DETERMINATION OF DIFFUSION COEFFICIENTS OF FOOD ADDITIVES IN DIFFERENT MEDIA AT 25˚C

BY

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DECLARATION

Declaration by the candidate

This thesis is my original work and has not been presented for degree in any other University. No part of this thesis may be reproduced without the prior written permission of the author and/or University of Eldoret.

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SC/PGC/017/09

Declaration by the supervisors

This thesis has been submitted for examination with our approval as the University Supervisors.

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DEDICATION

This thesis is dedicated to my loving mum Ruth Olenganai for her encouragement and support during the entire period of study.

ABSTRACT

Food additives play a vital role in today's bountiful and nutritious food supply and their transportation in water is a diffusion process governed by Fickian diffusion laws. The study looks into the rate of diffusion of ascorbic acid, citric acid, sodium nitrite, sodium citrate, and L-(+) - tartaric acid at 25˚C in water, HCl and NaOH solutions of different concentrations. The objective of this work was to determine the diffusion coefficients of each food additive using spectrophotometric and moving boundary methods and to compare the diffusion coefficients obtained with those calculated from limiting ionic conductance at infinite dilution. The absorbance at different height levels (x) were measured at a given time and at specific wavelength. The boundary heights (x) at a time t and concentration were also recorded. The diffusion coefficients were obtained from and square-root relationship respectively. The spectrophotometric diffusion coefficient ranged from -4.06798 x 10^{-05} to -1.82441 x 10^{-05} cm^2/sec, -3.87546 x 10^{-05} to -6.4791 x 10^{-06} cm^2/sec, -5.60548 x 10^{-05} to -1.59965 x 10^{-05} cm^2/sec, -3.09157 x 10^{-05} to -1.01433 x 10^{-05} cm^2/sec and -2.5445 x 10^{-05} to -7.96674 x 10^{-06} cm^2/sec and for moving boundary/indicator method were 1.1500 x 10^{-04} to 6.3728 x 10^{-05} cm^2/sec, 1.2250 x 10^{-04} to 7.4908 x 10^{-05} cm^2/sec, 1.3500 x 10^{-04} to 1.0286 x 10^{-04} cm^2/sec, 1.5652 x 10^{-04} to 1.2746 x 10^{-04} cm^2/sec and 1.2974 x 10^{-04} to 7.4908 x 10^{-05} cm^2/sec for ascorbic acid, citric acid, sodium nitrite, sodium citrate, and L-(+) - tartaric acid, respectively. Spectrophotometric diffusion coefficients were in close agreement with the expected D_o values unlike moving boundary/indicator method. This method is also cheap, accurate and applicable method, under ordinary laboratory conditions and within a short experimental time. This study will provide a set of reference data of diffusion coefficients of food additives and can be used for routine food safety analysis.

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LIST OF ACRONYMS

ADI	Acceptable Daily Intake (expressed in mg/kg bw)
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts System
EC	Enzyme Commission of IUBMB (for systematic nomenclature and numbering system of enzymes)
FDA	Food and Drug Administration
ISO	International Organization for Standardization
JECFA	Joint FAO/WHO Experts Committee on Food Additives

ACKNOWLEDGEMENT

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A special word of thanks also goes to the technical support of Mercy Surtan and Margaret Maina; University of Eldoret is gratefully acknowledged. For the time at Masinde Muliro University I owe a big thank you to Dr. Owino and all technicians at chemistry laboratory for giving me an opportunity to carry out the research using their facilities. The support, knowledge and experience I gained in the two weeks that I was there was invaluable and I cherish the memories inside and outside the laboratory.

This experience would not be memorable without my colleagues: Collins Anditii and Daniel Boit who were thoughtful and kind hearted, I am honoured to be able to call them my friends. I enjoyed working with them. My deep gratitude also extends to my mother, my brothers, my sister and my uncles their incomparable love, financial support and prayers.

Finally by no means least, I would like to thank the Almighty God for His sustaining grace and for blessing me with the capacity to undertake this research amid challenging moments.

To Him be the Glory.

CHAPTER ONE

INTRODUCTION

1.1 Background

The use of food additives have become more prominent in recent years, due to the increased production of prepared, processed, and convenience foods. Food additives are intentionally added to food and must be safe for a lifetime of consumption based on current toxicological evaluation. Food additives are used for the purpose of maintaining or improving the keeping quality, texture, consistency, appearance and other technological requirements (Directorate General of Health Services Ministry of Health and Family Welfare Government of India, 2012). This study considered the following food additives: ascorbic acid, citric acid, sodium citrate, sodium nitrite and L-tartaric acid.

Food and food additive are assimilated in the body through diffusion; a process that obeys Fick's laws of diffusion. This process is described by a diffusion coefficient (D) (Gooddy *et. al*., 2007) which is an important transport property in food processing operations (Mauro *and* Rodrigues, 2005). Diffusion is the process whereby ionic or molecular constituents move from an area of high concentration to an area of low concentration under influence of the kinetic motion of the constituent molecules or ions and it ceases when there is no longer a concentration gradient (Gooddy *et. al*., 2007). Determination of D provides a way by which safety and toxicological levels of food additives is estimated (Begley, 1997).

This study assessed diffusion coefficients of food additives based on Fick's second law and D was determined using simple spectrophotometric and basic acid-base

indicator techniques. D measures the mass of food additive transported in a given time under the influence of concentration gradient. The spectrophotometric method applies Beer-Lambert law and quantification of a food additive component that absorbs in the ultra-violet/visible region is achieved using calibration or standard curves; where the natural logarithm of absorbance (ln A) at the maximum wavelength (λ_{max}) of the sample (each additive) is plotted against square distance (x^2) at different time intervals (Irina, 1985). This gives a straight line graph whose slope equals $-(4Dt)^{-1}$ governed by the equation:

$$c = \frac{c_o}{2(\pi D_t \, t)^{1/2}} \exp\left(-\frac{x^2}{4D_t t}\right) \qquad 1.1$$

Where C is the activity (or concentration) of the tracer at a distance x from the surface, c_o is the activity originally present on the surface, and t is time of diffusion. D_t is the tracer diffusion coefficient. In the study water was used as a reference solution.

The moving boundary/indicator method involves monitoring the boundary height (x) between the basic and acidic solutions of each food additive in calibrated cuvette, using appropriate indicator, at different time intervals. Plots of square distance (x^2) versus time (t) give straight line graphs and the slope is equal to $4\pi D$. This relation is given by the square-root relationship $x/\sqrt{Dt}$.

In both methods, diffusion coefficients were determined at 25˚C and the introduction of food samples was done gently under this thermal and mechanical equilibrium condition. In the study D and D' refer to the experimental diffusion coefficient obtained from Ultra-violet/ visible spectrophotometric and moving boundary/indicator method, respectively, were compared with the calculated diffusion

coefficient at infinite dilution D_o given by the equation below:

$$\mathbf{D_o = \frac{8.936\times10^{-10}T(\gamma_1+\gamma_2)\lambda^\circ_1\lambda^\circ_2}{\gamma_{1Z_1}(\lambda_1+\lambda_2)}} \qquad 1.2$$

where *T* is the absolute temperature, γ_1 and γ_2 are the respective numbers of the cations and the anions from the dissolution of one molecule of the electrolyte, Z_1 is the cationic charge while λ_1 and λ_2 are the equivalent cationic and anion limiting conductances.

1.2 Statement of problem.

Though food additives are being used daily for flavor and appeal, food preparation and processing, freshness and safety in most food of varied kinds, consumers and scientists across the globe raise questions about the necessity and safety of these substances. It is important to realize that expensive analytical methods have often been used in the past, by various regulating bodies and various international organizations, to extensively investigate and carefully regulate food additives in order to ensure that, their addition to foods is safe. Safety and toxicological test have been conducted to ascertain migration across packing materials, moisture diffusion and diffusion in meat and plant tissues without considering the actual diffusion under room and stomach conditions; upon which these food additives are assimilated into the body. The diffusion coefficients generally remain unknown though they should be known and are measured in some type of kinetic experiments (Begley, 1997).

1.3 Rationale for the study

Food additives play a vital role in today's bountiful and nutritious food supply and must only be used in limited quantities in certain food stuffs (U.S. Dept. of Health and Human Services, Office of the General Counsel, 2008). However, the safety and

toxicological levels of these food additives remain a concern to both consumer and the regulatory federal authorities and international organizations. According to the Commission of European Communities (2006), the levels of food additives should be set at lowest level necessary to achieve the desired effect and the level should take into account the acceptable daily intake. Though the intake of food and food additives is basically a diffusion process, little research has been done to quantify levels and rate of diffusion. It is also notable that the transport phenomena of food and other important biological materials are a significant link between the processing of these materials, quality and safety of the products (Welti-Chanes *et al*., 2005).

This research sought to use moving boundary/indicator method and simple, rapid ultra-violet/ visible spectrophotometric method to obtain boundary heights and absorbance data and determine their respective diffusion coefficients from subsequent graphical plots which would be used as reference data since this method (ultra-violet/ visible spectrophotometry) is considered by Joint FAO/WHO Experts Committee on Food Additives (JECFA) (2006) to have high degree of accuracy, precision and sensitivity.

In an effort to overcome the inherent difficulties associated with migration testing and possibly simplify the process by which FDA evaluates migration of food additives (Begley, 1997), this study provides a simple procedure of determining diffusion within a short experimental time; less than a day. This study uses simple food-simulating liquids: water, NaOH and HCl solutions that have high solubility for most food additives. The diffusion coefficients obtained from this research forms a set of

database and provides an alternative method for laboratories not equipped with expensive materials and equipment especially in the developing countries.

1.4 Objectives of the study

1.4. 1 General objective

The main objective of this study was to determine the diffusion coefficients of five of the food additives (ascorbic acid, citric acid, sodium nitrite, sodium citrate and L-(+) - Tartaric acid) at thermal equilibrium condition of 25°C.

1.4. 2 Specific objectives

The specific objectives were:

- To determine diffusion coefficients of each food additives in water using spectrophotometric method
- To determine the rate of diffusion of food additives in acidic/basic solutions of different concentrations, using moving boundary/indicator method
- To compare the experimental diffusion coefficients in relation with those calculated from conductance at infinite dilution

CHAPTER TWO

LITERATURE REVIEW

2.1 Food additives

The Codex Alimentarious Commission (1997) has defined food additive as any substance not normally consumed as a food by itself and not normally used as a typical ingredient of the food, whether or not it has nutritive value, the intentional addition of which to food for a technological (including organoleptic) purpose in the manufacture, processing, preparation, treatment, packing, packaging transport or holding of such food results, or may be reasonably expected to result, (directly or indirectly) in it or its byproducts becoming a component or otherwise affecting the characteristics of such food. The term does not include contaminants or substances added to food for maintaining or improving its nutritive value.

Food and food additives are assimilated in the body basically through diffusion, this mass transfer in food systems is described by Fick's second law; which in many cases can be analytically solved if experimental data, as well as initial and boundary conditions provided, in order to yield an effective mass transfer coefficient (Danae *et al.*, 2000). There are several food additives but in this study only five additives were considered:

2.1.1 Ascorbic acid E300

L-Ascorbic acid, ($C_6H_8O_6$), CAS registry number 50-81-7, E300 (Figure 2.1) is a naturally occurring organic compound with antioxidant properties. Ascorbic acid is one form ("vitamer") of vitamin C. The name is derived from *a*- (meaning "no") and *scorbutus* (scurvy), the disease caused by a deficiency of vitamin C (Ascorbic acid,

Wikipedia, the free encyclopedia, 2012). Ascorbic acid is white or slightly yellow crystalline powder or colourless crystals; virtually odourless and which melts at but 190-192˚C with some decomposition. Ascorbic acid is also freely soluble in water to give mildly acidic solutions and sparingly soluble in ethanol.

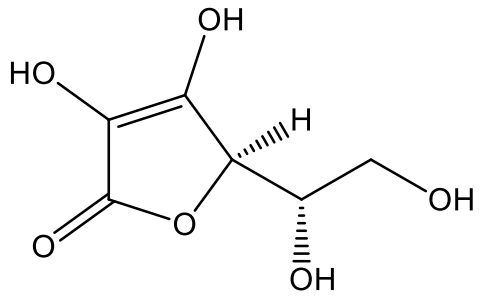

Figure 2.1: Structure of L-ascorbic acid

Vitamin C is the most important vitamin for human nutrition, is supplied by fruits and vegetables. Ascorbic acid is the main biologically active form of vitamin C and is probably the most commonly used vitamin (Gonzalez *et al.*, 1999) as it is highly soluble in water and functions as an effective reductant (Kojo, 2004). It is categorized as antioxidant, sequestrant and reducing agent (Smith and Hong-Shum, 2003). In the food industry it is used in the following areas: fruit juices, carbonated beverages, wine, beer, frozen fruits, canned fruits and vegetables, sausages, cured meat, milk powder and citrus oils. Ascorbic acid and its sodium, potassium, and calcium salts are commonly used as antioxidant food additives (Ascorbic acid, Wikipedia, the free encyclopedia, 2012).

Ascorbic acid is widely used in the meat industry as antioxidant. In cured meats ascorbic acid accelerates colour development and inhibits nitrosamine formation. When ascorbic acid is added to cured-meats, it is oxidized to dehydroascorbic acid. This

oxidation accelerates the reduction of nitrosomet-myoglobin to nitrosomyoglobin, which gives cured meat the characteristic colour ("Panel 1 Vitamin C in Food Processing" (n.d.)). Ascorbic acid reduces nitrate to nitrogen oxides which cannot react with amines to form nitrosamine. It also prevents oxidation and fading of colour in both cured and fresh meat during storage. Ascorbic acid addition is also common in the manufacture of beverages, especially those made from fruit juices. Ascorbic acid not only restores nutritional value lost during processing, but also contributes to the products' appearance and palatability (Smith and Hong-Shum, 2003).

Intestinal absorption of L-ascorbic acid is achieved by sodium dependent system. Its transport into ileum is a carrier-mediated process at low mucosal concentration of L-ascorbic acid. However, at high mucosal concentration of L-ascorbic acid into ileum is linearly dependent on its concentration and absorption occurs predominantly by simple diffusion. Its gastro-intestinal absorption is inversely dependent on its dosage (Tsao, 1997). Eighty percent of the world's supply of ascorbic acid is produced in China (Weiss and Rick, 2007).

2.1.2 Citric acid E330

Citric acid, ($C_6H_8O_7$), CAS registry number 77-92-9, E330 (Figure 2.2) is a white crystalline powder at room temperature. It can exist either in an anhydrous (water-free) form, or as a monohydrate that contains one water molecule for every molecule of citric acid. The anhydrous form crystallizes from hot water, while the monohydrate forms when citric acid is crystallized from cold water. The monohydrate can be

converted to the anhydrous form by heating it above 78 °C (Citric acid, Wikipedia, the free encyclopedia, 2011).

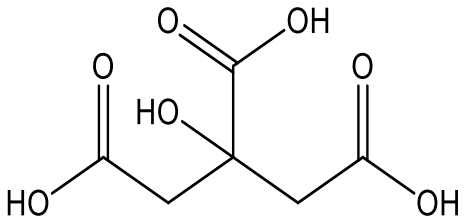

Figure 2.2: Structure of Citric acid.

Chemically, citric acid shares the properties of other carboxylic acids, (organic acids characterized by the presence of a carboxyl group). When heated above 175 °C, it decomposes through the loss of carbon dioxide and water. Citric acid also dissolves in absolute (anhydrous) ethanol (76 parts of citric acid per 100 parts of ethanol) at 15 °C (Citric acid, Wikipedia, the free encyclopedia, 2011). Citric acid is a water soluble organic solid. It is a natural substance that appears as an intermediate in the basic physiological citric acid or Krebs cycle in every eukaryote cell (OECD SIDS, 2001).

Citric acid exists in a variety of fruits and vegetables, but it is most concentrated in lemons and limes, where it can comprise as much as eight percent of the dry weight of the fruit. Citric acid is used widely as an acidulant, pH regulator, flavour enhancer, preservative and antioxidant synergist in many foods, like soft drinks, jelly sweet, baked nutrients, jam, marmalade, candy, tinned vegetable and fruit food (Gursoy, 2002). The dominant use of citric acid is as a flavouring agent and a preservative (Verhoff, 2005). Citric acid is a natural preservative and is also used to add an acidic (sour) taste to foods and soft drinks; as an environmentally benign cleaning agent; as an antioxidant (slows or prevents the oxidation of other chemicals); to keep fat globules separate in ice cream

among many uses. Worldwide, about one million tons of citric acid is commercially produced each year (Soccol *et al*., 2003).

The average daily intake of citric acid from natural sources in the diet and food additives is estimated at about 40 mg/kg for women, 130 mg/kg for infants and 400 mg/kg for individuals on slimming diets; maximum daily intake is reported to reach levels of 500 mg/kg bodyweight (bw). No formal acceptable daily intake (ADI) level has been specified for citric acid and its common salts by the JECFA or by the EC Scientific Committee for Food (OECD SIDS, 2001).

2.1.3 Sodium Citrate E331

Trisodium citrate ($Na_3C_6H_5O_7$), CAS registry number 68-04-2, E331(Figure 2.3) is sometimes referred to simply as sodium citrate, though sodium citrate can refer to any of the three sodium salts of citric acid. It possesses a saline, mildly tart flavour. It is white, odourless crystals or crystalline powder (Sodium citrate, Wikipedia, the free encyclopedia, 2011).

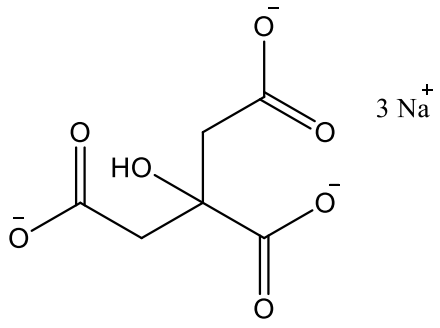

Figure 2.3: Structure of Sodium citrate.

Sodium citrate is chiefly used as a food additive for flavour or preservative. Sodium citrate is used to accelerate color fixation in cured meat and comminuted poultry

and poultry products to replace 50% ascorbic acid or sodium ascorbate that is used. It is also used as a chelating agent in conjunction with phosphate buffers to prepare non-caking meat-salt mixtures and to provide heat and storage stability in condensed, evaporated and sterile concentrated milks. Sodium citrate is also added to carbonated beverages to reduce the sharpness of acid taste and it imparts a cool, saline taste and aids in the retention of carbonation (Smith and Hong-Shum, 2003).

Daily ingestion of 6 g of sodium citrate in 10% aqueous solution over 4 days in 10 men affected the blood acid- base balance, with the urine becoming more alkaline and sodium excretion being increasing while magnesium and potassium excretion was decreased (OECD SIDS, 2001).

2.1.4 Sodium Nitrite E250

Sodium nitrite, CAS registry number 7632-00-0, E250 (Figure 2.4) is an inorganic compound with the chemical formula $NaNO_2$. It is a white to slight yellowish crystalline powder that is very soluble in water and is hygroscopic (Sodium nitrite, Wikipedia, the free encyclopedia, 19/02/2012). Sodium nitrate (or sodium nitrite) is used as a preservative, coloring and flavoring in bacon, ham, hot dogs, luncheon meats, corned beef, smoked fish and other processed meats (Sodium nitrite, Wikipedia, the free encyclopedia, 2011).

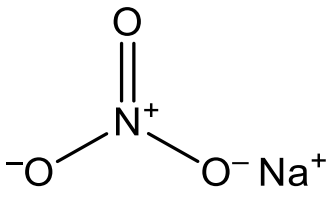

Figure 2.4: Structure of sodium nitrite

In the early 1900s, irregular curing was commonplace, this led to further research surrounding the use of sodium nitrite as an additive in food, standardizing the amount present in foods to minimize the amount needed while maximizing its food additive role (Jeffrey *et al*., 2012). Nitrite is responsible for the development of cured colour and flavour, serves as a strong antioxidant to protect flavour, and acts as a strong antimicrobial to control *Clostridium botulinum* outgrowth (Shahidi and Pegg, 1992). Nitrite controls and stabilizes the oxidative states of lipids in meat products (Shahidi and Hong, 1991a), thus preventing lipid oxidation and subsequent warmed-over flavours (Cornforth and Vasavada, 2005). According to Jeffrey *et al*., (2012), sodium nitrite has been found to inhibit growth of disease causing microorganisms; gives taste and colour to the meat; inhibits lipid oxidation that leads to rancidity and the ability of sodium nitrite to address the above mentioned issues has led to production of meat with improved food safety, extended storage life and improving desirable colour/taste.

This ingredient, which sounds harmless, is actually highly carcinogenic once it enters the human digestive system. There, it forms a variety of nitrosamine compounds that enter the blood stream and wreak havoc with a number of internal organs: the liver and pancreas in particular. Sodium nitrite is widely regarded as a toxic ingredient, and the United States Department of Agriculture (USDA) actually tried to ban this additive

in the 1970's but was vetoed by food manufacturers who complained they had no alternative for preserving packaged meat products. Why does the industry still use it? Simple: this chemical just happens to turn meats bright red. It's actually a colour fixer, and it makes old, dead meats appear fresh and vibrant (Sodium nitrite, Wikipedia, the free encyclopedia, 2011).

However, it is already well documented that N-nitroso compounds and nitrate induce tumor formation through it conversion into nitrite, an oxide destabilized, and leading to an increase in the production of free radicals to cell damage (Demeyer *et al*., 2008). Many other nitrosamines have been found to induce malignant tumours in various species of laboratory animals (Barnes and Magee, 1967). There is growing concern with regard to certain nitrosamines as etiological agents for cancer in the human environment (Epstein and Lijinsky, 1970).

According to *JECFA (1974),* it is evident that: nitrosamines are potent carcinogens in several species of animals; nitrosamines can be formed when nitrites and secondary amines are incubated at pH 1-3 (as exists in human gastric juice); nitrosamine formation from nitrites and amines occurs in vivo. Nitrosamines have been reported as reaction products of nitrites and components of foods; while there are indications of a dose-response relationship in nitrosamine-induced tumour development, a no-effect level for several nitrosamines has not yet been established. It must be remembered that the possibility of nitrosamine formation in foods or in the animal organism does not depend solely on added nitrite. Some nitrate occurs naturally in many foods and may be converted to nitrite by microorganisms. From consideration of the long-term studies it

can be concluded that this level will be somewhat below 100 mg/kg bw per day. Estimate of ADI for man is 0-0.2 mg/kg bw.

2.1.5 Tartaric acid (L-(+)) E334

L (+)-tartaric acid (E334) (Figure 2.5) is listed in European Parliament and Council (1995) and is therefore a generally permitted additive, allowed at *quantum satis* in all foods except those for which there is a defined list of permitted additives. Of this latter group, it is permitted in cocoa and chocolate products at a maximum permitted level of 0.5%, and in jams, jellies, marmalades, canned and bottled fruit and vegetables and fresh pasta at *quantum satis*. It may also be present in biscuits and rusks as weaning foods for infants and young children in good health, at a maximum level of 5 g/kg as a residue.

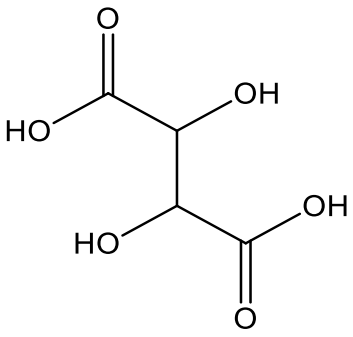

Figure 2.5: Structure of Tartaric acid.

L-Tartaric acid (CAS registry number 87-69-4) is soluble in water, methanol, ethanol, propanol, ether and glycerol, and is insoluble in chloroform.

Tartaric acid occurs naturally in fruits and wine (120-180 mg/100 ml) and L-tartaric acid and its salts are approved as food additives, with acidulant, antioxidant synergist, buffering and sequestrant functions. Typical products in which they are used

are baking powder, biscuits and jam. The ADI for tartaric acid is 0 – 30 mg/kg bw (JECFA, 1977, 1978).

2.2 Diffusion

According to Sun (2004) diffusion is a process that involves the random motion of particles and the concentration gradient dc/dx in the system. Diffusion takes place along a concentration gradient. A concentration gradient exists until the diffused substance is evenly distributed. The diffusion coefficient, which is the major concern, is a measure of the mass of solute transported in a given period of time under the influence of a known driving force. The driving force is essentially the concentration gradient.

Diffusion is a process leading to equalization of substance concentrations in a system or establishing in a system an equilibrium concentration; distribution that results from random migration of the system's elements (Mostinsky, 2011). The quantitative measurements of the rate at which a diffusion process occurs are usually expressed in terms of a diffusion coefficient and by definition, diffusion coefficient (D) is the rate of transfer of diffusing substance across unit area of a cross-section, divided by the space gradient of concentration at the section (Crank,1975).

A number of methods of studies have been carried out using various techiques to determine diffusion coefficients of various substances and have been reported (Irina, 1985, 1980). The Taylor dispersion method (Cussler, 1997) is the most commonly used method for determining molecular diffusion coefficient at infinite dilution due to its versatility and experimental simplicity. An optical technique, the laser-induced grating

method, was used by Butenhoff *et al*. (1996) to determine diffusion of concentrated solutions. This method is particularly interesting for measuring short-lived radicals in the solution or excited species (Terazima *et al*., 1995).

2.3 Diffusion mechanisms

2.3.1 Vacancy mechanism

In all crystals some of the lattice sites are unoccupied and these unoccupied sites are called vacancies (Shewmon, 2010). The diffusion is said to take place by the vacancy mechanism if an atom on a normal site jumps into an adjacent unoccupied lattice site (vacancy). This is illustrated schematically in Figure 2.6. It should be noted that the atoms move in the direction opposite the vacancies (Kaur *et al*., 1995).

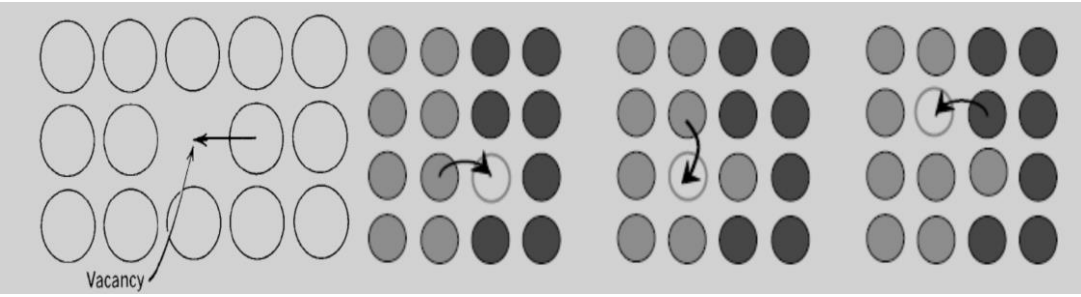

Figure 2.6: An illustration of vacancy diffusion in solids

Diffusion in ionic compounds occurs by vacancy mechanism and in order to maintain charge neutrality in ionic compounds, ionic vacancies occur in pairs, they form in nonstoichiometric compounds and they are created by substitution-impurity-ions having different charge states than the host ions. It follows that the rate of diffusion of these charged couples is limited by the diffusion rate of the slowest moving species.

2.3.2 Interstitial mechanism

If an atom on an interstitial site moves to one of the neighbouring interstitial sites, the diffusion occurs by an interstitial mechanism. This is schematically shown in Figure 2.7.

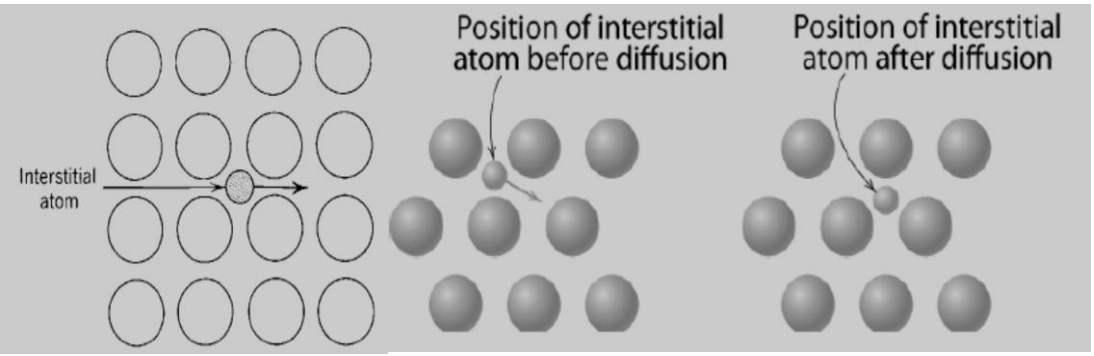

Figure 2.7: An illustration of interstitial diffusion in solids.

Such a movement or jump of the interstitial atom involves a considerable distortion of the lattice, and this mechanism is probable when the interstitial atom is smaller than the atoms on the normal lattice positions. Diffusion of interstitially dissolved light atoms, for example. H, C, N, and O in metals provides the best known examples of this mechanism (Kaur *et al*., 1995).

Finally, even large anions may diffuse interstitially if the anion sub-lattice contains structurally empty sites in lines or planes which may serve as pathways for interstitial defects.

2.3.3 Interstitialcy mechanism

If the distortion becomes too large to make the interstitial mechanism probable, interstitial atoms may move by another type of mechanism. In the interstitialcy

mechanism an interstitial atom pushes one of its nearest- neighbour on a normal lattice site into another interstitial position and itself occupies the lattice site of the displaced atom. This mechanism is illustrated schematically in Figure 2.8 (Kaur *et al*., 1995). One jump gives less distortion and the distortion involved in displacement is quite small, so it occurs with relative ease (Shewmon, 2010).

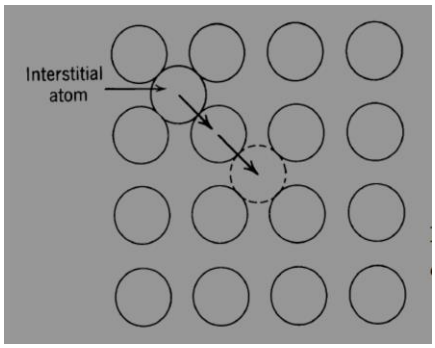

Figure 2.8: An illustration of interstitialcy diffusion in solids

In the interstitialcy mechanism one may distinguish between two types of movements. If the atom on the normal lattice site is pushed in the same direction as that of the interstitial atom, the jump is termed collinear (Figure 2.8). If the atom is pushed to one of the other neighbouring sites so that the jump direction is different from that of the interstitial atom, the jump is termed non-collinear (Kaur *et al*., 1995).

2.3.4 Other mechanisms

In elemental solids also other mechanisms have been proposed. The Crowdion is a variant of the interstitialcy mechanism. In this case it is assumed that an extra atom is crowded into a line of atoms, and that it thereby displaces several atoms along the line from their equilibrium positions. The energy to move such a defect may be small, but it can only move along the line or along equivalent directions (Kaur *et al*., 1995).

2.4 Theory of mass transfer

2.4.1 Definition

Mass transfer by diffusion is the transport of molecules caused by a random molecular motion in the region where composition gradient exists (Welti-Chanes *et. al*., 2003).

2.4.2 Steady-State Diffusion: Fick's first law

Diffusion is a time dependent process where the quantity of an element being transported within another is a function of time. The rate of diffusion is expressed as diffusion flux (J), defined as the mass (equivalent number of atoms) M diffusing through and perpendicular to a unit cross section area of solid per unit time. In mathematical form, this may be represented as

$$\boldsymbol{J = \frac{M}{At}} \qquad 2.1$$

where *A* denotes area across which diffusion is occurring and *t* is the elapsed diffusion time. In differential form, this expression becomes

$$\boldsymbol{J = \frac{1}{A}\frac{dM}{dt}} \qquad 2.2$$

The SI units for *J* are kilograms or atoms per metre squared per second (kg/m^2s or $atoms/m^2s$). If the diffusion flux does not change with time, a steady-state condition exists.

Fick in by analogy to Fourier's law of heat conduction, proposed the law of mass diffusion which is stated as, "the mathematical theory of diffusion in isotropic substances is based on the hypothesis that the rate of transfer of diffusing substances

through unit area of a section is proportional to the concentration gradient measured normal to the section" (Crank, 1968). Fick's first law of diffusion expressed the change of concentration (of solute) with respect to coordinates and is mathematically expressed as:

$$\boldsymbol{J = -D\frac{dC}{dX}} \qquad 2.3$$

where *J* is called flux or diffusion flux or flow which is the rate of transfer per unit area of section (in kg/m^{2}s), *C* is the concentration of diffusing substances (in g/ml or g/ 100 ml), and x is the space co-ordinate measured normal to the section and D is diffusion coefficient (in cm^2/sec). This is shown in Figure 2.9.

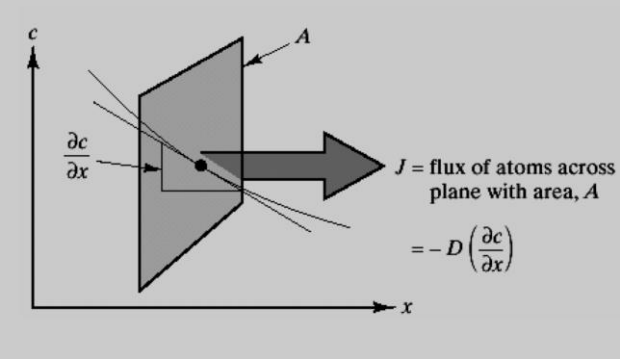

Figure 2.9: Fick's first law

The concentration gradient is often called the driving force in diffusion (but it is not a force in the mechanistic sense).The minus sign in the equation means that diffusion is down the concentration gradient; opposite to that of the increasing concentration. This Fick's first law on diffusional transport rate was also expressed as:

$$\boldsymbol{\frac{dm}{dt} = -DA\left(\frac{dc}{dx}\right)_t} \qquad 2.4$$

where dm is the amount of solute transported in the direction of x through the area A of a cross section perpendicular to x. The amount m can be given in the unit of substance and the concentration c must take in the same units per unit volume.

2.4.3 Non steady-State Diffusion: Fick's second law

Once the mass-balance of an element is taken into account, equation 2.3 can be used to derive the fundamental differential equation of diffusion; equation 2.5.

$$\frac{\partial C}{\partial x} = D\left(\frac{\partial^2 c}{\partial x^2} + \frac{\partial^2 c}{\partial y^2} + \frac{\partial^2 c}{\partial z^2}\right) \qquad 2.5$$

In polymeric and non-homogeneous systems, the diffusion coefficient largely depends on the concentration. The diffusion coefficient in polymeric and non-homogeneous systems varies from point to point and equation 2.5 is more accurately expressed as equation 2.6

$$\frac{\partial C}{\partial x} = \frac{\partial}{\partial x}\left(D\frac{\partial C}{\partial x}\right) + \frac{\partial}{\partial y}\left(D\frac{\partial C}{\partial y}\right) + \frac{\partial}{\partial z}\left(D\frac{\partial C}{\partial z}\right) \qquad 2.6$$

Where *D* is a function of *x*, *y*, *z* and C. In most applications diffusion is restricted to one direction for example, many times a gradient of concentration is present and diffusion only occurs along the *x*-axis. In these cases, equations 2.6 and 2.7 can be reduced to: equation 2.8

$$\frac{\partial C}{\partial x} = D\frac{\partial^2 c}{\partial x^2} \qquad 2.7$$

and

$$\frac{\partial C}{\partial x} = \frac{\partial}{\partial x}\left(D\frac{\partial C}{\partial x}\right) \qquad 2.8$$

respectively. Equations 2.8 and 2.9 are commonly referred to as Fick's second law of diffusion.

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x}\left(D\frac{\partial C}{\partial x}\right) = D\frac{\partial^2 c}{\partial x^2} \qquad 2.9$$

Fick's second law allows the concentration function, C (x) to be calculated as a function of time (Scotten, 2000) and a solution of this equation is concentration profile as function of time, C (x,t):

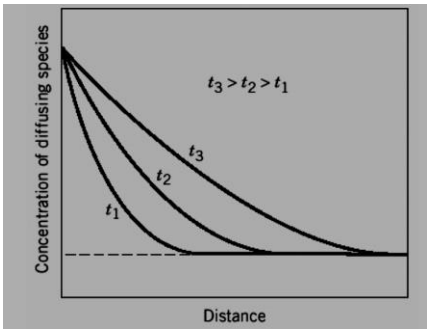

Figure 2.10: Fick's second law

Fick's second law (equation 2.7) relates the rate of change of composition with time to the curvature of the concentration profile: Concentration increases with time in those parts of the curvature and decreases where curvature is negative. The phenomenological description based on the Fick's laws is valid for any atomic mechanism of diffusion. It may be solved explicitly under certain boundary conditions which may be closely approximated experimentally (Crank, 1975). A couple of examples of this are given Figure 2.11.

The concentration gradient changes with time. Furthermore, if the diffusion is homogenous (that is taking place by lattice diffusion), the concentration of the diffusing tracers normal to the plane is through solution of Fick's second law with appropriate boundary conditions (Moore, 1955) given in equation 1.1.

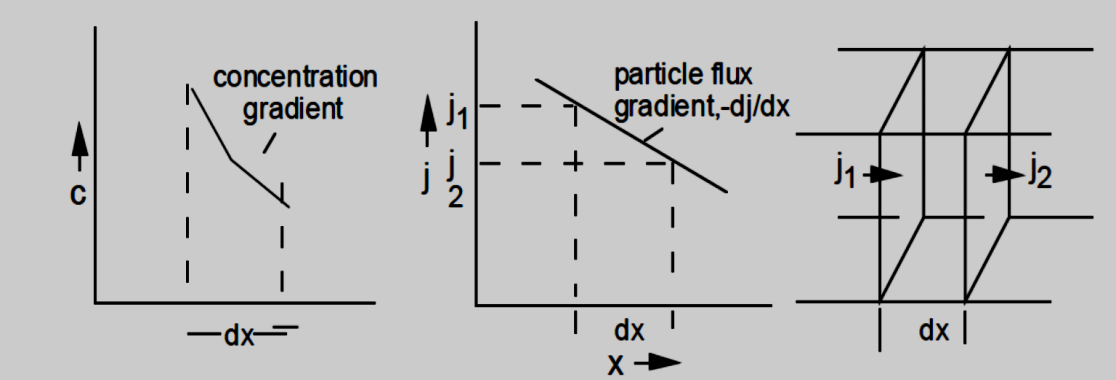

Figure 2.11: Schematic illustration of Fick's second law

Following equation 1.1 it is determined by plotting ln c versus x^2, in which case the resultant straight line has the slope -$\frac{1}{4Dt}$. According to Vany´sek (2012) the diffusion coefficient D of ions in dilute aqueous solution at 25 ˚C is related to λ through the equation:

$$\boldsymbol{D} = \left(\frac{\boldsymbol{RT}}{\boldsymbol{F^2}}\right)\left(\frac{\boldsymbol{\lambda}}{|\boldsymbol{Z}|}\right) \qquad 2.10$$

Where *R* is the molar gas constant, *T* the temperature, *F* the faraday constant, and *Z* the charge on the ion.

2.5 Electrolytic diffusion coefficient

The binary diffusion coefficient (D) of the electrolyte, can be expressed in terms of the diffusion coefficients of the ionic species (Cussler, 1997).The diffusion coefficient for a salt, D_{salt} may be calculated from the D_+ and D_- values of the constituent ions by the relation

$$\boldsymbol{D_{salt}} = \frac{(\boldsymbol{z_+}+|\boldsymbol{z_-}|)\boldsymbol{D_+}\boldsymbol{D_-}}{\boldsymbol{z_+}\boldsymbol{D_+}+|\boldsymbol{z_-}|\boldsymbol{D_-}} \qquad 2.11$$

The diffusion coefficient is concentration dependent and at infinite dilution its value is the tracer diffusion coefficient D^o. For solutions of simple, pure electrolytes (one

positive and one negative ionic species) such as NaCl, equivalent ionic conductivity Λ° which is the molar conductivity per unit concentration of charge, is defined as

$$\Lambda^{\circ} = \lambda_{+} + \lambda_{-} \qquad 2.12$$

Where λ_{+} and λ_{-} are equivalent ionic conductivities of the cation and anion. The more general formula is:

$$\Lambda^{\circ} = \nu_{+}\lambda_{+} + \nu_{-}\lambda_{-} \qquad 2.13$$

Where ν_{+} and ν_{-} refer to the corresponding number of moles of cations and anions which one mole of electrolyte gives rise to in the solution.

In electrolytic solutions, salts dissociate and diffuse as ions and molecules depending on the degree of dissociation. The theory of salt diffusion is elaborated mainly for dilute solutions in which the degree of dissociation is close to one. Thus, the diffusion coefficient for a simple salt that is infinitely diluted can be found using the Nernst-Heckell equation.

$$D_{AB} = \frac{RT}{Fa^2}\frac{1/n_{+} + 1/n_{-}}{1/\lambda_{+}^{0} 1/\lambda_{-}^{0}} \qquad 2.14$$

where D_{AB} is the diffusion coefficient, defined as the proportionality factor between the molecular flow of dissolved salt and the gradient of its molecular concentration, cm^2/s; T, the temperature, K; Fa, the Faraday number, n_{+} and n_{-}, the cation and anion valences; λ_{+}^{0} and λ_{-}^{0}, the limit (under an infinite dilution) ionic conductions of cation and anion, cm^2/Ω mol (Mostinsky, 2011).The diffusion coefficient for a strong electrolyte at infinite dilution is calculated by the formula (Harned and Owen, 1958) in equation 1.2.

2.6 The root-mean squared distance relationship

For Warrens (2001), the probability of a diffusing molecule M λ from its starting point after N collisions is mathematically exactly the same as a coin toss probability of M more heads than tails. The root-mean squared distance travelled from the starting point will be:

$$\left(\overrightarrow{\boldsymbol{M^2}}\right)^{1/2} \boldsymbol{\lambda} = \sqrt{\boldsymbol{N\lambda}} \qquad 2.15$$

This is proportional to the square root of the number of steps, or equivalently proportional to the square root of the travel time. If all molecules start at time t = 0 at the position x = 0, the concentration distribution of C (x, t) at later time is given by Gaussian expression: $[\boldsymbol{c(x,t)} \infty \boldsymbol{exp}\left(-\frac{\boldsymbol{x^2}}{\boldsymbol{4Dt}}\right)]$. Where D is the diffusion constant and on comparing to the standard form of the Gaussian equation for a purely random process, it gives a normal distribution:

$$\boldsymbol{P(M)dM} = \frac{\boldsymbol{1}}{\boldsymbol{\sigma\sqrt{2\pi}}} \boldsymbol{e^{-M^2/2\sigma^2}} \boldsymbol{d}\mathbf{M} \qquad 2.16$$

The standard deviation σ (the width of distribution) is equivalent to

$\left(\overrightarrow{\boldsymbol{x^2}}\right)^{1/2}$ $(RMS\ distance\ travelled\ from\ start)$ so that $\boldsymbol{\sigma} = \sqrt{\boldsymbol{2Dt}}$

2.17

Diffusion into a semi-infinite medium involving the dimensionless parameter, the distance of penetration of any given concentration is proportional to the square root of time and the time required for any point to reach a given concentration is proportional to the square of its distance from the surface and varies inversely as the diffusion

coefficient. This is best expressed by the square-root relation ($\frac{x}{2\sqrt{(Dt)}}$) (Crank, 1975). Where x is the distance of a given concentration of a substance at the time t and D is diffusion coefficient. Einstein derived that the root mean-square distance covered in a given direction, Δ1, will follow the relation

$$\Delta 1 \equiv < x^2 >^{1/2} = \sqrt{Dt} \qquad 2.18$$

Where t is the time. It may be noted that the equation considers the absolute value of the distance in a given direction; a projection of the real distance covered on a straight line of given orientation (one-dimensional) (Pieter, 2003).

2.7 Mass Diffusion

Materials consist of chemical species (molecules, atoms, ions).The rate at which a chemical species diffuses from a higher to a lower concentration region depends on its mass diffusivity. This property depends on both the species and the medium through which it diffuses. As Pieter (2003) states; the rate at which mass diffusion occurs is generally described by Fick's laws. Fick postulated in his first law that the diffusional transport rate is proportional to the concentration gradient according to equation 2.4.

Total mass transport and concentration profiles as a function of time can be obtained from these differential equations. The solution greatly depends on the boundary conditions; the geometrical constraints. For the fairly simple case of diffusion through an infinite plane surface, on one side of which a constant concentration C_1 is maintained, whereas at the other side initially C = 0**,** the amount of mass transported is given by

$$\boldsymbol{m = 2Ac_1\sqrt{\frac{Dt}{\pi}}} \qquad 2.19$$

The concentration as a function of the distance x from the surface then is given by

$$\boldsymbol{c(x) = c_1(1 - \mathrm{erf}\, y) = c_1\left[1 - \frac{2}{\sqrt{\pi}}\int_0^y exp(-z^2)dz\right]}$$

$$\boldsymbol{y = \frac{x}{2\sqrt{(Dt)}}} \qquad 2.20$$

Where *Z* is an integration variable; *erf y*, the error function (Pieter, 2003).

The following factors affect D:

2.7.1 Concentration dependence of diffusion coefficient.

The translational diffusion coefficient is considered to be a constant only for particles in dilute solution. If solution is not dilute, D is dependent on concentration and may be expressed as ($\boldsymbol{D - D_o\,(1 + k_D C + \ldots)}$). Where D_o is the diffusion coeefficient at infinite dilution and k_D is hydrodynamic and thermodynamic combined factor.

The dependence of the diffusion coefficient on concentration of diffusing substance in Mostinsky (2011), is a consequence of the fact that diffusion flow depends on the difference (gradient) of the thermodynamic potential of the system rather than concentration and the formula must allow for activity of the diffusing substance at constant volume,

$$\boldsymbol{D = D_O\frac{\partial\, ln\, a}{\partial\, ln\, c}D_O\left(1 + \frac{\partial\, ln \vee}{\partial\, ln\, c}\right)} \qquad 2.21$$

where, D_0 and D are the diffusion coefficients, respectively, in an infinitely-dilute solution and in a solution with finite concentration *c; a* and *c*, the activity and the concentration of diffusing substance; and *n*, the activity coefficient of this substance.

2.7.2 Temperature dependence of diffusion coefficient

The dependency of diffusion coefficient on temperature is described by the Arrhenius equation by (Naylor, 1988),

$$\boldsymbol{D = D_0 exp\left(-\frac{E}{RT}\right)} \qquad 2.22$$

here D_0 is a hypothetical diffusion coefficient at very high temperature (m^2s^{-1}), E is the activation energy of diffusion (J mol^{-1}), *R* the gas constant (J mol^{-1}) and T the temperature (K).

2.8 Mass Diffusion of food additives and contaminants.

In the recent years, scientists focused on the following four main areas:

2.8.1 Migration of additives and contaminants across the packing materials

Packed food, as consumed, may contain different substances which are undesirable and migration of such substances into foodstuffs is a subject of increasing interest and an important aspect of food packaging (Haldimann *et al*., 2013). Researchers have attempted to explain specific mechanisms by which diffusion occurs in polymeric systems, but there is no unified theory to explain this phenomenon (Stern, 1994).

An enormous number of scientific attempts related to various applications of diffusion equation are presented for describing the transport of penetrant molecules through the polymeric membranes or kinetic of sorption/desorption of penetrant in/from the polymer bulk (Karimi. (n.d)). Based on the focus of these empirical studies, either microscopic (molecular) or macroscopic (continuum) theories are employed (Stannett, 1978). Several studies for example, have examined the leaching of Sb from polyethylene

terephthalate (PET) bottles into mineral water or juices (BAG, 2005; Rusz and Pergantis, 2006; Shotyk *et al*., 2006; Westerhoff *et al*., 2008).

In some ready meals prepared in PET trays that were exposed to high temperatures, the Sb concentration found in food exceeded the specific migration limit (Haldimann *et al*., 2007). In their study Haldimann *et al*., (2013), say that, due to the low diffusivity of most migrants in PET, the determination of diffusion coefficients requires long-term experiments. The diffusion coefficients were calculated at various temperatures and the Arrhenius parameters, activation energy and pre-exponential factor were determined.

2.8.2 Infusion

Infusion, the transfer of solutes from a liquid into a solid is an important food processing operation which is used to transfer colours, flavours and curing and conditioning agents into foods. Smoking, salting, and the addition of certain additives (for example, sodium nitrite for sausage) are diffusion applications that play important roles in meat processing (Behrouz *et al*., 2010). In his study numerically the diffusion of salt into the potato tissues using Fick's second law equations of unsteady state was solved.

2.8.3 Moisture diffusivity/ drying process

The sensitivity of effective moisture diffusivity to water content has been widely studied (Zogzas *et al*., 1996). Water migration in food products is the consequence of a number of coexisting moisture transport mechanisms, enhanced by the complexity of the food structure and its composition (Guillard *et al.*, 2006). Molecular diffusion is however the main water transport mechanism and to predict the water transfer in food

materials diffusion models based on Fick's second law are used (Guillard *et al.*, 2006 and Isa *et al*., 2013).

Studies have shown that effective diffusivity increases with moisture content in corn based extruded pasta (Andrieu *et al.*, 1988), bread, biscuits and muffins (Tong and Lund, 1990) and in sponge cake (Lostie *et al*., 2002a,b). For example, the effective diffusion coefficient of water in a commercially available marshmallow product was determined by using a combined experimental-computational approach and the results indicated that the experimental drying process is limited by internal diffusion (Huang *et al.*, 2012).

Drying of moist materials is a complicated process involving simultaneous heat and mass transfer (Isa *et al*., 2013) and when drying process is controlled by the internal mass transfer, mainly in the falling rate period, modeling of drying is carried out through diffusion equations based on Fick's second law (Midilli and Kucuk, 2003; Wang *et al*., 2007; Sarimeseli, 2011 and Evin, 2011). An apparent moisture diffusion coefficient, the effective moisture diffusivity (D_{eff}), is used to describe the overall moisture transport phenomena in the food system studied (Guillard *et al.*, 2006).

2.8.4 Diffusion in plant tissues and meat

Telis *et al*. (2004) determined the apparent diffusion coefficients for sucrose, NaCl and water during osmotic dehydration of tomatoes. Pajonk *et al*. (2003) conducted an experimental study and modeling of NaCl diffusion coefficients during hard-cheese brining.

These studies employ a number of techniques: dynamic light scattering (Malvern Nanosizer ZS), Fourier transform infrared spectroscopy (FTIR), secondary in mass spectrometry (SIMS), matrix surface morphology by scanning electron microscopy (SEM), isotope dilution ICP-MS and other techniques, which are sample selective, require a lot of sample preparation and are also expensive. This calls for a simple, quicker and cost-effective method which this research proposes.

CHAPTER THREE

EXPERIMENTAL

3.1 Apparatus

Spectrophotometric measurements were carried out using Spectro-UV11 spectrophotometer (MRC Laboratory equipment manufacturers, Israel) of Masinde Muliro University and UV-1600 series spectrophotometers with 1 cm matching quartz cell (Government Chemist of Kenya). Crystal pellets were made using Infrared crystal pellet maker. The pH values were determined using pH 211- Microprocessor pH meter (HANNA Instruments, Mauritius) of University of Eldoret.

3.2 Reagents

Ascorbic acid, citric acid, sodium nitrite, sodium citrate, L-(+) - tartaric acid and sodium hydroxide were purchased from Sigma-Aldrich. Hydrochloric acid, phenol red, bromothymol blue, bromophenol blue and absolute ethanol was supplied by Loba-Chemie. All the reagents were of analytical grade and were used without further purification. Redistilled and deionized water was used in preparing solutions.

3.3 Research design

The research was carried out in a classical experimental design (casual research) which focuses on two variables: the independent variables (they cause intervention) and dependent variables (outcome). The purpose to the design was to remove influence of other variables so that the effect of other interventions can be seen clearly, (Vaus, 2001). The study considered absorbance and boundary height as dependent variables, temperature, pH and time as independent variables, solubility of food additives as

intervening variables and viscosity of water, hydration, dissociation and solvation of food additives as extraneous variables.

Pre-test and post-test measurements of absorbance and boundary height were carried out at the start and at the end (at infinity), respectively. A cuvette was randomly assigned as the control group and the remaining tree as the experimental groups. The experimental and the control groups were subjected to the same environment except for the interventions.

To optimize the results, only samples with high solubility in water and subject to Beer-Lambert Law were considered. However, the experimental procedures were conducted in thermal equilibrium conditions and specific acid-base indicators were carefully chosen for each food additive with respect to the pH of diffusing medium and pH of food additives at 25 ˚C. Diffusion coefficients were determined using linear regression plots of dependent variables against time.

3.4 Procedure

3.4.1 Preparation of standard solutions

A stock solution of 0.1 M NaOH was prepared by dissolving 0.4 g in 1 litre of redistilled water. Concentrations (0.002-0.008 M) were made by suitable dilution of the stock. Stock solution of 0.02 M hydrochloric acid was prepared by micro pipetting 0.171 ml of HCl into 100 ml of redistilled water. Lower concentrations (0.002-0.010 M) were made by suitable dilution of stock. All solutions were sonicated for ten minutes and all solutions were incubated at 25 ˚C. Fresh solutions were prepared for each set of experiments.

3.4.2 Preliminary sample preparation

Crystal pellets of each additive (citric acid, sodium nitrite, sodium citrate and L-(+) - tartaric acid) were made by pressing 0.25 g of each additive using the infrared crystal maker while ascorbic acid was recrystallized twice in distilled water. The crystals and pellets were dried in a vacuum at 101 ˚C before use.

3.4.3 Ultra-violet/ visible spectrophotometric method

The solutions of ascorbic acid, citric acid, sodium nitrite, sodium citrate and L-(+) - tartaric acid of 0.006 M were prepared by dissolving 1.0567 g, 1.1527 g, 0.5088 g, 1.5483 g and 0.9004 g in 100 ml redistilled water. Three millilitres of each food additive solution was scanned using UV1600 spectrophotometer in 1cm matching quartz cell to obtain respective maximum absorption wavelength of each additive.

Spectro-UV11 spectrophotometer in single beam was used to measure the absorbance of solutions. Using a sharp pencil, a reference line was drawn on the ground glass side of 1 cm quartz sample cell along the edge of the spectrophotometer. Four other horizontal lines were drawn at 2 mm intervals parallel; two on top and two below the reference line. The temperature of the sample and the reference compartment were kept constant at 25.0 $\pm$0.2 ˚C.

Three millilitres of distilled water was pipeted into each of the samples and reference cells and then the contents were allowed to stand for one hour to gain thermal and mechanical equilibrium. The maximum absorption wavelength (252nm) was set on spectrophotometer and then the crystal of L-ascorbic acid of a known mass was dropped into the sample cell. At an interval of one hour, the absorbances at 5 different levels were recorded. These readings were obtained by sliding the sample cell up and down

the compartment and aligning the pencil mark on the ground glass side with the top edge of the partion between the sample and the reference compartments. The absorbances of the sample at infinity were obtained from a homogenous solution of the sample cell.

This procedure was repeated for each of the remaining four food aditives at their specific wavelength (citric acid-210 nm, sodium nitrite-346 nm, sodium citrate-210 nm and L-(+) - tartaric acid-210 nm).

3.4.4 Moving +boundary/indicator method

The solutions of ascorbic acid, citric acid, sodium nitrite, sodium citrate and L-(+) - tartaric acid of varied concentrations (0.002, 0.004, 0.006, 0.008 and 0.010 M) were prepared by dissolving 1.7612 g, 1.9212 g, 0.8480 g, 2.5806 g and 1.5008 g in 100 ml water to make 0.01 M respectively followed by suitable dilutions to get the lower concentrations. The solutions were transferred into 250 ml beakers, then sonicated for ten minutes and respective pH values were measured at 25 ± 0.1 ˚C using pH 211-Microprocessor pH meter the average pH value of each additive recorded in Table 4.1.

A set of 0.002 M to 0.010 M NaOH solutions was prepared and to 3 mls of each solution in a calibrated 1 cm plastic cuvette, two drops of phenol red indicator were added, mixed well and allowed to stand for over night while closed using a fitted stopper at 25˚C so as to attain thermal and mechanical equilibrium after which sample pellet of ascorbic acid of given weight were dropped into each cuvette and start time was recorded. At different time intervals, boundary heights between basic and acidic solution were recorded. The entire procedure was repeated using citric acid (using phenol red indicator) and tartaric acid (using bromothymol blue) sample pellets.

A set of 0.002 M to 0.01 M HCl solutions were prepared and to 3mls of each solution in a calibrated 1cm plastic cuvette, two drops of thymol blue indicator was added, mixed well and allowed to stand over night while closed using a fitted stopper at 25˚C. The sample pellet of sodium citrate of given weight was dropped into each cuvette and start time recorded. At different time intervals, boundary heights between basic and acidic solution were recorded. The entire procedure was repeated using sodium nitrite sample pellets using bromophenol blue indicator.

3.5 Data collection and statistical analysis

The data collected from the laboratory where the research was carried out, were analyzed by plotting scatter graphs and managed using the Microsoft Excel 2013. The measured results in the photometric measurement mode were saved automatically, and were numbered in the measurement order.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.2 Ultra-violet/ visible spectrophotometric method

The centre beam for the sample compartment was 1.40 cm from the inside of the bottom of the holder. This gave the maximum value of the monitoring distance x (from the plane were diffusion began) to be equal to 1.40 cm. The displacement of the cell upwards was limited to 1cm because greater displacements showed anomalies of the cells and refracted source beam. To ensure that Beer Lambert Law was observed, masses of sample pellets which would give concentration within Beer-Lambert range (0.001 - 0.10 M) were used.

The experimental conditions are for an amount of diffusing substance deposited at the time $t = 0$ in the plane $x = 0$ approximating to those of a reflection boundary. Hence at t = 0, $x = 0$, $c = c_o$; but for all values of $x > 0$, $c = 0$. For these conditions, Moore (1955) showed that

$$\frac{c}{c_o} = \boldsymbol{D_t}^{1/2}\, \boldsymbol{e}^{-x^2/4D_t} \qquad 4.1$$

Where *C* is the concentration of the tracer at a distance x from the surface, c_o is the concentration originally present on the surface, and t is time of the diffusion anneal. D_t is the tracer diffusion coefficient. For dilute solutions, the amount of light absorbed at a specific wavelength was directly proportional to the concentration of the solution since absorbance A was directly proportional to concentration. The graphs of ln A versus x^2 gave straight line graphs whose slope equals $-(\boldsymbol{4D_t})^{-1}$.

4.2.1 Ascorbic acid

Appendix I shows absorbance from diffusion of ascorbic acid at maximum absorption wavelength (λ_{max}) of 252 nm for the first 90 minutes. The diffusion process was rapid and at time (t) ≥ 60 minutes, absorbance ≥ 2.548 were recorded and were close to the maximum absorbance of (3.0) which Spectro-UV11 spectrophotometer could record.

The inter-level distance, distance between adjacent calibration marks on the cuvette, was 2 mm and in order to have a minimum of 4 plot points, absorbance between adjacent levels (at x = 0.5, 0.7, 0.9 cm) were also measured at t ≥ 60 minutes. The diffusion coefficient was obtained by plotting, for a given time, graph of ln A versus x^2 as shown in Figure 4.1. The plots were all linear with correlation coefficient (R^2) of 0.941, 0.92 and 0.984 at t = 20, 60 and 90 minutes, respectively. Only absorbance less than 1.5 were used in plotting the graphs. The standard deviation (sd) and the relative standard deviation (rsd) were determined using Microsoft excel 2013 and were within the acceptable range and are given a long side the equation of each line.

Experimental D were determined using equation 4.1 and values of the linear equations. The determined D were: -4.06798 x 10^{-05} cm^2/sec, -2.22194 x 10^{-05} cm^2/sec and -1.82441 x 10^{-05} cm^2/sec at 20, 60 and 90 minutes, respectively. This showed that D varied inversely with the concentration of ascorbic acid.

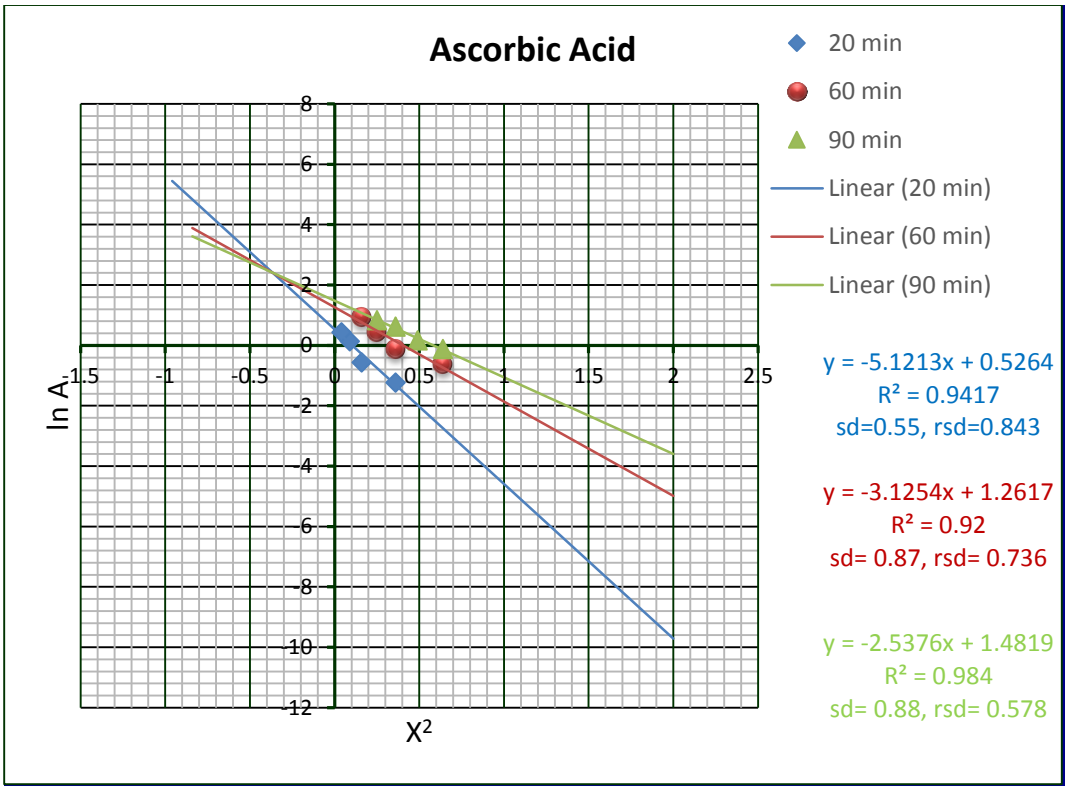

Figure 4.1: Graph of natural log of absorbance of 0.0022 g ascorbic acid against square boundary height (x^2) at 252nm λ_{max}.

4.2.2 Citric acid

Absorbance of diffusing of citric acid in water were measured at 210 nm λ_{max} at t = 20, 60, 120 and 170 minutes and were recorded in Appendix II. The rate of diffusion process was relatively slow as compared to ascorbic acid.

At t ≥ 60 minutes Spectro-UV11 spectrophotometer recorded absorbance ≥ 2.5 for $x \leq 0.2$ cm and in order to have a minimum of 5 plot points, absorbance at midpoint of calibration marks were also measured. The plots of ln A versus x^2 are shown in Figure 4.2 illustrating a linear relationship. R^2 values ranged between 0.958 and 0.9985;

a good indication of linearity. The respective sd (either 0.31.or 0.29) and rsd (between 0.372 and 0.393) were low and within the acceptable range.

The experimental D values were calculated using values from respective graphical equations in equation 4.1 and were: -3.87546 x 10^{-05} cm^2/sec, -1.15644 x 10^{-05} cm^2/sec, -7.1392 x 10^{-06} cm^2/sec, and -6.4791 x 10^{-06} cm^2/sec. for t = 20, 60, 120 and 170 minutes respectively. Which shows a decrease in the rate of diffusion with time and an inverse relationship between D and the concentration of citric acid.

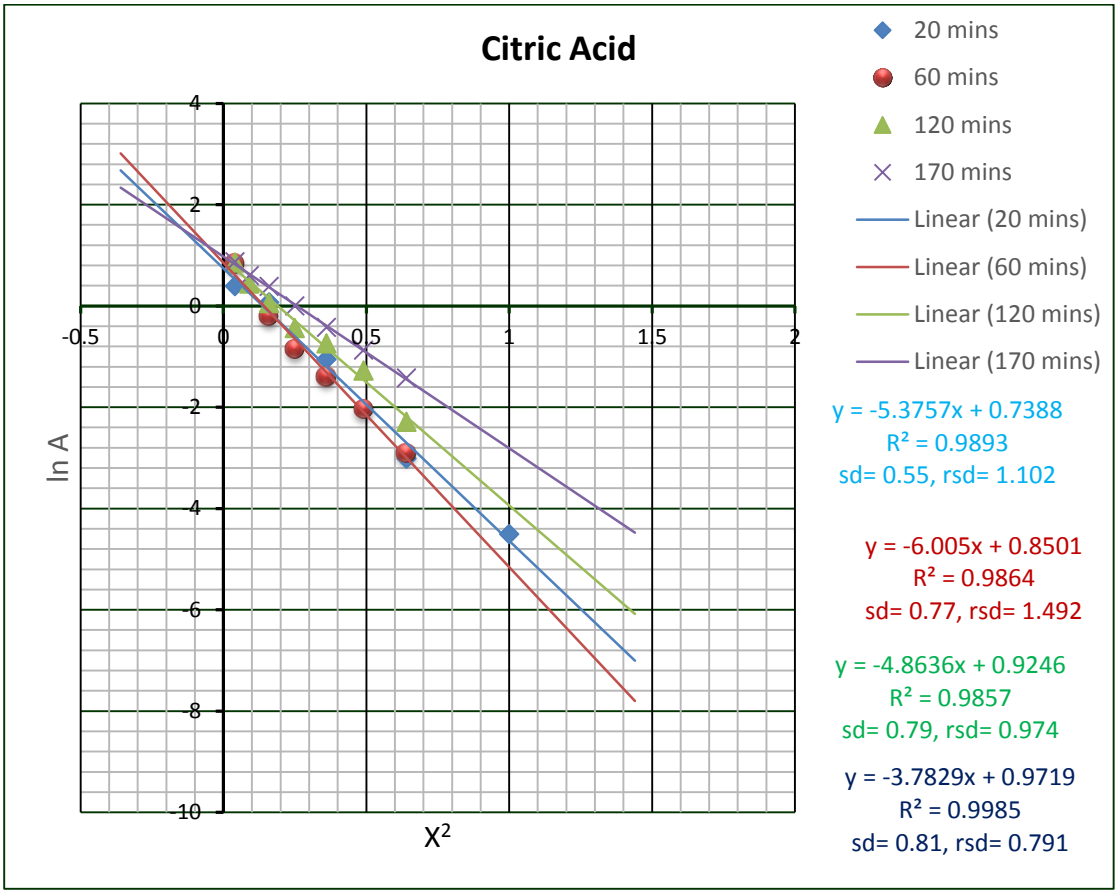

Figure 4.2: Graph of natural log of absorbance of 0.003 g citric acid against square boundary height (x^2) at 210 nm λ_{max}

4.2.3 Sodium citrate

Absorbance of diffusion of sodium citrate in water at 210 nm λ_{max} are recorded in Appendix III for t = 20, 60, 120 and 170 minutes. Like citric acid, the rate of diffusion was relatively faster. It is notable that at x = 0.2 cm, sodium citrate recorded the highest absorbance of 1.758 at t = 20 minutes and there was the need to measure the absorbance at x = 0.3, 0.5 and 0.7 cm in order to have enough plot points. The plots of ln A versus x^2 are shown in Figure 4.3. The graphs illustrated linear relationship between ln A and x^2.

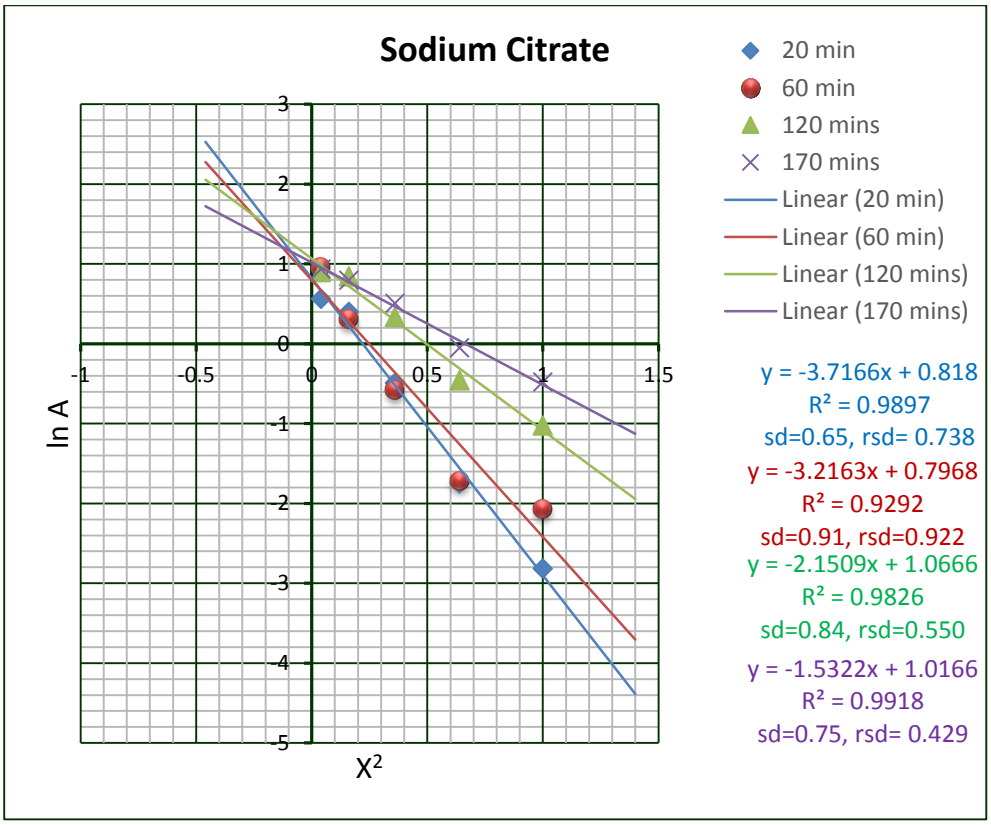

Figure 4.3: Graph of natural log of absorbance of 0.0041 g sodium citrate against square boundary height (x^2) at 210 nm λ_{max}

The experimental D values were calculated using values of graphical equations and equation 4.1 and were: -5.60548 x 10^{-05} cm^2/sec, -2.15914 x 10^{-05} cm^2/sec, -1.61431

x 10^{-05} cm^2/sec, and -1.59965 x 10^{-05} cm^2/sec. at t = 20, 60, 120 and 170 minutes respectively. R^2 values ranged between 0.9292 and 0.9918; a good indication of linearity. The respective sd were between 0.65 and 0.91 while rsd were in the range of 0.442 and 0.922.

4.2.4 Sodium nitrite

Typical data for measurements of $NaNO_2$ at 346 nm λ_{max} for 90 minutes are given in Appendix IV**.** The diffusion process was considerably slow. The plot of ln A versus x^2 is shown in Figure 4.4(a). The graphs are non-linear with R^2 of 0.9409, 0.8945 and 0.9287 at t = 30, 60 and 90 minutes respectively.

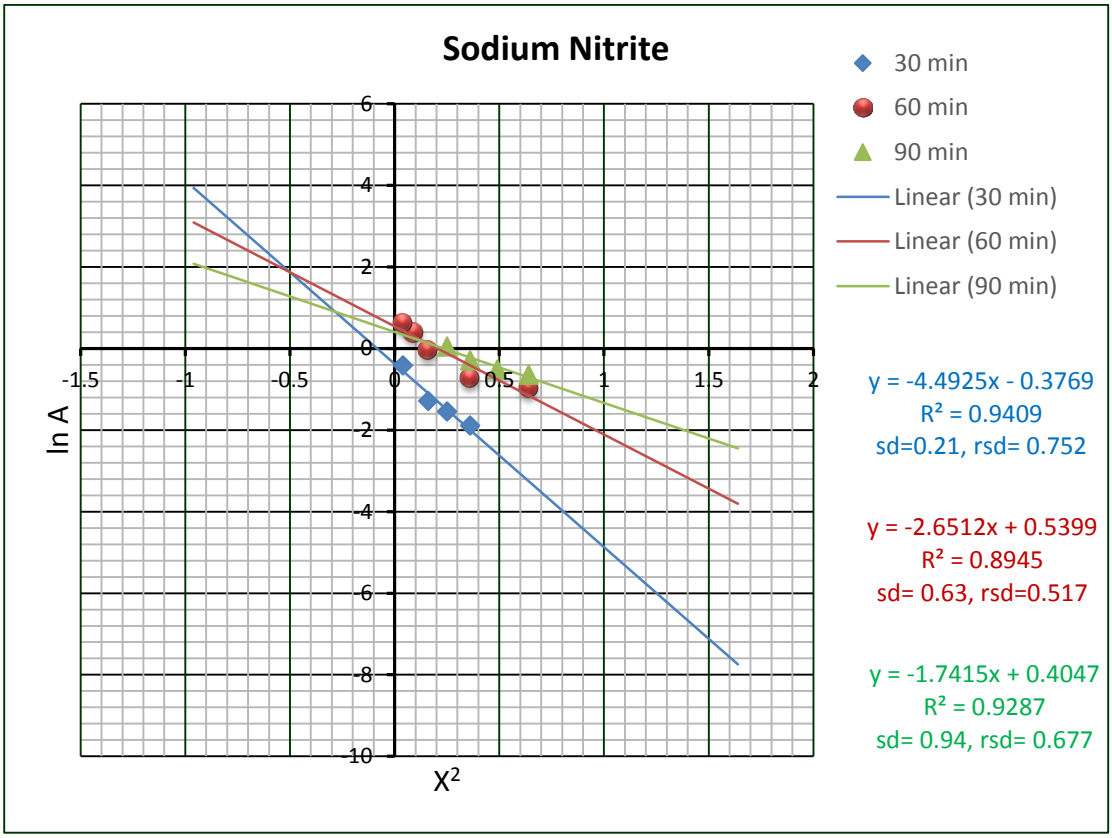

Figure 4.4(a): Graph of natural log of absorbance of 0.0024 g $NaNO_2$ against square boundary (x^2) height at 346 nm λ max

In all determinations, the absorbance at a given displacement (x) increased with increase in time and the higher the absorbance, the higher the diffusion coefficient, which contradicts the generally known trend between diffusing material and diffusion coefficient. The experimental D values were -3.09157 x 10^{-05} cm^2/sec, -2.61936 x 10^{-05} cm^2/sec and -2.65841 x 10^{-05} cm^2/sec at t = 30, 60 and 90 minutes respectively. These could not be relied on as they were obtained from non-linear relations.

The determination of diffusion of sodium nitrite was repeated over a longer experimental time period (260-1150 minutes) and Appendix V shows typical data of absorbance and time. In all determinations, the absorbance at a given displacement (x) decreased with increase in time. The plot of ln A versus x^2 is shown in Figure 4.4(b) illustrating linear relationship.

R^2 ranged between 0.9935 and 0.9977; a very good indication of linearity. The respective sd were between 0.03 and 0.49 while rsd ranged between 0.112 and 0.673. The experimental D values were calculated using graphical equations and equation 4.1 and were: -8.44922 x 10^{-06} cm^2/sec, -1.05847 x 10^{-05} cm^2/sec, -1.40382 x 10^{-05} cm^2/sec and -1.01433 x 10^{-05} cm^2/sec for t = 260, 500, 995 and 1150 minutes respectively. It is notable that the higher the absorbance, the lower the diffusion coefficient.

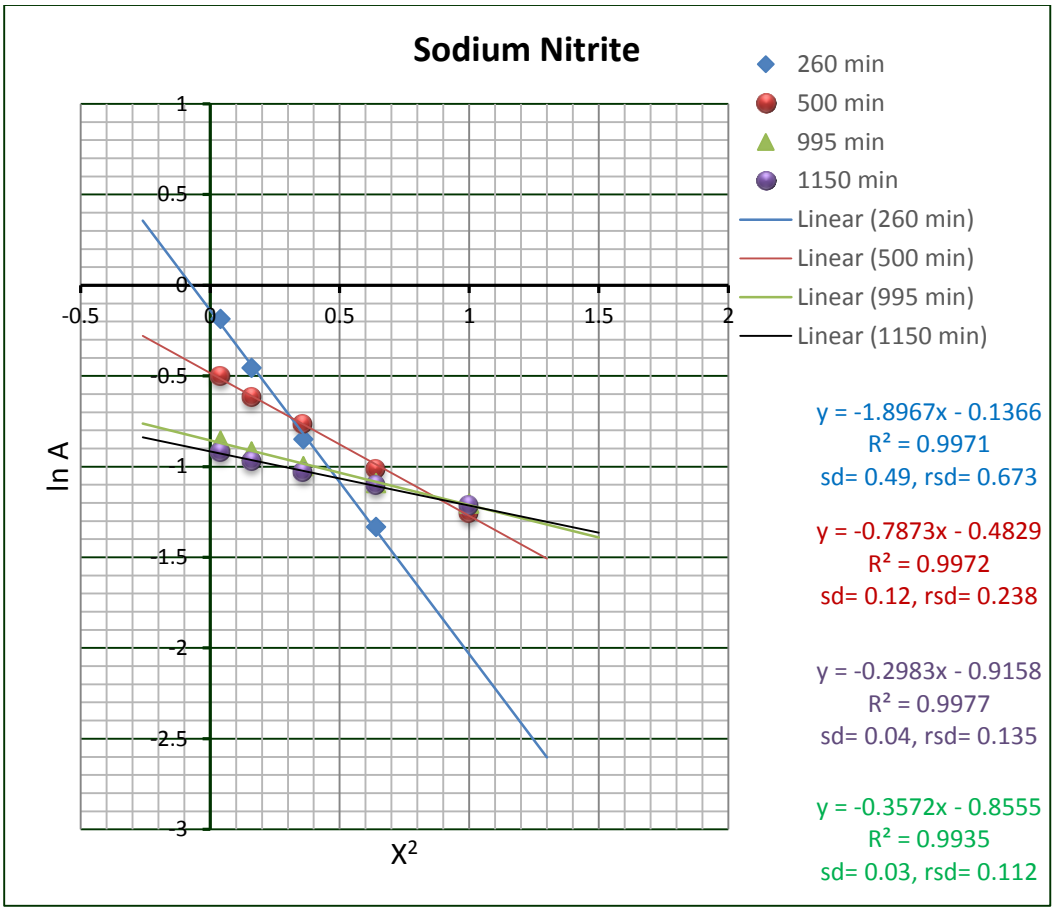

Figure 4.4(b) Graph of natural log of absorbance of 0.0024g $NaNO_2$ against square boundary height (x^2) at 346 nm λ_{max}.

4.2.5 Tartaric acid

Absorbance of diffusion of tartaric acid in water at 210 nm λ_{max} are recorded in Appendix VI at t = 20, 60, 120 and 170 minutes. The plot of ln A versus x^2 is shown in Figure 4.5 and it illustrates a linear relationship. It was observed that absorbance at a given displacement (x), decreased with increase in time.

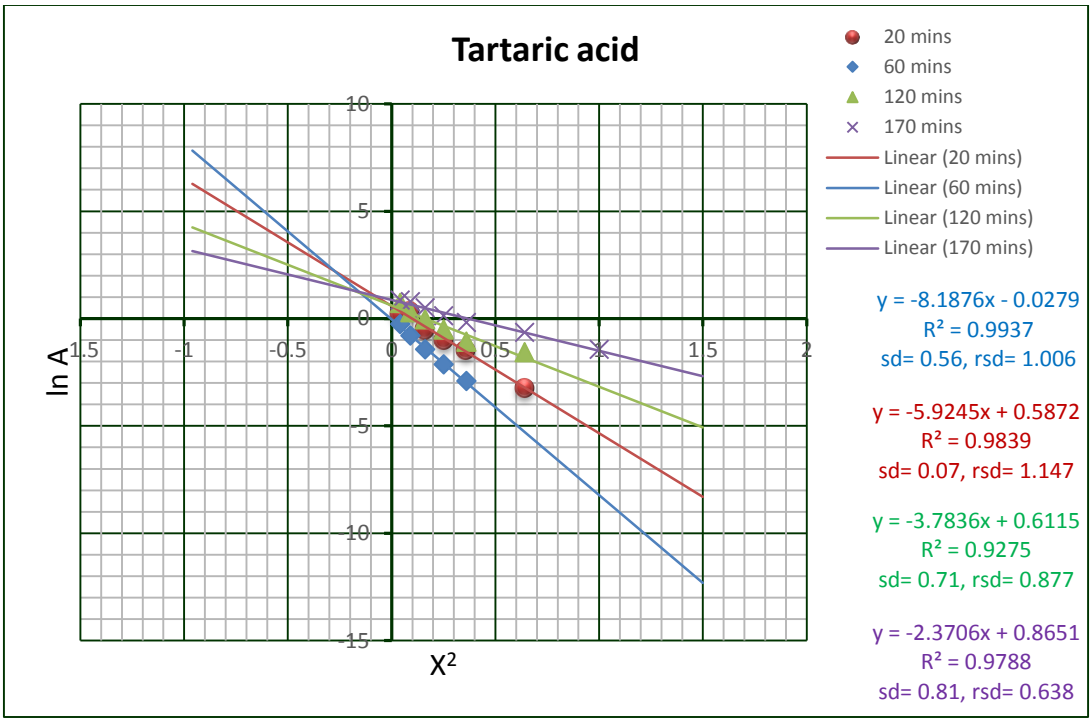

Figure 4.5: Graph of natural log of absorbance of 0.0025 g tartaric acid against square boundary height (x^2) at 210 nm λ_{max}

The experimental D values were calculated using values of graphical equations and equation 4.1 and were: -2.5445 x 10^{-05} cm^2/sec, -1.17216 x 10^{-05} cm^2/sec, -9.17703 x 10^{-06} cm^2/sec, and -1.03391 x 10^{-05} cm^2/sec. for t = 20, 60, 120 and 170 minutes, respectively. R^2 ranged between 0.9275 and 0.9937; an indication of linearity. The respective sd were between 0.27 and 0.81 while rsd were between 0.877 and 1.006.

4.3 Moving boundary/indicator method

Table 4.1 shows the average pH value for each food additive at 25 ˚ C. these values agreed with those in literature. This was aimed at getting the appropriate indicator which would give a sharp end point. From to the respective pH values of food additives, the following indicators were considered: phenol red indicator for ascorbic acid and

citric acid, thymol blue indicator for sodium citrate, bromophenol blue indicator for sodium nitrite and bromothymol blue indicator for tartaric acid.

Table 4.1: Experimental pH values of food additives at 25 ° C

Number	Name of the sample	pH value at 25 ° C
1	Ascorbic Acid (0.002-0.010 M)	6.28
2	Citric Acid (0.002-0.010 M)	6.20
3	Sodium Citrate (0.002-0.010 M)	7.10
4	Sodium Nitrite (0.002-0.010 M)	6.96
5	Tartaric Acid (0.002-0.010 M)	6.21

It was observed that the rate of respective food additive was proportional to time and was dependent on the concentration of diffusing medium; either HCl or NaOH and the mass of the sample pellet (food additive). The plots of the square boundary heights against time gave straight lines passing close to the origin. From the graphs, the slopes were found to be dependent on the concentration of the diffusing medium (HCl or NaOH).

The rate at which food additives diffused in NaOH /HCl decreased with increase in concentration of HCl or NaOH and this agrees with the expectation of diffusion with chemical reaction. The results also agree with the square-root relationship for diffusion into a semi-infinite medium involving the dimensionless parameter ($\frac{\boldsymbol{x}}{\mathbf{2}\sqrt{(\boldsymbol{Dt})}}$) (Crank, 1975) in two aspects:

a. The distance obtained by any given concentration (indicated by the sharp blue/colorless or yellow/red boundary in this experiment) was proportional to the square root of the time.

b. The time needed for any point to reach a given concentration is proportional to the square of its distance from the surface where diffusion begins.

The experimental conditions are for an amount of diffusing substance deposited at the time t = 0 in the plane $x = 0$ approximating to those of a reflection boundary. Bockris and Reedy (1970) reported that these experimental conditions are expressed as

$$\boldsymbol{n = \frac{n_{total}}{(\pi D t)^{1/2}} e - x^2/4D_t} \qquad 4.2$$

or

$$\boldsymbol{\frac{n}{n_{total}} = \frac{1}{(\pi D t)^{1/2}} e - x^2/4D_t} \qquad 4.3$$

Where n is the number of ions at a distance x at a time t and n_{total} is the total number of ions placed in the plane x = 0 at the time t = 0. In the present work, n and n_{total} are constant. Therefore the plot of x^2 with t gives a straight line whose slope equals -4D', where D' is the diffusion coefficient at a given acid/ base concentration. Hence:

$$\boldsymbol{D = \frac{slope}{4}} \qquad (4.4)$$

The masses of the samples used exceeded those required to neutralize with either HCl or NaOH solutions, which allowed the square-root for diffusion into semi-infinite medium involving the dimensionless expression ($\frac{x}{2\sqrt{(Dt)}}$) to be observed.

4.3.1 Ascorbic acid

Appendix VII shows the typical data of boundary heights of diffusing ascorbic acid in a range of NaOH solution (0.002 M to 0.01 M) with respect to time using phenol red indicator. It was observed that the rate of raise of ascorbic acid is proportional to

time and is dependent on both the concentration of diffusing medium (NaOH) and the mass of the sample (ascorbic acid crystal).The plot of the square boundary height against time gave straight line graphs passing close to the origin. The plot of square boundary height with time is given in Figure 4.6**.**

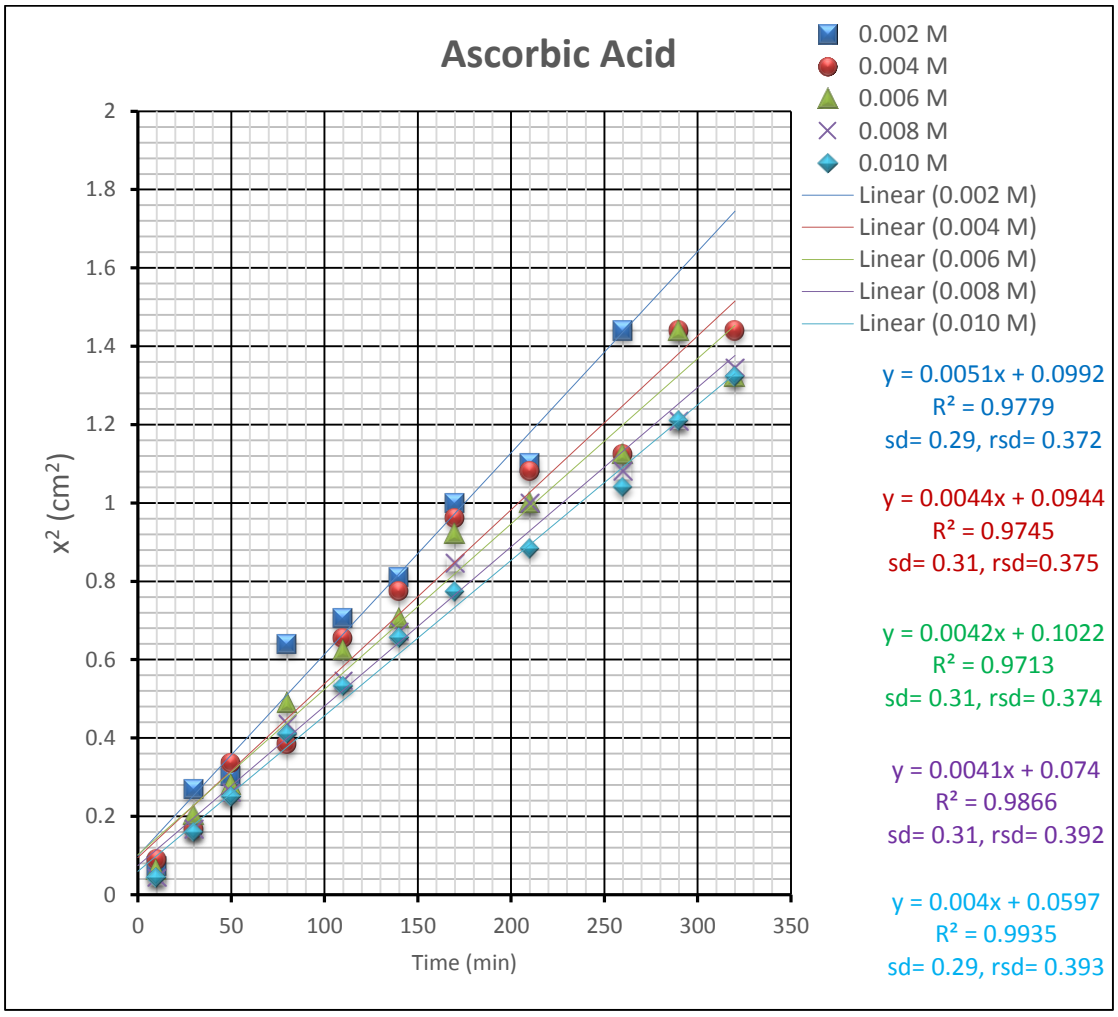

Figure 4.6: Graph of square boundary height x^2 (cm^2) of ascorbic acid against time in NaOH solution using phenol red indicator

The rate at which ascorbic acid diffused decreased with increase in concentration of NaOH solution due to decrease in decrease in concentration of ascorbic acid in the

diffusion matrix. This is in agreement with diffusion involving chemical reaction. The boundary height (x) at any given concentration was indicated by the sharp yellow/red boundary and was proportional to the square-root of the time.

Using equation 4.4, the D' values for ascorbic acid in basic concentration 0.002, 0.004, 0.006, 0.008 and 0.01 M NaOH (Appendix 7) are 5.7020 x 10^{-05} cm^2/sec, 6.9570 x 10^{-05} cm^2/sec, 8.1333 x 10^{-05} cm^2/sec, 9.1679 x 10^{-05} cm^2/sec and 1.0000 x 10^{-04} cm^2/sec, respectively and were obtained by multiplying D values with square root of molar concentrations of NaOH solution (diffusing medium). The average D' was 7.99 x 10^{-05} cm^2/sec. The correlation coefficient for the graphs were between 0.9713 and 0.9935.

4.3.2 Citric acid

Appendix IIX shows the typical data of boundary heights of diffusing citric acid in a range of NaOH solution (0.002 M to 0.01 M) with respect to time using phenol red indicator. It was observed that the rate of raise of citric acid is proportional to time and is dependent on both the concentration of diffusing medium (NaOH) and the mass of the sample (citric acid pellet). The plots of the square boundary heights against time gave straight line passing close to the origin. The plot of square boundary height with time is given in Figure 4.7.

The rate at which citric acid diffused, decreased with increase in the concentration of NaOH solution (diffusing medium), which agrees with diffusion involving chemical reaction. The boundary height (x) at any given concentration was marked by sharp yellow/red boundary

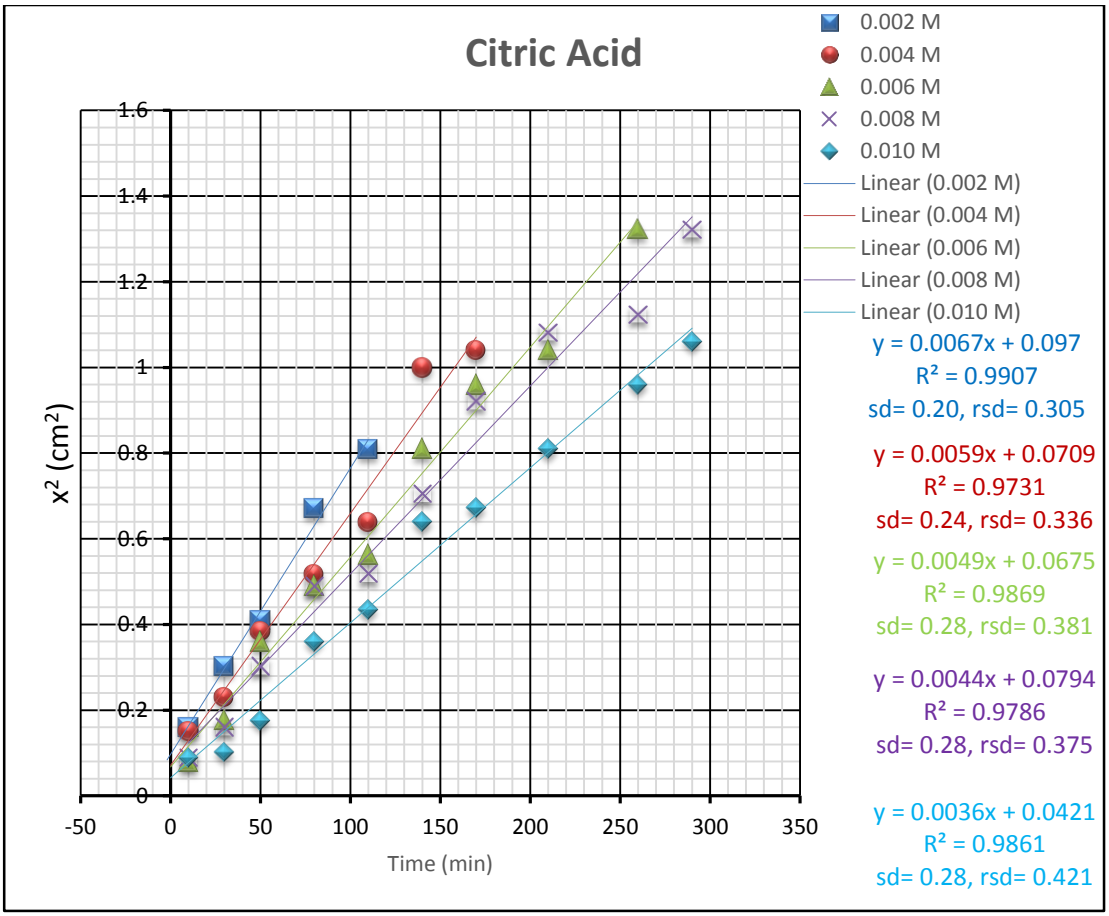

Figure 4.7: Graph of square boundary height x^2 (cm^2) of citric acid against time (min) in NaOH solution using phenol red indicator

Using equation 4.4, the D' values for citric acid in basic concentration 0.002, 0.004, 0.006, 0.008 and 0.01M NaOH (Appendix 8) are 7.4908 x 10^{-05} cm^2/sec, 9.3287 x 10^{-05} cm^2/sec, 9.4888 x 10^{-05} cm^2/sec and 9.8387 x 10^{-05} cm^2/sec, respectively and were obtained by multiplying experimental D values with square root of molar concentrations of NaOH solution (diffusing medium). The average D' was 9.03 x 10^{-05} cm^2/sec. The correlation coefficients for the graphs were between 0.8397 and 0.9937.

4.3.3 Sodium citrate

Typical data of boundary heights of diffusing sodium citrate in a range of HCl solution (0.002 M to 0.01 M) with respect to time using thymol blue indicator is shown in Appendix IX. It was observed that the rate of sodium citrate, is proportional to time and is dependent on both the concentration of diffusing medium (HCl) and the mass of the sample (sodium citrate pellet). The plot of the square boundary height against time gave straight line graphs passing close to the origin. The plot of square boundary height with time is given in Figure 4.8.

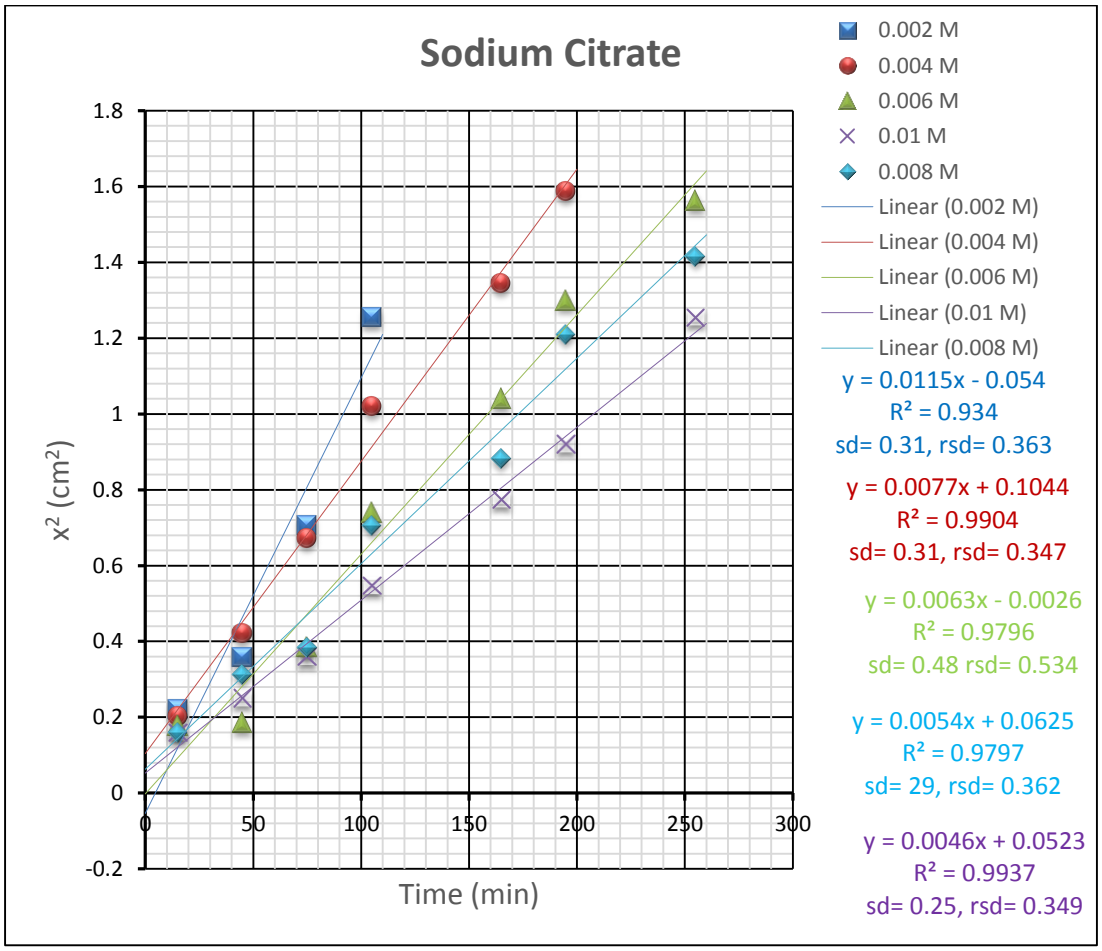

Figure 4.8: Graph of square boundary height x^2 (cm^2) of sodium citrate against time (min) in HCl solution using thymol blue indicator

The rate at which sodium citrate diffused, decreased with increase in concentration of HCl solution (diffusing medium) which agrees with diffusion involving chemical reaction. The boundary height (x) at any given concentration was indicated by the sharp yellow/blue boundary in the experiment; which was proportional to the square root of the time.

Using equation 4.4, the D' values for sodium citrate in acidic concentration 0.002, 0.004, 0.006, 0.008 and 0.01 M HCl (Appendix 9) are 1.3416 x 10^{-04} cm^2/sec, 1.2175 x 10^{-04} cm^2/sec, 1.0651 x 10^{-04} cm^2/sec, 1.0286 x 10^{-04} cm^2/sec and 1.3500 x 10^{-04} cm^2/sec respectively. These values were obtained by multiplying experimental D values with square root of molar concentrations of HCl solution (diffusing medium). The average D' was 1.20 x 10^{-04} cm^2/sec. The correlation coefficient for the graphs ranged between 0.8397 and 0.9937.

4.3.4 Sodium nitrite

Typical data of boundary heights of diffusing sodium nitrite in a range of HCl solution (0.002 M to 0.01 M) with respect to time using bromophenol blue indicator is shown in Appendix X. It was notable that the rate of raise of sodium nitrite is proportional to time and is dependent on both the concentration of diffusing medium (HCl) and the mass of the sample (sodium nitrite pellet). The plot of the square boundary height against time gave straight line graphs passing close to the origin. The plot of square boundary height with time is given in Figure 4.9.

Using equation 4.4, the D' values for sodium citrate in acidic concentration 0.002, 0.004, 0.006, 0.008 and 0.01M HCl (Appendix 10) are 1.2746 x 10^{-04} cm^2/sec,

1.2807 x 10^{-04} cm^2/sec, 1.4330 x 10^{-04} cm^2/sec, 1.5652 x 10^{-04} cm^2/sec and 1.4500 x 10^{-04} cm^2/sec, respectively. They were obtained by multiplying experimental D values with square root of molar concentrations of HCl solution (diffusing medium). The average D' was 1.1996 x 10^{-04} cm^2/sec. The correlation coefficient for the graphs were between 0.9795 and 0.9954.

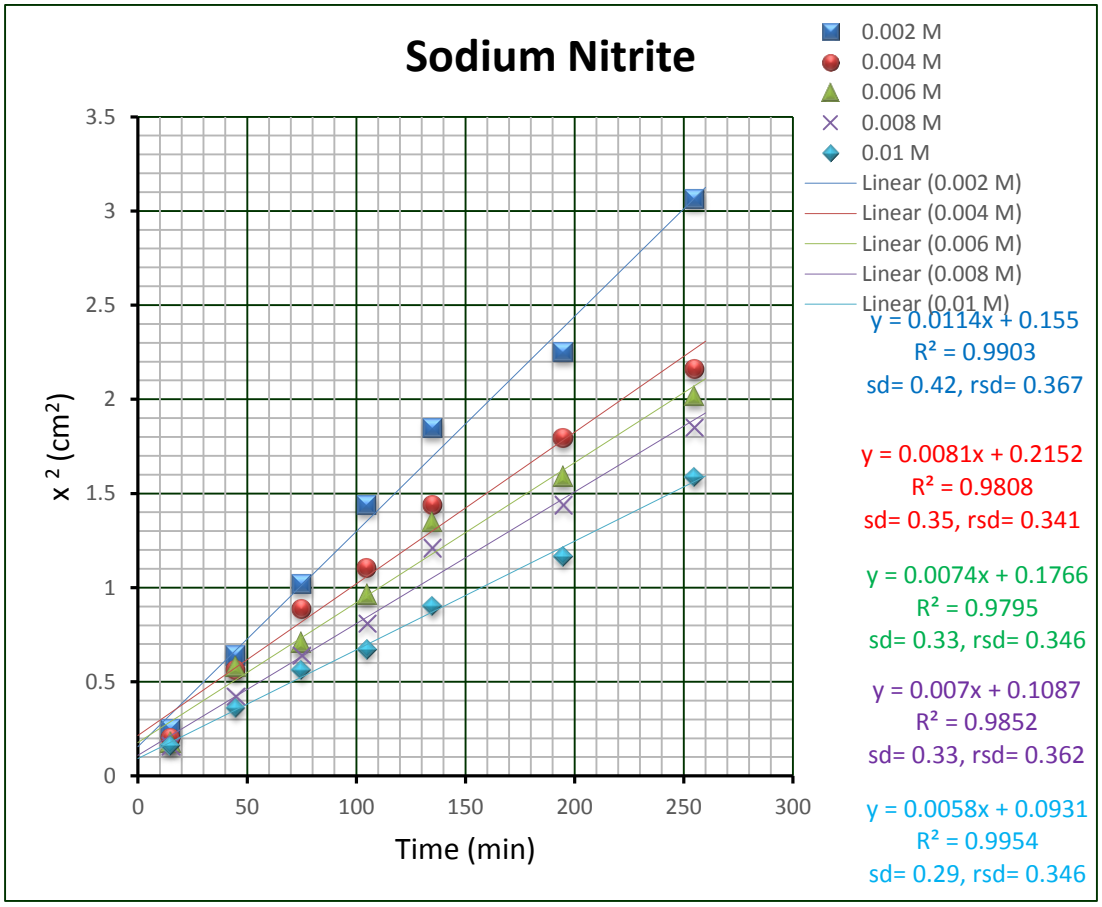

Figure 4.9: Graph of square boundary height x^2 (cm^2) of sodium nitrite against time (min) in HCl solution using bromophenol blue indicator

4.3.5 Tartaric acid

Appendix XI shows the typical data of boundary heights of diffusing tartaric acid in 0.002 M to 0.01 M range of NaOH solution with respect to time using

bromothymol indicator. It was observed that the rate of diffusion of tartaric acid is proportional to time and is dependent on both the concentration of diffusing medium (NaOH) and the mass of the sample (tartaric acid pellet).

The plot of the square boundary height against time gave straight line graphs passing close to the origin. The plot of square boundary height with time is given in Figure 4.10. The rate at which tartaric acid diffused decreased with increase in concentration of NaOH solution, this agrees with diffusion involving chemical reaction. The boundary height (x) at any given concentration was marked by sharp yellow/blue boundary; which was proportional to the square root of the time.

Using equation 4.4, the D' values for tartaric acid in basic concentration 0.002, 0.004, 0.006, 0.008 and 0.01 M NaOH (Appendix 11) were 7.4908 x 10^{-05} cm^2/sec, 9.3287 x 10^{-05} cm^2/sec, 1.0263 x 10^{-04} cm^2/sec, 1.0510 x 10^{-04} cm^2/sec and 1.0000 x 10^{-04} cm^2/sec respectively and were obtained by multiplying experimental D values with square root of molar concentrations of NaOH solution. The average D' was 9.5185 10^{-05} cm^2/sec. The correlation coefficients for the graphs ranged between 0.9731 and 0.9910.

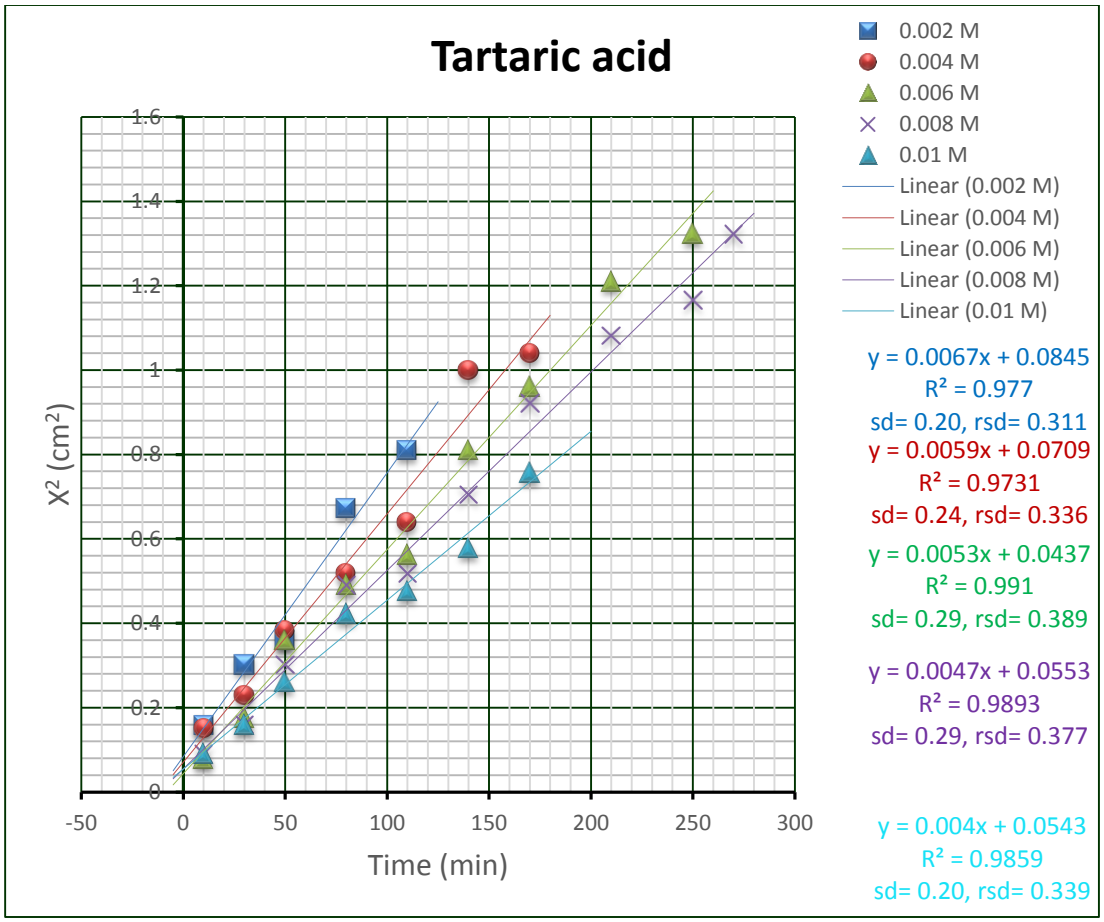

Figure 4.10: Graph of square boundary height x^2 (cm^2) of tartaric acid against time (min) NaOH solution using bromothymol blue indicator

4.4 Discussion

Diffusion of food additives is a time dependent process and it decreases with increase in time, this is in agreement with Fick's second law which states that, diffusion is inversely proportional with time. The diffusion coefficients for strong electrolytes at infinite dilutions D_o, obtained using equivalent cationic and anionic limiting conductance values (Vany´sek, 2012) of each food additive in the formula given in equation 1.2, are given in Table 4.2.

Table 4.2: D_o values calculated from limiting conductance at 25 ˚C

Food additive	λ°_1	λ°_2	γ_1	γ_2	Z_1	calculated D_o
Ascorbic acid						
Citric acid	394.65	70.2	3	1	1	2.11609E-05
Sodium citrate	50.08	70.2	3	1	1	1.03778E-05
Sodium nitrite	50.08	71.8	1	1	1	1.57125E-05
Tartaric acid	394.65	59.6	2	1	1	2.0683E-05

Table 4.3 shows the experimental D and D' values from the present work and the D_o values as calculated from limiting conductance. D values were within the range and were in agreement with the expected D_o values. The rate of diffusion of citric acid and tartaric acid was rapid. The average of D value for citric acid at t = 20 minutes and t = 60 minutes is -2.5159 x 10^{-05} cm^2/sec which is ± 0.39981 x 10^{-05} cm^2/sec of the expected value (2.11609 x 10^{-05} cm^2/sec). The average of D value for tartaric acid at t = 20minutes and t = 60 minutes is -1.85833 x 10^{-05} cm^2/sec; which is ± 0.20997 x 10^{-05} cm^2/sec the expected value (2.0683 x 10^{-05} cm^2/sec).

For sodium nitrite, the average of D values of at t = 90, t = 260 and t = 500 minutes gives a value of 1.5206007 x 10^{-05} cm^2/sec, which was close to the expected 1.57125 x 10^{-05} cm^2/sec (±0.0081007 x 10^{-05} cm^2/sec) and the D value for sodium citrate at 170 minutes was -1.59965 x 10^{-05} cm^2/sec; which is (±0.562185 x 10^{-05} cm^2/sec) the expected (1.03778 x 10^{-05} cm^2/sec). Absorbance of sodium citrate at the time beyond 170 minutes were all greater than 1 (Appendix 3) and could be considered in Ln A vs x^2 plots. There are no reported limiting ionic conductance for ascorbic acid and therefore, D_o for ascorbic acid could not be determined.

Table 4.3: A comparison of experimental D and D' values from the present work and the D_o values calculated from limiting conductance

	Ultra-violet/ visible spectrophotometric method		Moving boundary/indicator method		Limiting conductance
Food additive	Time (Min)	D	Concentra-tion (M)	D'	D_o
Ascorbic acid	20	-4.06798 x 10^{-05}	0.002	5.7020 x 10^{-05}	
	60	-2.22194 x 10^{-05}	0.004	6.9570 x 10^{-05}	
	90	-1.82441 x 10^{-05}	0.006	8.1333 x 10^{-05}	
			0.008	9.1679 x 10^{-05}	
			0.010	1.0000 x 10^{-04}	
Citric acid	20	-3.87546 x 10^{-05}	0.002	7.4908 x 10^{-05}	2.1161 x 10^{-05}
	60	-1.15644 x 10^{-05}	0.004	9.3287 x 10^{-05}	
	120	-7.13920 x 10^{-06}	0.006	9.4888 x 10^{-05}	
	170	-6.47910 x 10^{-06}	0.008	9.8387 x 10^{-05}	
			0.010	9.0000 x 10^{-05}	
Sodium citrate	20	-5.60548 x 10^{-05}	0.002	1.3416 x 10^{-04}	1.0378 x 10^{-05}
	60	-2.15914 x 10^{-05}	0.004	1.2175 x 10^{-04}	
	120	-1.61431 x 10^{-05}	0.006	1.0651 x 10^{-04}	
	170	-1.59965 x 10^{-05}	0.008	1.0286 x 10^{-04}	
			0.010	1.3500 x 10^{-04}	
Sodium nitrite	30	-3.09157 x 10^{-05}	0.002	1.2746 x 10^{-04}	1.5713 x 10^{-05}
	60	-2.61936 x 10^{-05}	0.004	1.2807 x 10^{-04}	
	90	-2.65841 x 10^{-05}	0.006	1.4330 x 10^{-04}	
			0.008	1.5652 x 10^{-04}	
	260	-8.44922 x 10^{-06}	0.010	1.4500 x 10^{-04}	
	500	-1.05847 x 10^{-05}			
	995	-1.40382 x 10^{-05}			
	1150	-1.01433 x 10^{-05}			
Tartaric acid	20	-2.54450 x 10^{-05}	0.002	7.4908 x 10^{-05}	2.0683 x 10^{-05}
	60	-1.17216 x 10^{-05}	0.004	9.3287 x 10^{-05}	
	120	-9.17703 x 10^{-06}	0.006	1.0263 x 10^{-04}	
	170	-1.03391 x 10^{-05}	0.008	1.0510 x 10^{-04}	
			0.010	1.0000 x 10^{-04}	

The moving boundary/indicator method involved the use of dilute solutions which is easier to understand in physical terms, the experimental D' values were remarkably large and most were in the range of 10^{-5} cm^2/sec which was in agreement

with Cussler, (1997) that diffusion coefficients in liquids cannot be reliably estimated and cluster around a value 10^{-5} cm^2/sec. it was further noted that, at 25 ^{o}C almost none are less than 10 x 10^{-5} cm^2/sec and those significantly below 10^{-5} cm^2/sec are macromolecules. Most D' values, at low concentration, were close to 10^{-5} cm^2/sec, with exception of the basic sodium citrate and sodium nitrite. This could be due to formation of macromolecules between their dissociated ions of sodium citrate and sodium nitrite with respective indicators.

The diffusion of ascorbic acid, citric acid and tartaric acid were rapid when compared to sodium nitrite and sodium citrate as a result of high mobility of hydrogen ions, which are small in size, as compared to low mobility of the large sodium ions in their dissociated forms. The D' values of sodium nitrite were the highest followed by sodium citrate. They both have Na^+ as cations in their dissociated form, which are larger than H^+ ions within the diffusion matrix resulting in low mobility and large D' values. Both Na^+ and H^+ ions have a charge of +1 and are in the same thermal condition (25 ˚C) but Na^+ ions have large ionic radii, which result in small hydration force and high ionic mobility as determined by Coulombs law. H^+ ions are very small, more hydrated and with very low mobility resulting in D' values smaller than those with Na^+ ions.

Sodium nitrite is dibasic base while sodium citrate is tribasic base. Ascorbic acid and tartaric acid are dibasic acids and they recorded larger D' values as compared to D' values of citric acid which is tribasic acid. On dissociation, tribasic additives give more conjugate base (more ions) into the diffusion media than dibasic additives which

explains why the D' values of dibasic additives were greater than those of tribasic additives.

The moving boundary in the moving boundary/indicator method became less distinct (faded away) with time and the boundary heights became more of approximation which affected the certainty of D'values. This was due to unaccounted pH change during the neutralization reaction and the results could have been altered by vibration within the laboratory. The research was conducted in an elevated laboratory (second floor) which experiences, the influence of vibrations.

4.5 Statistical analysis

Linearity was assessed by repeated measurements (n = 3) for spectrophotometric method and (n = 6) for moving boundary/indicator method; for five concentration levels (0.002-0.010 M) of HCl and NaOH solution. Acceptability of linearity of data was judged by examining the correlation coefficient (R^2) of graphical lines. A R^2 value of greater than 0.950 was considered to be sufficient to demonstrate linearity of the method. The equation of the regression lines are given in each plot together with calculated respective standard deviation (sd) and their percentage relative standard deviation (rsd).

The precision of the method was ascertained by carrying out the analysis as per the proposed method. The precision was investigated through repeatability and reproducibility. The analysis was repeated six times on the same day. The calculations of the respective standard deviation (sd) and their percentage relative standard deviation (rsd) gave low values indicating acceptable level of precision. The % rsd are also less

than 2% as required by U.S. Pharmacopeia and International Conference on Harmonization guideline.

The accuracy was assessed by comparing the values of D obtained from Ultra-violet/ visible spectrophotometry and moving boundary/indicator method (D'), with those from limiting conductance at infinite dilution (D_o). Ultra-violet/ visible spectrophotometry D values were in close agreement with D_o values while D' values from moving boundary/indicator method differed.

Specificity of the method was demonstrated by recording Ultra-violet/ visible spectra for (0.006 M) standard solution of each food additive, this showed single peaks between 200 nm and 390 nm. No spectral interference could be noticed from the excipients at their respective wavelengths (in nm). Further analysis was done at respective maximum wavelengths (λ_{max}). Specific indicators were used for each additive in the moving boundary/indicator method.

4.6 Errors and assumptions

In the calculations, D/ D' has been assumed to be independent of the concentration of diffusing solution (either water, HCl or NaOH) since equation 4.1 applies only when D/ D' is independent of concentration. There was a marked variation in absorbance at infinity, this brought variation of C_o values at $x = 0$, an evidence that D/ D' varied with concentration. The calculations also assumed viscous friction in pure water; the interaction forces between water molecules. The presence of the sample in the system modifies friction by introducing the solvent-ion and ion-ion interactions which could increase or decrease the D and D' values.

The initial disturbance when the dropping of the crystals and the convective effects of the cells were neglected. The sliding of the sample cells up and down caused no significant side disturbance. The used sample pellets/ crystals were obtained by mechanically breaking the large crystals and it was difficult to obtain samples of equal masses and samples of close weights were used.

The moving boundary analysis was carried out in a typical acidic/basic medium and introduction of sample resulted in ionic dissociation. The presence of dissociated and un-dissociated forms results in different ionic mobility, affecting D/ D' . The experiments were also carried out under pH conditions greater than 2 which could considerably affect diffusion.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

From the research findings, it is concluded that ultra-violet/ visible spectrophotometric method describes a simple, rapid and valid method of determining diffusion coefficients of food additives. This method gives D values that are close to those calculated from limiting conductance within experimental error. The moving boundary/indicator method gives reasonable but rough estimation of diffusion coefficients of food additives. The experimental D' values varied from those from limiting conductance. Spectrophotometric method was specific, linear, accurate and stable. No doubt this method and the data generated by this study forms a set of reference.

5.2 Recommendation

It should be noted that the present study considered only five food additives and this gives a preliminary outline upon which diffusion coefficients of other food additives may be determined. The study overlooked the effect viscosity, hydration energy of food additives, ionic sizes, ionic interactions and interactive forces in the diffusion matrix which should be factored in in determination of D. The present method should be used to test food additives with low solubility in water and weak ionic properties under different experimental conditions and across diffusion membranes particularly those of living tissues; the gastrointestinal membrane.

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APPENDICES

Appendix I: Record of measured absorbance (A) of ascorbic acid with respect to time (min) and boundary height *x* (cm)

Mass of ascorbic acid 0.0022 g										
Time (min)		20			60			90		
X	X^2	T	A	ln A	T	A	ln A	T	A	ln A
1.0	1.00	62.1	0.207	-1.5750	37.2	0.430	-0.8440	20.6	0.687	-0.3754
0.9	0.81							17.1	0.768	-0.2640
0.8	0.64	61.1	0.214	-1.5418	29.2	0.535	-0.6255	12.9	0.889	-0.1177
0.7	0.49							6.5	1.187	0.1714
0.6	0.36	50.9	0.293	-1.2276	13.1	0.885	-0.1222	1.3	1.873	0.6275
0.5	0.25				2.7	1.572	0.4523	0.5	2.295	0.8307
0.4	0.16	26.8	0.571	-0.5604	0.9	2.548	0.9353	0.0	3.000	1.0986
0.3	0.09	7.2	1.142	0.1328						
0.2	0.04	2.9	1.540	0.4318						
∞		4.9	1.306							

Appendix II: Records of measured absorbance (A) of citric acid with respect to time (min) and boundary height *x* (cm)

Mass of citric acid 0.003 g													
Time (min)		20			60			120			170		
X	X^2	T	A	ln A	T	A	ln A	T	A	ln A	T	A	ln A
1.2	1.44	98.2	0.008	-4.8283	97.2	0.012	-4.4228	89.5	0.571	-0.5604	77.4	0.114	-2.1716
1	1	97.5	0.011	-4.5099	97.6	0.01	-4.6052	82.4	0.086	-2.4534	69.1	0.164	-1.8079
0.8	0.64	89.4	0.049	-3.0159	88.2	0.055	-2.9004	79.6	0.101	-2.2926	58.4	0.24	-1.4271
0.7	0.49				74.6	0.13	-2.0402	53.7	0.278	-1.2801	39.4	0.418	-0.8723
0.6	0.36	46	0.347	-1.0584	57.2	0.249	-1.3903	34.8	0.476	-0.7423	23.8	0.652	-0.4277
0.5	0.25				38.9	0.424	-0.8580	24.3	0.644	-0.4401	11.8	0.999	-0.0010
0.4	0.16	10.4	1.067	0.0649	16.8	0.82	-0.1985	10.6	1.058	0.0564	5.4	1.465	0.3819
0.3	0.09				6.3	0.1274		4.8	1.55	0.4383	3.5	1.824	0.6010
0.2	0.04	5.3	1.472	0.3866	2.5	2.312	0.8381	2.4	2.362	0.8595	2.4	2.385	0.8692
∞		54.6	0.267										

Appendix III: Records of measured transmittance (T) and absorbance (A) of sodium citrate with respect to time (min) and boundary height *x* (cm)

Mass of Sodium Citrate 0.0041 g													
Time (min)		20			60			120			170		
X	X^2	T	A	ln A	T	A	ln A	T	A	ln A	T	A	ln A
1	1	87.2	0.06	-2.8134	75.1	0.127	-2.0636	44.4	0.36	-1.0217	25	0.619	-0.4797
0.8	0.64	67.4	0.174	-1.7487	66.7	0.179	-1.7204	24.2	0.633	-0.4573	12.3	0.951	-0.0502
0.7	0.49	38	0.429	-0.8463	54.3	0.27	-1.3093	14.3	0.877	-0.1312	6	1.31	0.2700
0.6	0.36	25.3	0.613	-0.4894	28.4	0.561	-0.5780	5.2	1.397	0.3343	3.3	1.665	0.5098
0.5	0.25	11.2	0.995	-0.0050	15	0.86	-0.1508	2.7	1.816	0.5966	2	2.068	0.7266
0.4	0.16	4.4	1.493	0.4008	5.5	1.36	0.3075	1.7	2.33	0.8459	1.8	2.215	0.7953
0.3	0.09	6.2	1.203	0.1848	2.3	1.971	0.6785	1.6	2.43	0.8879	1.4	2.71	0.9969
0.2	0.04	2.9	1.758	0.5642	1.4	2.619	0.9628	1.5	2.459	0.8998	1.5	2.552	0.9369
∞		2.8	1.803										

Appendix IV: Records of measured transmittance (T) and absorbance (A) of sodium nitrite with respect to time (min) and boundary height *x* (cm)

Mass of sodium nitrite 0.0024 g										
Time (min)		30			60			90		
X	X^2	T	A	ln A	T	A	ln A	T	A	ln A
0.8	0.64	73.6	0.133	-2.0174	41.7	0.380	-0.9676	29.9	0.524	-0.6463
0.7	0.49							25.4	0.594	-0.5209
0.6	0.36	70.6	0.151	-1.8905	32.8	0.485	-0.7236	17.9	0.748	-0.2904
0.5	0.25	61.5	0.213	-1.5465				9.0	1.047	0.0459
0.4	0.16	53.0	0.275	-1.2910	11.0	0.959	-0.0419	42.9	1.545	0.4350
0.3	0.09				3.4	1.471	0.3859	0.5	2.293	0.8299
0.2	0.04	25.0	0.658	-0.4186	1.3	1.871	0.6265	0.1	3.000	1.0986
∞		1.1	2.956							

Appendix V: Records of measured transmittance (T) and absorbance (A) of sodium nitrite with respect to time (min) and boundary height *x* (cm)

Mass of Sodium Nitrite 0.0024 g													
Time (min)		260			500			995			1150		
X	X^2	T	A	ln A	T	A	ln A	T	A	ln A	T	A	ln A
1.0	1.00	70.5	1.548	0.4370	52.2	0.285	-1.2553	50.5	0.300	-1.2040	50.8	0.297	-1.2140
0.8	0.64	54.7	0.264	-1.3318	43.6	0.364	-1.0106	46.5	0.336	-1.0906	67.0	0.332	-1.1026
0.6	0.36	37.7	0.428	-0.8486	34.6	0.466	-0.7636	43.1	0.369	-0.9970	44.4	0.357	-1.0300
0.4	0.16	23.7	0.634	-0.4557	29.3	0.540	-0.6162	40.1	0.400	-0.9163	42.1	0.380	-0.9676
0.2	0.04	15.3	0.830	-0.1863	25.2	0.606	-0.5009	38.0	0.425	-0.8557	40.4	0.398	-0.9213
∞		67.0	0.332										

Appendix VI: Data of transmittance (T) and absorbance (A) of tartaric acid with respect to time (min) and boundary height *x* (cm)

Mass of Tartaric acid: 0.0025 g													
Time (min)		20			60			120			170		
x	x^2	T	A	ln A	T	A	ln A	T	A	ln A	T	A	ln A
1	1	97.5	0.011	-4.5099	94.7	0.024	-3.7297	75	0.125	-2.0794	57.7	0.24	-1.4271
0.8	0.64	91.2	0.04	-3.2189	91.7	0.037	-3.2968	62.1	0.208	-1.5702	38.9	0.526	-0.6425
0.6	0.36	58.1	0.237	-1.4397	88	0.055	-2.9004	45.7	0.342	-1.0729	15	0.834	-0.1815
0.5	0.25	43	0.368	-0.9997	76.1	0.119	-2.1286	25.4	0.6	-0.5108	7.5	1.148	0.1380
0.4	0.16	25.2	0.604	-0.5042	57.9	0.238	-1.4355	11.2	0.966	-0.0346	2.6	1.658	0.5056
0.3	0.09	4.4	1.401	0.3372	35.2	0.456	-0.7853	4.9	1.344	0.2957	1	2.189	0.7834
0.2	0.04	6	1.252	0.2247	17.2	0.772	-0.2588	1.2	2.086	0.7352	0.8	2.359	0.8582
∞		7.2	1.164										

Appendix VII: Time t (min), boundary height *x* (cm) and square boundary height x^2 (cm^2) for ascorbic acid in 0.002 M to 0.01 M NaOH solutions

Indicator used: Phenol red										
Mass (g)	0.0041		0.0043		0.0044		0.0054		0.0059	
Concentration (M)	0.002		0.004		0.006		0.008		0.010	
Time (min)	X	X^2	X	X^2	X	X^2	X	X^2	X	X^2
10	0.27	0.0729	0.30	0.0900	0.25	0.0625	0.21	0.0441	0.21	0.0441
30	0.52	0.2704	0.41	0.1681	0.45	0.2025	0.41	0.1681	0.40	0.1600
50	0.55	0.3025	0.58	0.3364	0.53	0.2809	0.51	0.2601	0.50	0.2500
80	0.80	0.6400	0.62	0.3844	0.70	0.4900	0.66	0.4356	0.64	0.4096
110	0.84	0.7056	0.81	0.6561	0.79	0.6241	0.74	0.5476	0.73	0.5329
140	0.90	0.8100	0.88	0.7744	0.84	0.7056	0.82	0.6724	0.81	0.6561
170	1.00	1.0000	0.98	0.9604	0.96	0.9216	0.92	0.8464	0.88	0.7744
210	1.05	1.1025	1.04	1.0816	1.00	1.0000	1.00	1.0000	0.94	0.8836
260	1.20	1.4400	1.06	1.1236	1.06	1.1236	1.04	1.0816	1.02	1.0404
290			1.20	1.4400	1.10	1.2100	1.10	1.2100		
320					1.16	1.3456	1.15	1.3225		

Appendix IIX: Time t (min), boundary height *x* (cm) and square boundary height x^2 (cm^2) for citric acid in 0.002 M to 0.01 M NaOH solutions

Indicator used: Phenol red										
Mass (g)	0.0041		0.0043		0.0044		0.0054		0.0056	
Concentration (M)	0.002		0.004		0.006		0.008		0.010	
Time (min)	X	X^2	X	X^2	X	X^2	X	X^2	X	X^2
10	0.40	0.1600	0.39	0.1521	0.28	0.0784	0.30	0.0900	0.30	0.0900
30	0.55	0.3025	0.48	0.2304	0.42	0.1764	0.40	0.1600	0.22	0.0484
50	0.64	0.4096	0.62	0.3844	0.60	0.3600	0.55	0.3025	0.42	0.1764
80	0.82	0.6724	0.72	0.5184	0.70	0.4900	0.70	0.4900	0.60	0.3600
110	0.90	0.8100	0.80	0.6400	0.75	0.5625	0.72	0.5184	0.66	0.4356
140	1.00	1.0000	0.90	0.8100	0.84	0.7056	0.80	0.6400		
170	1.02	1.0404	0.98	0.9604	0.96	0.9216	0.82	0.6724		
210			1.02	1.0404	1.04	1.0816	0.90	0.8100		
260			1.15	1.3225	1.06	1.1236	0.98	0.9604		
290					1.15	1.3225	1.03	1.0609		

Appendix IX: Time t (min), boundary height *x* (cm) and square boundary height x^2 (cm^2) for sodium citrate in 0.002 M to 0.01 M HCl solutions

Indicator used: Thymol Blue										
Mass (g)	0.0088		0.0090		0.0091		0.0095		0.0097	
Concentration (M)	0.002		0.004		0.006		0.008		0.010	
Time (min)	X	X^2	X	X^2	X	X^2	X	X^2	X	X^2
15	0.47	0.2210	0.45	0.2025	0.42	0.1764	0.40	0.1600	0.40	0.1600
45	0.60	0.3600	0.65	0.4225	0.43	0.1849	0.56	0.3136	0.50	0.2500
75	0.84	0.7056	0.82	0.6724	0.62	0.3844	0.62	0.3844	0.60	0.3600
105	1.20	1.4400	1.01	1.0201	0.86	0.7396	0.84	0.7056	0.74	0.5476
165			1.16	1.3456	1.02	1.0404	0.94	0.8836	0.88	0.7744
195			1.26	1.5876	1.14	1.2996	1.10	1.2100	0.96	0.9216
255					1.25	2.5625	1.19	1.4161	1.12	1.2544

Appendix X: Time t (min), boundary height *x* (cm) and square boundary height x^2 (cm^2) for sodium nitrite in 0.002 M to 0.01 M HCl solutions

Indicator used: Bromophenol Blue										
Mass (g)	0.0100		0.0104		0.0106		0.0106		0.0107	
Concentration (M)	0.002		0.004		0.006		0.008		0.010	
Time (min)	X	X^2	X	X^2	X	X^2	X	X^2	X	X^2
15	0.50	0.2500	0.45	0.2025	0.42	0.1764	0.40	0.1600	0.40	0.1600
45	0.80	0.6400	0.75	0.5625	0.76	0.5776	0.65	0.4225	0.60	0.3600
75	1.01	1.0201	0.94	0.8836	0.84	0.7056	0.80	0.6400	0.75	0.5625
105	1.20	1.4400	1.05	1.1025	0.98	0.9604	0.90	0.8100	0.82	0.6724
135	1.36	1.8496	1.20	1.4400	1.16	1.3456	1.10	1.2100	0.95	0.9025
195	1.50	2.2500	1.34	1.7956	1.26	1.5876	1.20	1.4400	1.08	1.1664
255	1.75	3.0625	1.47	2.1609	1.42	2.0164	1.36	1.8496	1.26	1.5876

Appendix XI: Time t (min), boundary height *x* (cm) and square boundary height x^2 (cm^2) for tartaric acid in 0.002 M to 0.01 M NaOH solutions

Indicator used: Bromotymol blue										
Mass (g)	0.0041		0.0043		0.0047		0.0049		0.0050	
Concentration. (M)	0.002		0.004		0.006		0.008		0.010	
Time (min)	X	x^2	X	X^2	X	X^2	X	X^2	X	X^2
10	0.40	0.1600	0.39	0.1521	0.28	0.0784	0.30	0.0900	0.30	0.0900
30	0.55	0.3025	0.48	0.2304	0.42	0.1764	0.40	0.1600	0.40	0.1600
50	0.60	0.3600	0.62	0.3844	0.60	0.3600	0.55	0.3025	0.51	0.2601
80	0.82	0.6724	0.72	0.5184	0.70	0.4900	0.70	0.4900	0.65	0.4225
110	0.90	0.8100	0.80	0.6400	0.75	0.5625	0.72	0.5184	0.69	0.4761
140			1.00	1.0000	0.90	0.8100	0.84	0.7056	0.76	0.5776
170			1.02	1.0404	0.98	0.9604	0.96	0.9216	0.87	0.7569
210					1.10	1.2100	1.04	1.0816		
250					1.15	1.3225	1.08	1.1664		
270							1.15	1.3225		