|-----------------------------|----------------------------------|-----------------------------|
| 1.70 | +83.3 | 0.90 | +49.7 |
| 1.39 | +72.2 | 0.75 | +43.8 |
| 1.12 | +55.5 | 0.62 | +35.1 |
| 0.85 | +44.4 | 0.44 | +26.3 |
| 0.57 | +27.8 | 0.26 | +17.5 |
| 0.29 | +11.1 | 0.15 | +8.8 |
| 0.05 | +0.0 | 0.03 | +2.9 |

Dextrose-Glycine Interaction

At aqueous dextrose concentration of 0.3 M from Figure 5-3 the specific rotation was observed to be +58°. When the glycine concentration was added to 0.1 M the specific rotation dropped from +58° to +48° and to 0.7 M of glycine, the specific rotation was +29°.

The specific rotation of aqueous dextrose concentration of 0.6 M from Figure 5-4, obtained from the experiment, is +48°. When 0.1 M of glycine concentration was added to the dextrose solution, the specific rotation changed from +48° to +43° and at 0.7 M of glycine, the specific rotation of the solution drastically reduced to +32°.

In Figure 5-5, At 0.8 M the aqueous dextrose concentration the specific rotation shown is +46°. When glycine concentration was increased from 0.1 M to 0.7 M, the change of specific rotation was from +43° to +35°.

Sucrose-Glycine Interaction

Evaluation of the sucrose-glycine combination was conducted in a similar fashion to dextrose-glycine. Unlike dextrose, which had conditions properly stated by the manufacturers the specific rotation (*Dextrose, Anhydrous, Reagent Chemical Grade, n.d.*), sucrose did not have much information (*Sucrose (Crystalline/Certified ACS), Fisher Chemical | Fisher Scientific,*

n.d.) so, ambiguities can be expected on the specific rotation differences with experimentally determined pure aqueous sucrose solutions to that of literature results.

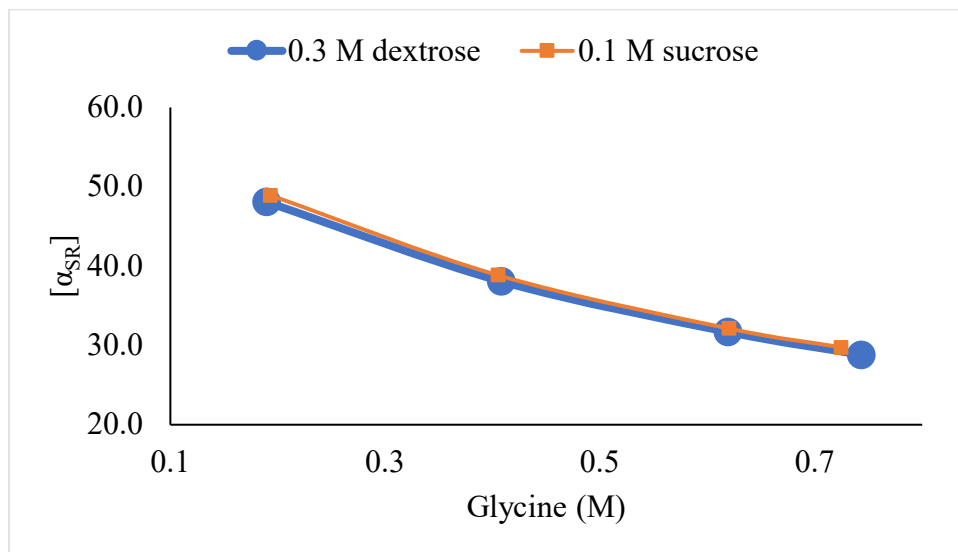


Figure 5-3: Specific rotation of 0.3 M dextrose and 0.1 M sucrose solutions with glycine added in concentrations from 0.1 M to 0.7 M.

At 0.1 M sucrose the specific rotation was found to be $+60^\circ$ and is shown in Figure 5-3. When the glycine concentration was added from 0.1 M to 0.7 M, the specific rotation showed a similar change observed in aqueous dextrose solution obtained was $+49^\circ$ and $+30^\circ$ respectively.

At 0.3 M aqueous sucrose the specific rotation from the experiment is $+65^\circ$. The specific rotation obtained is close to the values mentioned in the literature works. Shown in Figure 5-4, when 0.1 M of glycine was added to aqueous sucrose, the specific rotation reduced from $+65^\circ$ to $+59^\circ$. The specific rotation continued to reduce as glycine concentration was increased. The specific rotation observed to 0.7 M of glycine in 0.3 M aqueous sucrose is $+34^\circ$ as seen in Figure 5-4.

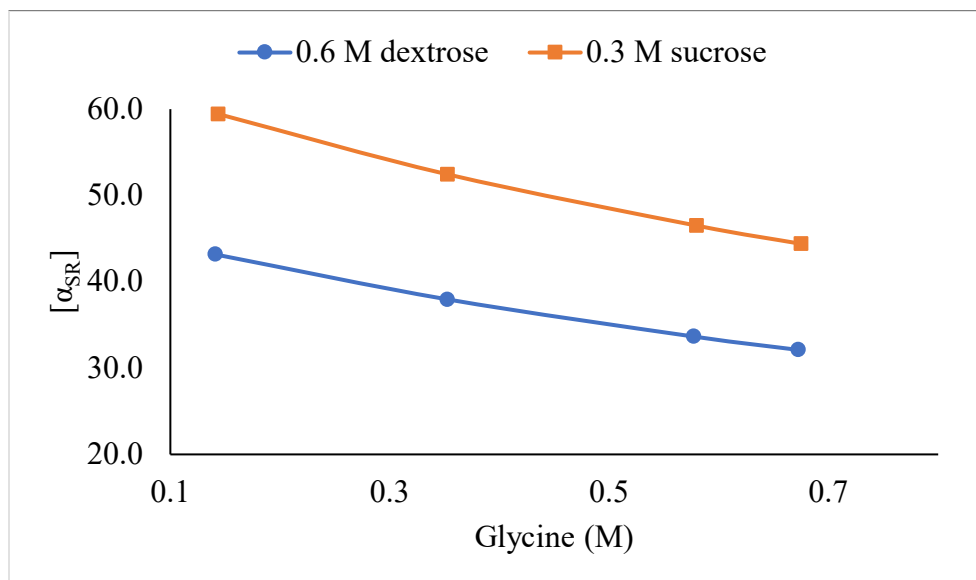


Figure 5-4: Specific rotation of 0.6 M dextrose and 0.3 M sucrose solutions with glycine added in concentrations from 0.1 M to 0.7 M.

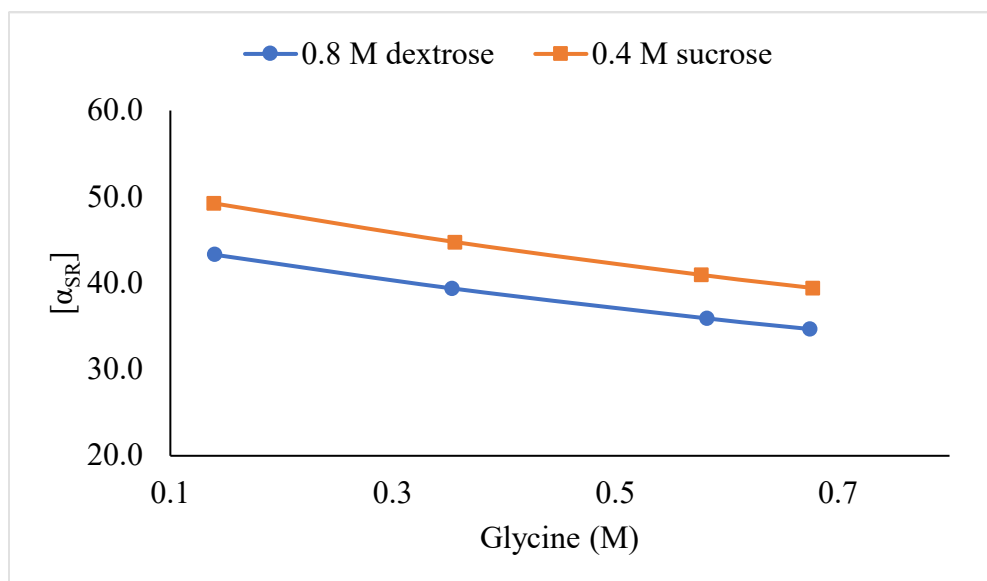


Figure 5-5: Specific rotation of 0.8 M dextrose and 0.4 M sucrose solutions with glycine added in concentrations from 0.1 M to 0.7 M.

The aqueous sucrose of 0.4 M shown in Figure 5-5 is not significantly different from the solution of concentration of 0.3 M showing a specific rotation of $+59^\circ$ (not shown in Figure 5-5). As the glycine concentration was increased, initially a difference of specific rotation was by $+10^\circ$ was noted, the specific rotation was $+49^\circ$ at 0.1 M glycine. At 0.7 M glycine concentration, the specific rotation reached $+39^\circ$.

Dextrose -L-alanine Interaction

Another amino acid, L-alanine was chosen for this experiment to check for the specific rotation characteristics of aqueous sugar under the influence of amino acids. At 0.3 M dextrose the specific rotation was $+59^\circ$ (not shown in Figure 5-6). When 0.1 M of L-alanine was added to aqueous dextrose solution, the specific rotation changed to $+49^\circ$ is shown in Figure 5-6. When the concentration of L-alanine was increased, at 0.7 M the specific rotation changed from $+49^\circ$ to $+28^\circ$.

Similarly, for 0.6 M dextrose the specific rotation for this experiment trial is provided in Figure 5-7 and is found to be $+37^\circ$. At about 0.1 M of L-alanine concentration added to the solution, the specific rotation showed slight increase, the experimental value obtained was $+43^\circ$.

Sucrose-L-alanine Interaction

The specific rotation of sucrose at 0.1 M is found to be $+59^\circ$ (not shown in Figure 5-6). With a 0.1 M L-alanine concentration in this solution the specific rotation changed from $+59^\circ$ to $+49^\circ$ and is show in Figure 5-6. Further increase of concentration to 0.7 M of L-alanine, the specific rotation reduced to $+28^\circ$.

For 0.3 M of sucrose, showed specific rotation of $+65^\circ$. However, in this experimental trial the specific rotation is $+50^\circ$. At 0.1 M of L-alanine added to this solution shown in Figure 5-7 the specific rotation suddenly increased to $+54^\circ$.

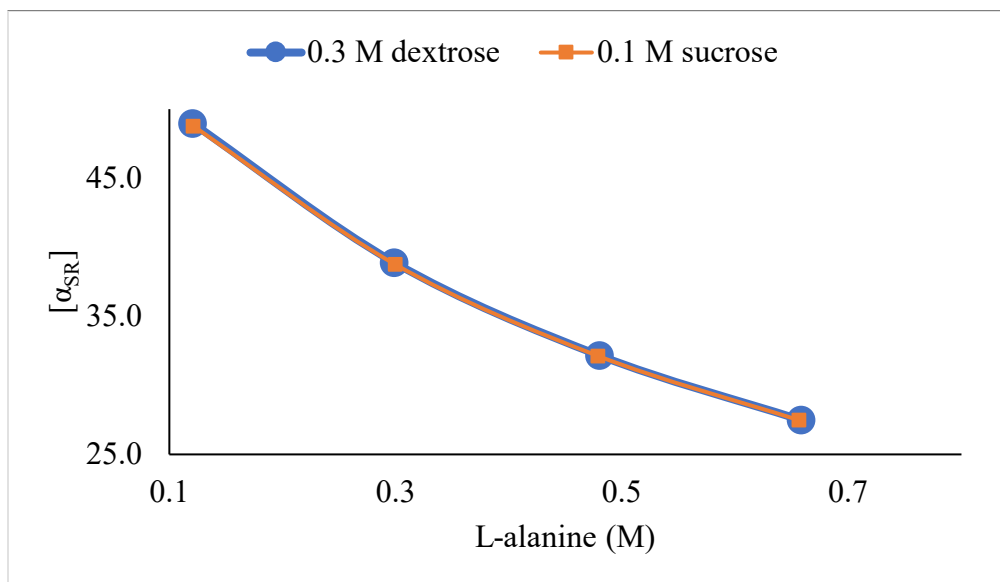


Figure 5-6: Specific rotation of 0.3 M dextrose and 0.1 M sucrose solutions with L-alanine added in concentrations from 0.1 M to 0.7 M.

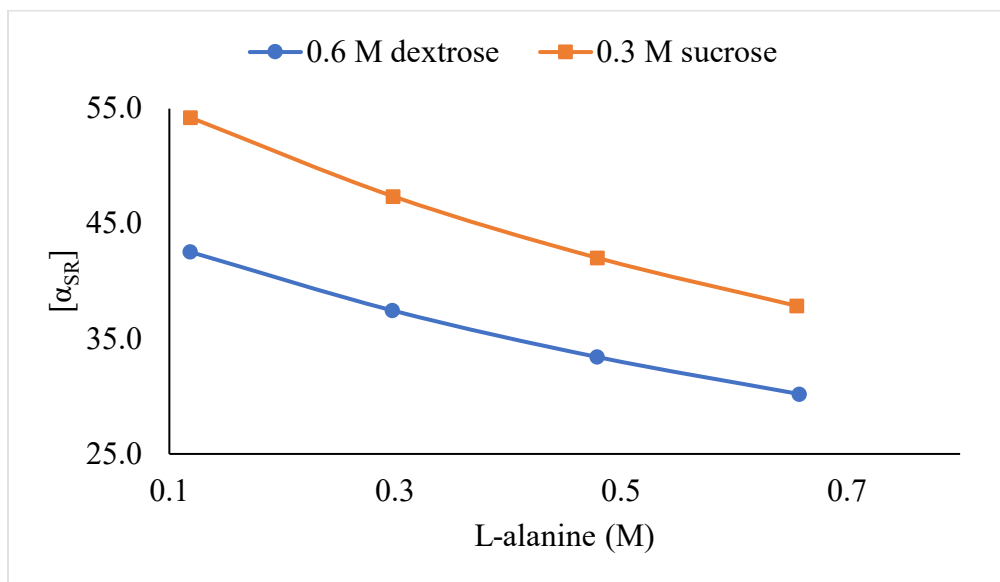


Figure 5-7: Specific rotation of 0.6 M dextrose and 0.3 M sucrose solutions with L-alanine added in concentrations from 0.1 M to 0.7 M.

However, at each subsequent increase of L-alanine concentration, the specific rotation reduced continuously. By 0.7 M of L-alanine, the specific rotation was reduced +38°.

For 0.5 M sucrose shown in Figure 5-8, the specific rotation was $+64^\circ$ (not shown in Figure 5-8) $+54^\circ$ at 0.1 M of L-alanine and $+42^\circ$ at 0.7 M L-alanine added.

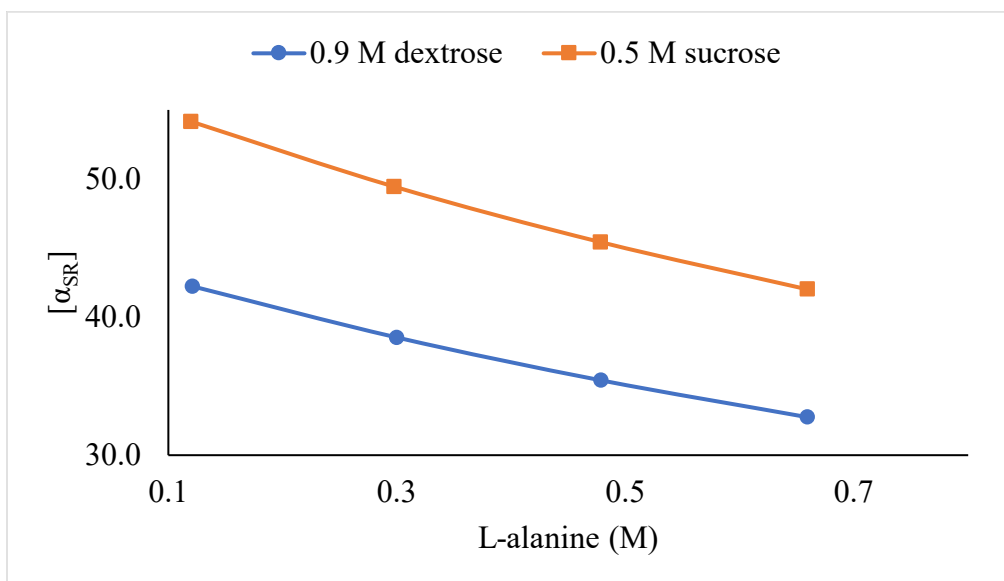


Figure 5-8: Specific rotation of 0.9 M dextrose and 0.5 M sucrose solutions with L-alanine added in concentrations from 0.1 M to 0.7 M.

Chapter 6: Discussion

The idea of considering polarimetry as a detection of sugars technique stands on two reasons:

Principles of polarimetry are already applied in detection of blood glucose. This a non-invasive approach to detect the glucose levels is performed in two different ways. One way is to apply the polarimetry sensor on the arm, and another is to measure the polarization through the aqueous humor of the eye. The success of such sensing devices already in the market, despite so many issues such as thickness of skin tissues, turbidity of the solution, and presence of other chiral compounds, indicate that this research offers a viable approach to see if it is possible to use polarimetry to detect for sugar compounds by understanding the specific rotation of sugar compounds under the influence of amino acids.

Sugars upon exposure to atmospheric carbon dioxide can decompose easily or upon interaction with water can alter the pH of the solution thereby making it difficult to pinpoint the source of pollution in the water bodies. Polarimeters are robust, require little power, easy to operate, and are cost efficient. Polarimetry can help in detection of sugars on-site in real time situation making it suitable for quick decision management process.

Experimental results upon sugar-amino acid combination have shed some insight and are discussed below:

Aqueous Dextrose and Aqueous Sucrose

Optical rotation of the solution is directly proportional to the concentration as well as the path length of the light. The optical rotation of dextrose shows a linear relation to the

concentration. The Optical rotation of sucrose also shows a similar linear trend. Increasing in the concentration of the sucrose concentration increases the optical rotation. The polarized light is left-handed projection that is refracted to the left therefore the light is polarized clockwise when viewed from the analyzer perspective. The dipole moments of aqueous dextrose are 8.68 D (ECHEMI, 2022) and sucrose is 2.35 D. Given that there are large number of OH groups in sucrose, and large dipole moment of dextrose, both molecules have relatively high molecular polarity. The combination of the polarity and the chiral center results in the polarization of light to the right.

Aqueous Dextrose and Glycine Interaction

Glycine is one of the two amino acids that is optically inactive. It does not have a chiral center as its side chain is attached to hydrogen atom resulting in isomers that are superimposable mirror images. However, glycine with its dipole moment value being 17.0 D (R. Pethig, 1984) strongly interacts with aqueous dextrose which has dipole moment of 8.68D (ECHEMI, 2022) Therefore, it is a possibility that for this reason, it does not cancel out the optical rotation. Specific rotation of the aqueous dextrose in its interaction with glycine trends downward at increasing glycine concentrations.

Aqueous Sucrose and Glycine Interaction

Another interaction of glycine with sugar was with sucrose. Sucrose is a disaccharide sugar. Compared to aqueous dextrose dipole moment value, aqueous sucrose has a lesser dipole moment: 2.35D (Gopinath et al., 2015). Glycine has high polarity compared to sucrose but then due to this, instead of canceling out the optical rotation of aqueous sucrose, interaction of glycine with aqueous sucrose has only led to the specific rotation of aqueous sucrose to trend downward.

Aqueous Dextrose and L-alanine Interaction

L-alanine is another organic acid that was used to study the specific rotation of sugars. L-alanine is optically active amino acid. Its symbol 'L' denotes that it rotates the plane polarized being refracted to the right. Similar to glycine, L-alanine too has higher dipole moment. Whereas acids tend to decompose sugars, the amphoteric nature of L-alanine is not acidic enough to initiate decomposition. The observed pH for our concentrations was between 5.0 and 6.0 and the amino acid L-alanine did not cause any internal reactions with dextrose. Another important observation is that L-alanine being a levorotatory, it did not cancel the optical rotation of aqueous dextrose. From the experimental results, it is observed that the specific rotation of aqueous sucrose decreases at increasing L-alanine concentrations.

Aqueous Sucrose and L-alanine Interaction

In this interaction, L-alanine being levorotatory did not cancel the optical rotation of the aqueous sucrose. Given that dipole moment of L-alanine being 17.4 D (R. Pethig, 1984) compared to sucrose which is 2.35 D (Gopinath et al., 2015), the molecules are highly polar and also since it has larger dipole moment, there is larger polarizability therefore the L-alanine has chiral center that rotates the direction of polarization of light to the left, however in the Specific rotation of aqueous sucrose in its interaction with L-alanine, did not cancel the optical rotation. From the experimental results, specific rotation decreased with increasing L-alanine concentrations.

Therefore, the dipole moments of aqueous dextrose and sucrose, and amino acids glycine and L-alanine indicates that when placed in an external electric field orient themselves in the direction of that field. When this electric field is passed through quartz plate i.e., polarizer, the light because of its physical property to polarize, absorbs the magnetic field and electric field

scattered in different directions and allow electric and magnetic wave at a particular angle to pass through the polarizer, the tetravalence carbon atom present in the solution because of chiral property rotates the plane of polarized light to the right which is detected by the analyzer based on the intensity of incoming polarized light. The dipole moments of amino acids glycine and L-alanine are 17.0 D and 17.4 D, compared to that of dipole moments of dextrose and sucrose molecules which are 8.68 D and 2.35 D respectively, the magnitude of dipole moment of dextrose is closer to amino acids than sucrose. Therefore, dextrose and amino acid molecules have stronger interactions.

Dipole moment of water is around 1.85 D, since dipole moment of sucrose is 2.35 D, compared to amino acid glycine and L-alanine dipole moments which are around 17.0 D and 17.4 D respectively, therefore even though amino acid having strong dipole moment, the interaction between water-sucrose is stronger than compared to interaction between sucrose and amino acids. Specific rotation of dextrose at different concentration levels under the influence of amino acids at increasing concentrations, ranged between $+53^\circ$ to $+28^\circ$ (not shown). Specific rotation of sucrose at different concentration levels under the influence of amino acids at increasing concentrations, ranged between $+65^\circ$ to $+28^\circ$ (not shown).

In the context of detection of sugar using polarimeter device, it offers insight as to how a specific rotation of sugars can possibly behave in the presence of amino acids in water. The results provided can be compared to other results that might be obtained on-site field conditions.

Chapter 7: Conclusion

This work involves the determination of specific rotation of aqueous dextrose and sucrose in the presence of the amino acids: glycine and L-alanine. The results from the experiment show that the optical rotation of pure aqueous dextrose and sucrose increase linearly with increasing concentration. Specific rotation of aqueous sugars was determined with amino acids of concentration ranging between 0.1 M - 0.7 M. The specific rotation of aqueous dextrose in its interactions with glycine an amino acid that is optically inactive, trend downward with increasing concentration of glycine. Specific rotation of aqueous dextrose in its interaction with L-alanine, an amino acid that is optically active, also followed a similar trend as dextrose – glycine solution. In a similar fashion the specific rotation of aqueous sucrose, upon its interaction with glycine and also L-alanine followed a decreasing trend. Based on this work, it is believed that the influence of interactive molecular forces such as dipole-dipole significantly reduces the specific rotation of the sugars. One of the significant observations from the results obtained is that the presence of amino acids such as L-alanine being a levorotatory in aqueous dextrose and sucrose that rotates the plane of polarized light to the right does not cancel out optical rotation. Considering the results obtained in this study, the plausibility of utilizing polarimetry for detection of sugars in treated and untreated waters seems promising. Noting the influence of amino acids such as glycine and L-alanine in changing the special rotation of sugars obtained in this study, this interaction also needs to be understood at a more in-depth level to determine and improvise the efficacy of polarimetry-based devices.

Appendices

Appendix A: Physio-chemical Properties

| | | Physio-chemical properties | | | | | | |
|-------------|----------------------|----------------------------|--------------------------------------|---|---|--------------------------|-------|---------------------------|
| Name | Types | Classification | Other names | IUPAC name | Molecular formula | Molecular weight (g/mol) | Color | Physical form |
| Sugars | Glucose ^a | Monosaccharide | Dextrose/grape sugar/corn sugar | D-Glucopyranose | C ₆ H ₁₂ O ₆ | 180.16 | White | Powdery |
| | Sucrose ^b | Disaccharide | Table sugar/Cane sugar/Beet sugar | β-D-Fructofuranosyl-α-D-glucopyranoside | C ₁₂ H ₂₂ O ₁₁ | 342.30 | White | Crystalline/lumps/powdery |
| Amino acids | Glycine ^c | Aliphatic | Glycocoll | 2-Aminoacetic acid | C ₂ H ₅ NO ₂ | 75.07 | White | Crystalline |
| | Alanine ^d | Aliphatic | L-alanine L-α-Aminopropionic acid | (2S)-2-aminopropanoic acid | C ₃ H ₇ NO ₂ | 89.09 | White | Orthorhombic crystals |

| | | Physio-chemical properties | | | | | | |
|-------------|------------------------|----------------------------|--|---|------------------------------|--|-----------------------------|---------------------------------------|
| Name | Types | Taste | Odor | Decomposition (°C) | Density (g/cm ³) | Solubility (in H ₂ O) (g/ mL at 25°C) | Specific rotation | Refractive Index |
| Sugars | Glucose ^a | Sweet | Excess of glucose begins to smell fruity | 146 for anomer α -D-Glucose, and 150 for anomer β -D-Glucose | 1.540 | 0.91 ^e g/mL, between 25 - 30 °C | +52.48 ^g at 25°C | 1.36 at 20% glucose solution in water |
| | Sucrose ^b | Sweet | Odorless | 186 | 1.600 | 2.12 g/mL | +66.5 ^h at 20°C | 1.53 |
| Amino acids | Glycine ^c | Sweet | Odorless | 233 | 1.161 | 0.24 g/mL | 0.0 | 1.46 |
| | L-Alanine ^d | Sweet | odorless | 297 | 1.432 | 0.16 ^f g/mL | +2.42 at 25°C | 1.46 |

^aPubChem. "D-Glucose." Accessed September 29, 2022. <https://pubchem.ncbi.nlm.nih.gov/compound/5793>.

^bPubChem. "Sucrose." Accessed September 29, 2022. <https://pubchem.ncbi.nlm.nih.gov/compound/5988>.

^cPubChem. "Glycine." Accessed September 29, 2022. <https://pubchem.ncbi.nlm.nih.gov/compound/750>.

^dPubChem. "Alanine." Accessed November 28, 2022. <https://pubchem.ncbi.nlm.nih.gov/compound/5950>.

^e“Supersaturation.” Accessed November 28, 2022. https://preparatorychemistry.com/Bishop_supersaturated.htm.

^fChemicalBook. “L-Alanine | 56-41-7.” Accessed November 22, 2022.
https://www.chemicalbook.com/ChemicalProductProperty_EN_CB4350297.htm.

^gBates, Frederick, F.P. Phelps, and C.F Snyder. “Saccharimetry, the Properties of Commercial Sugars and Their Solutions.” International critical tables of numerical data, physics, chemistry, and technology, n.d.

^hBates, Frederick, and Richard F. Jackson. “Constants of the Quartz -Wedge Saccharimeter and the Specific Rotation of Sucrose.” *Bulletin of the Bureau of Standards* 13, no. 1 (1915).

Appendix B: Experimental Data of Dextrose-Glycine Mixtures

| Weight of Dextrose (M) | Weight of Glycine (M) | pH | Temperature (°C) |
|-------------------------------|------------------------------|-----------|-------------------------|
| 0.3 | 0.1 | * | 21.7 |
| 0.3 | 0.4 | | 21.6 |
| 0.3 | 0.6 | | 22.0 |
| 0.3 | 0.7 | | 22.7 |
| 0.6 | 0.1 | | 21.7 |
| 0.6 | 0.4 | | 21.7 |
| 0.6 | 0.6 | | 21.7 |
| 0.6 | 0.7 | | 21.7 |
| 0.8 | 0.1 | | 21.2 |
| 0.8 | 0.4 | | 21.5 |
| 0.8 | 0.6 | | 21.5 |
| 0.8 | 0.7 | | 21.7 |

*Not recorded

Appendix C: Experimental Data of Sucrose-Glycine Mixtures

| Weight of Sucrose (M) | Weight of Glycine (M) | pH | Temperature (°C) |
|--------------------------------------|--------------------------------------|-----------|-----------------------------|
| 0.1 | 0.1 | * | 22.7 |
| 0.1 | 0.4 | | 22.6 |
| 0.1 | 0.6 | | 22.6 |
| 0.1 | 0.7 | | 22.1 |
| 0.3 | 0.1 | | 22.0 |
| 0.3 | 0.4 | | 22.0 |
| 0.3 | 0.6 | | 22.0 |
| 0.3 | 0.7 | | 21.9 |
| 0.4 | 0.1 | | 21.8 |
| 0.4 | 0.4 | | 21.8 |
| 0.4 | 0.6 | | 21.8 |
| 0.4 | 0.7 | | 21.6 |

*Not recorded

Appendix D: Experimental Data of Dextrose-L-alanine Mixtures

| Weight of Dextrose (M) | Weight of L-alanine (M) | pH | Temperature (°C) |
|---------------------------------------|--|-----------|-----------------------------|
| 0.3 | 0.1 | 7.6 | * |
| 0.3 | 0.3 | 5.2 | 23.5 |
| 0.3 | 0.5 | 5.3 | 23.8 |
| 0.3 | 0.7 | 5.3 | 24.0 |
| 0.6 | 0.1 | 5.3 | 23.3 |
| 0.6 | 0.3 | 5.3 | 23.4 |
| 0.6 | 0.5 | 5.3 | 23.3 |
| 0.6 | 0.7 | 5.3 | 23.3 |
| 0.9 | 0.1 | 5.3 | 22.6 |
| 0.9 | 0.3 | 5.1 | 22.2 |
| 0.9 | 0.5 | 5.1 | 22.7 |
| 0.9 | 0.7 | 5.5 | 22.8 |

*Not recorded

Appendix E: Experimental Data of Sucrose-L-alanine Mixtures

| Weight of sucrose (M) | Weight of L-alanine (M) | pH | Temperature (°C) |
|-----------------------|-------------------------|-----|------------------|
| 0.1 | 0.1 | * | 24.3 |
| 0.1 | 0.3 | * | 24.2 |
| 0.1 | 0.5 | 4.9 | 23.1 |
| 0.1 | 0.7 | * | 24.2 |
| 0.3 | 0.1 | 5.2 | 23.2 |
| 0.3 | 0.3 | 5.3 | 23.2 |
| 0.3 | 0.5 | 5.1 | 23.1 |
| 0.3 | 0.7 | 5.4 | 22.8 |
| 0.5 | 0.1 | 5.7 | 22.5 |
| 0.5 | 0.3 | 5.4 | 22.2 |
| 0.5 | 0.5 | 5.3 | 22.8 |
| 0.5 | 0.7 | 5.6 | 22.6 |

*Not recorded

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