"ELECTROCHEMICAL AND PHYSICOCHEMICAL STUDY OF PYRIDOXINE HYDROCHLORIDE (VITAMIN B_6)"

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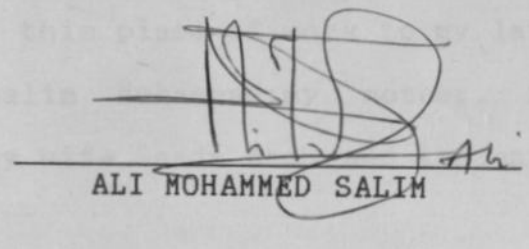
A thesis submitted in partial fulfillment for the degree of MASTER OF SCIENCE of the University of Nairobi.

April, 1991.

DECLARATION

This is my original work and has not been presented to any other university.



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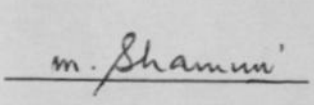
This work has been submitted for examination with our approval as university supervisors.



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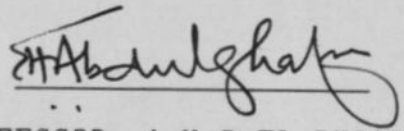

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DEDICATION

I dedicate this piece of work to my late father, Muhammad Salim Muhammad;my mother, Siraj Ali Bwanaheri; my wife Saada Abdu and the entire Muslim world.

ACKNOWLEDGMENT.

My first and foremost gratitude goes to The All-Mighty Allah for giving me the energy to do and complete this piece of work.

I greatly appreciate the active assistance and guidance I received from my supervisor Dr.M.Shamim. It is the guidance bestowed to him by God that made this work possible. My sincere gratitude and prayers goes to Dr Abdurahaman El-Sumaity of Kuwait for paying the required dues to the university. I also thank Professor A.H.S. El-Busaidy, Professor R.M Munavu and the Director of The Board of Postgraduate Studies for having made it possible for me to receive monthly stipend during my studies.

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TABLE OF CONTENTS

ABSTRACT

Vitamin B_6, 5 - Hydroxy - 6 - methyl 3, 4- Pyridine dimethanol - commonly occurs as hydrochloride. It exists as a cation, a zwitterion and an anion depending on the pH of the medium.

Despite the wide scope of studies on Vitamin B_6, no work had been done on density, refraction, conductivity and depression of freezing point. Nevertheless, a lot of work had been done on pK_a of the phenolic and pyridine hydrogens, but not on the dissociation that leads to the cationic structure in acidic media. Studies on these four aspects have, therefore, been undertaken.

The work has produced equations that relate concentration to the above mentioned four properties. The partial molar volume, $\overline{V}$, of fully dissociated Vitamin B_6 Hydrochloride has been found to be 79.00 cm^3mol^{-1} while that of Vitamin B_6 moeity with an attached proton to be 50.49 dm^3Mol^{-1}. Extrapolation of the density data yielded the density of solid Vitamin B_6 as 1.44 gcm^{-3}. Refraction measurements gave n- the refractive index of Vitamin B_6 Hydrochloride as 2.8421 and its molar refraction, $\overline{R}_D$, as 54.2624. Conductance measurements have yielded Λ^o = 142.58, $\Lambda_{VB_6H^+}$ = 66.23 Scm^2equiv^{-1} and K_a = 7.2867 x 10^{-2}.

LIST OF SYMBOLS

A	Constant in Kohlrausch equation
A	Cross-sectional area
å	Distant of closest approach of ions, Å
B_1	Parameter of conductance.
B_2	Parameter of conductance.
B_3	Parameter of conductance.
b	Constant in DHO extended Limiting equation
c	Concentration, Mol l^{-1}
C	Concentration of solute in gl^{-1}
D	Constant in DHO extended Limiting equation
D	Constant in equation 2.2.9
d	Density of solution
d_1	Density of solute
d_o	Density of solvent
F_{rel}	Ionic field
$f_{\pm}^2$	Square of mean activity coefficient
I	Ionic strength
I	Current in Ampere
J	Cell Constant.
K	Constant in equation 2.1.5
K	Equilibrium constant
K_D	Dissociation constant
K_d	Density increment
K_f	Molal freezing depression constant

κ	Specific conductance
l	Ionic mobility
l_+, l_-	Ionic mobility of Positive , Negative ion
L	Effective length
M_1	Molecular weight of solvent
M_2	Molecular weight of solute
m	Mass
m	Molality, Mol kg^{-1}
m'	Effective Molality
η	Constant in equation 2.2.9.
n	Refractive index of solution
n	Effective number of species
n_i	Number of moles of subscripted component
n_1	Refractive index of solute
$\overline{R}$	Resistance in Ohms
$\overline{R}_1$	Molar Refraction of solvent.
$\overline{R}_2$	Molar Refraction of solute.
$\overline{R}_D$	Molar Refraction of solution.
r_1	Specific refraction of solvent
S	Amount of ice separated at equilibrium
S_v	Constant in Masson's equation
$S(Z)$	A function in 2.2.24
T	Correct temperature of a component
T_c	Lowest temperature attainable.
T_f	Freezing point of the solute in a solvent.
T_o	Freezing point of pure solvent

V	Volume
V	Potential difference in Volts
$\overline{V}^{\circ}$	Partial Molar Volume at standard state
$\overline{V}_i$	Partial Molar Volume of component i
V_o	Volume of pure solvent
VB_6	Vitamin B6 moiety.
W_1	Actual weight of solute
W_2	Actual weight of solvent
X'	Mole fraction of solvent
X	Mole fraction of solute
Z_+, Z_-	Algebraic charge number of positive and negative ions.
Z_i	Algebraic charge number of ion i
α	Degree of dissociation.
α_1, α_2	Angle of incidence and refraction respectively.
ε	Correction factor, 0.54×10^{-3}
Δ	Delta, a change in quantity
ΔT_f	Freezing point depression
Λ	Conductance.
Λ°	Conductance at infinite dilution
ρ	Resistivity
ρ	Density
ϕ_v	Apparent (fictive) Molar Volume
ϕ_v°	Apparent Molar Volume at Infinite dilution
$\phi_{v(c)}$	Apparent Molar Volume on molar scale.

$\phi_{v(m)}$ Apparent Molar Volume on Molal scale.

LIST OF TABLES.

LIST OF FIGURES

APPENDICES

CHAPTER 1

INTRODUCTION

1.1.0 LITERATURE SURVEY

A literature survey for the Vitamin B_6 and its analogs was carried out covering the period extending from 1917 to 1988. The survey revealed that no work had been done on viscosity, partial molar volume, refractive index, conductance and freezing point depression. The widely studied aspect is the thermodynamic dissociation constants using spectrophotometric methods namely U.V and I.R. The survey also revealed that all the studies have confirmed the existence of Vitamin B_6 in three forms depending on whether the solution is acidic, neutral or alkaline. Given that HCl is a strong electrolyte, it was anticipated that the Vitamin B_6.HCL would behave as a strong electrolyte in water as solvent.

1.2.0 OBJECTIVE

The objective of the study was to investigate some physico-chemical aspects of Vitamin B_6.HCL at 25°C. Accordingly depression of freezing point, conductance, density and refractive index measurements were undertaken in aqueous solutions.

It is expected that the study would help understand the Chemistry of Vitamin B_6 in a situation resembling that in the human body, since the human body is a complex system of solution with different pH´s in different regions. For example Pyridoxal 5´-Phosphate is the co-enzyme for a variety of reaction involving transamination, decarboxylation and other transformation of amino acids. Detailed understanding of the mechanism of the action of these co-enzyme requires a knowledge of their ionization constant. Such information can be used to interpret the effect of pH on the binding of co-enzyme to either metals, apoenzymes or the substrate.[1]

1.3.0 CHEMISTRY OF VITAMIN B_6

1.3.1 IMPORTANCE, HISTORY & STRUCTURE

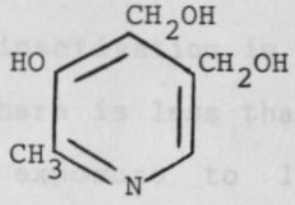
Vitamins are organic molecules that are needed in small amounts in the diet of higher animals to sustain life. They are broadly divided into two major groups. The fat-soluble ones are designated by the letters A, D, E & K and the water-soluble ones by the letters B and C. Most of the B types are components of co-enzymes. This is attributed to their ability to take part in reversible redox processes.[2,3,4]

Vitamin B_6 was first discovered as an essential nutrient by Birch and Gjorgy in 1936 and was isolated and identified in 1938-1939 by several group workers.[5,6,7,8,9,10] For a few years after the discovery, the names Alderman (in Germany) and Pyridoxine (in Britain and America) were used for Vitamin B_6. Evidence of occurrence in natural product of substances surpassing Pyridoxine in growth promoting activity for Lactic Acid Bacteria led to the discovery of two other related substances Pyridoxal and Pyridoxamine. The term Vitamin B_6 in chemical literature refers to any one of the three.[11]

The author´s subject of research is Pyridoxine which is also known as Pyridoxol. Its IUPAC name is

5-hydroxy-6-methyl 3,4-Pyridine dimethanol or 2-methyl-3-hydroxy-4-5-dihydroxymethanol Pyridine[12]

Figure: 1.3.1A



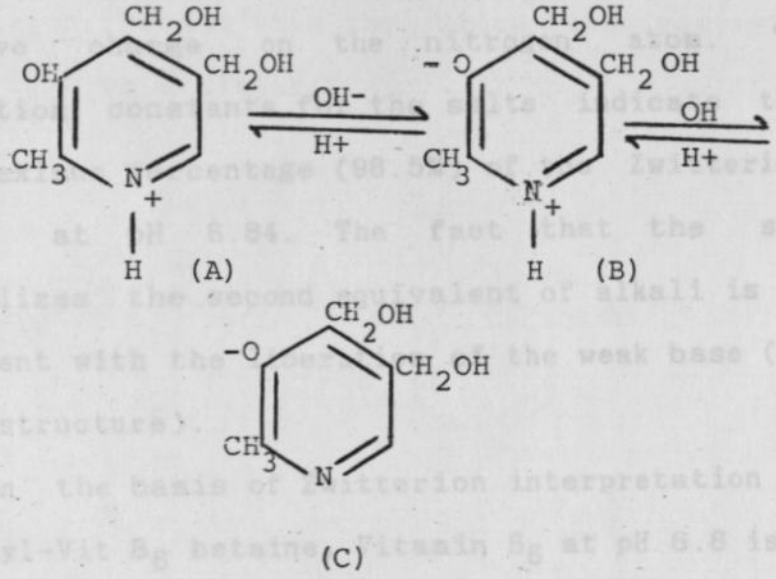
Vitamin B_6 has broad distribution through out plant and animal kingdom. It is also found as a 5′-Phosphate ester. Most of it i.e. 60-80% is chemically bound more or less tightly to protein or starch.[13,14,15] It has an empirical formula $C_8H_{11}NO_3$, a molecular weight of 169.28 and melting point of 160°C. It is a colourless crystalline powder commonly occurring as salt such as pyridoxine hydrochloride (one used in this work). The pyridoxine hydrochloride is a white substance with molecular weight of 205.64. It forms platelets or thick birefringent rods from alcohol which melt with decomposition at 205°C - 212°C. One gram of the salt dissolves in about 4.5 cm^3 of water or 90 cm^3 of ethanol, the pH of a 10% solution in water is 3.2. It is soluble in propylene glycol,sparingly soluble in acetone and insoluble in ether or

chloroform. Acidic solutions of the salt are stable and may be heated for thirty minutes at 120°C without decomposition.[16] Both the base and the salt are optically inactive, they sublime readily and dialyse very easily. Pyridoxine is destroyed by light. The inactivation is pH dependent. In acidic solution there is less than 10% inactivation even on longer exposure to light. In neutral and alkaline solution there is marked instability i.e. over 90% is rendered inactive. This photolysis is not affected by the presence of air. It is stable to strong acids like hydrochloric and sulphuric at 100°C and strong alkalis. However HNO_3 destroys pyridoxine presumably because of its oxidative action. However in acid solution it is destroyed by manganese dioxide. Hydrogen peroxide does so only at elevated temperatures.[17] It is also destroyed by prolonged heating in the presence of protein and by U.V. radiation in neutral or alkaline solution.[18,19,20] All the three oxygens of the molecule are present in the form of hydroxyl group. Analysis indicates that one of the three OH's is phenolic (or enolic) and the other are aliphatic OH groups.[21,22]

The salt neutralises two equivalents of alkali in the pH range of 3 to 11.5. The mid point occurs at pH 4.72 - 8.92 respectively. The

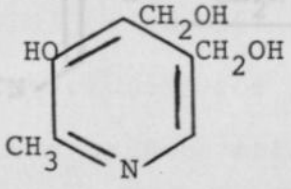
neutralization of the first equivalent of alkali by the salt indicated alkali-binding groups were involved. Accordingly, the equilibria

Figure: 1.3.1B The ionic structures of vitamin B_6



are assumed to represent the successive stages of acidic ionization of Pyridoxine Hydrochloride. In neutral medium there is small amount of uncharged form

Figure: 1.3.1C The uncharged structure of vitamin B_6

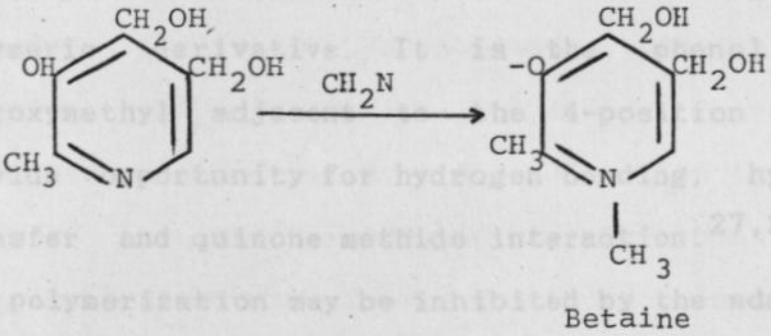


In alcoholic solution the uncharged form predominates.[23]

The enhancement of the phenolic hydrogen in this compound is readily explained qualitatively in terms of electrostatic effect arising from the net positive charge on the nitrogen atom. Two ionization constants for the salts indicate that the maximum percentage (98.5%) of the Zwitterions exists at pH 6.84. The fact that the salt neutralizes the second equivalent of alkali is in agreement with the liberation of the weak base (the third structure).

On the basis of Zwitterion interpretation of N-methyl-Vit B_6 betaine, Vitamin B_6 at pH 6.6 is to be regarded as existing essentially as the Zwitterion (B)

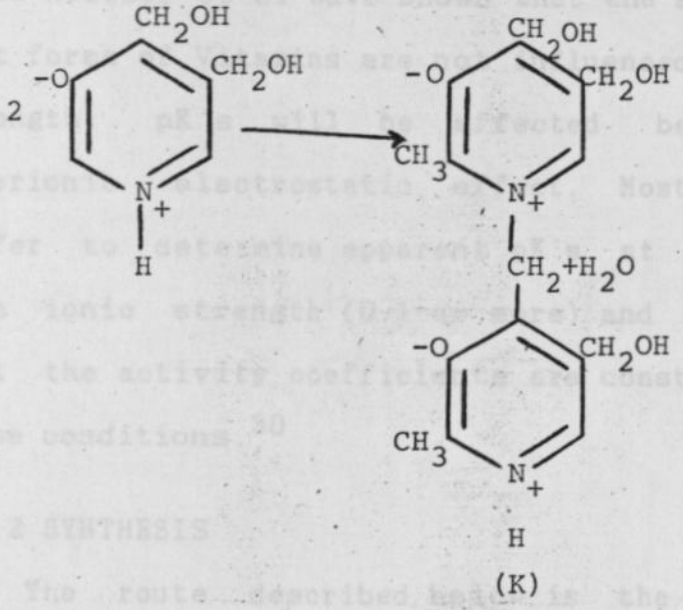
Figure: 1.3.1D



Betaine

The maxima at 2550 Å at pH 6.6 shifts progressively to 2470 Å at pH 10.2. The changes represent structural change from (B) to (C)[24,25,26] Polymerization has been found to occur only in neutral solution. It is thought probably that the Zwitterion reacted with the active 4-hydroxymethyl group to give a dimmer K.

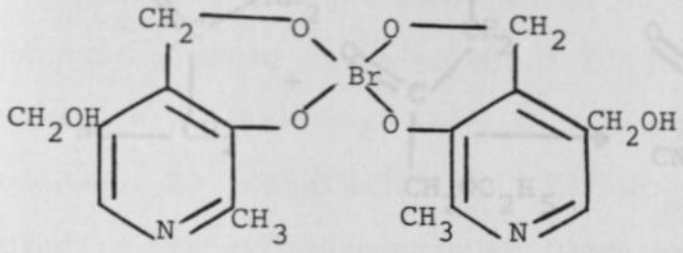
Figure: 1.3.1E



(K)

K is capable of further condensation to give a polymeric derivative. It is the phenol and hydroxymethyl adjacent to the 4-position that provide opportunity for hydrogen bonding, hydride transfer and quinone methide interaction.[27,28] The polymerization may be inhibited by the addition of borate which ties up the hydroxyl group in the 3 and 4 position of Pyridoxol.[29]

Figure: 1.3.1F The inhibited zwitterion

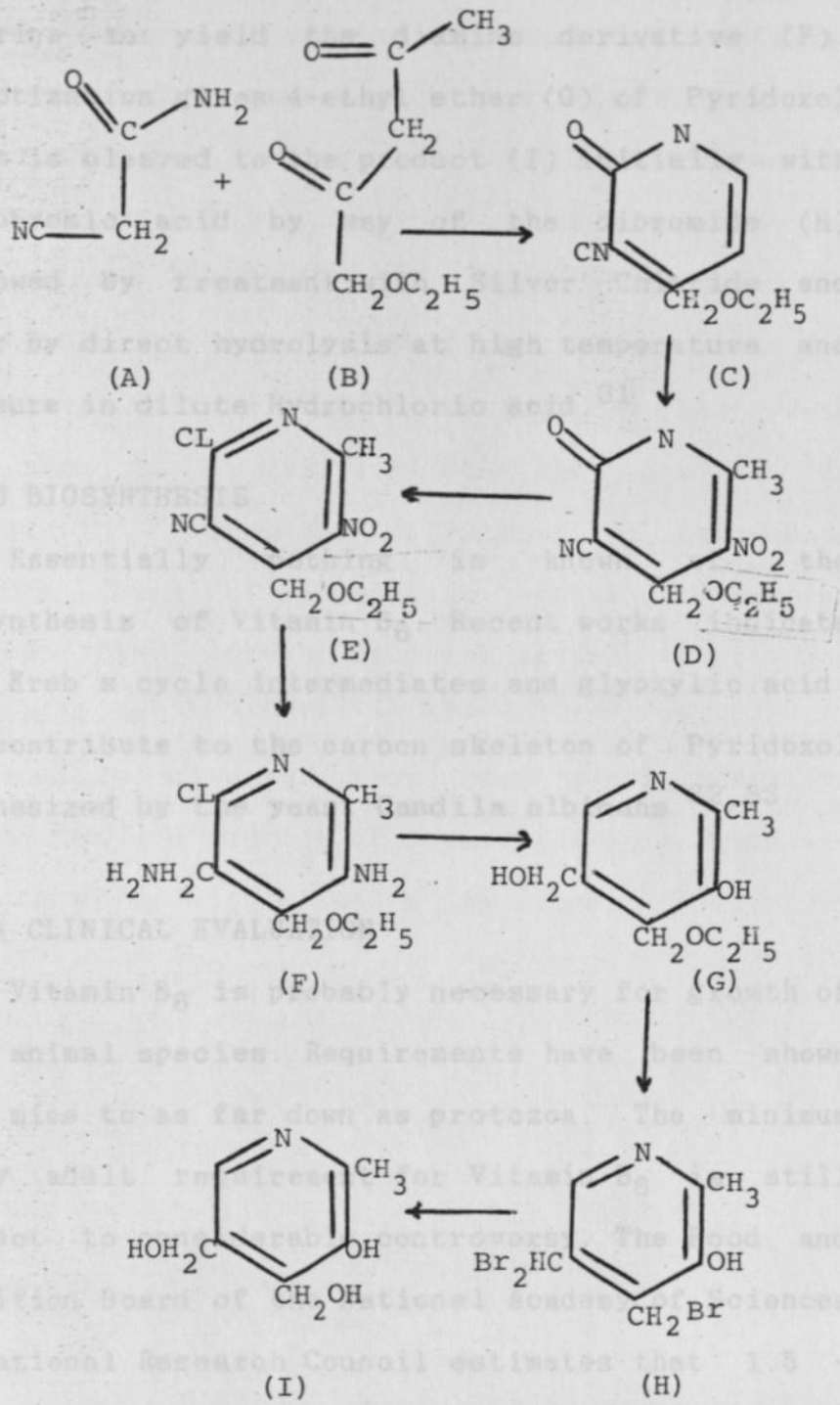


While Morozor et al have shown that the spectra of most forms of Vitamins are not influenced by ionic strength, pK's will be affected because of interionic electrostatic effect. Most workers prefer to determine apparent pK's at relatively high ionic strength (0.1 or more) and to assume that the activity coefficients are constant under these conditions.[30]

1.3.2 SYNTHESIS

The route described below is the basis of commercial synthesis. Condensation of ethoxyacetylacetone (B) with Cyanoacetamide (A) in the presence of Piperidine yields 3-cyano-4-ethoxymethyl-2(1H)-Pyridone (C). Nitration to D is effected in Acetic Anhydride and treatment with PCL_5 in Chlorobenzene yields the Chloro derivative (E). Catalytic hydrogenation in two steps reduces the nitro group, then attacks the nitrile and the

Figure: 1.3.1G



chlorine to yield the diamino derivative (F). Diazotization gives 4-ethyl ether (G) of Pyridoxol which is cleaved to the product (I) initially with hydrobromic acid by way of the dibromide (H) followed by treatment with Silver Chloride and later by direct hydrolysis at high temperature and pressure in dilute Hydrochloric acid.[31]

1.3.3 BIOSYNTHESIS

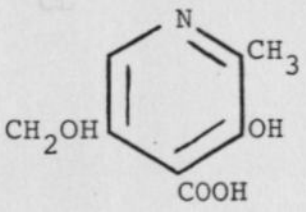
Essentially nothing is known of the biosynthesis of Vitamin B_6. Recent works indicate that Kreb´s cycle intermediates and glyoxylic acid may contribute to the carbon skeleton of Pyridoxol synthesized by the yeast **Candila albicans.**[32,33]

1.3.4 CLINICAL EVALUATION

Vitamin B_6 is probably necessary for growth of all animal species. Requirements have been shown for mice to as far down as protozoa. The minimum daily adult requirement for Vitamin B_6 is still subject to considerable controversy. The Food and Nutrition Board of the National Academy of Sciences - National Research Council estimates that 1.5 - 2.0mg per day is reasonable allowance although there is some feelings that this is too low.[33,34] The functions of Vitamin B_6 are closely related to protein metabolism i.e. synthesis or breakdown of

amino acids e.g. the 5´phosphate catalyse transamination, racemization and decarboxylation, dehydration of serine and desulfahydration of cystein. Some Vitamin B_6 is also required in the production of antibodies, the conversion of tryptophan to niacin, the formation of heme in hemoglobin. More Vitamin B_6 is required when diets are high in protein than when they are low in protein.[35,36] Symptoms of Vitamin B_6 deficiency include loss of appetite, vomiting, nervous irritability, anemia, fits and mental retardation in children[37,38,39,40,41,42,43,44,45]. Vitamin B_6 is readily absorbed from the gastrointestinal tract. The end product of metabolism is 4-Pyridoxic Acid which is eliminated in the urine.[46,47]

Figure 1.3.1I



(4-Pyridoxic acid)

3-hydroxy-5-hydroxymethyl-2-methylsonicotinic acid

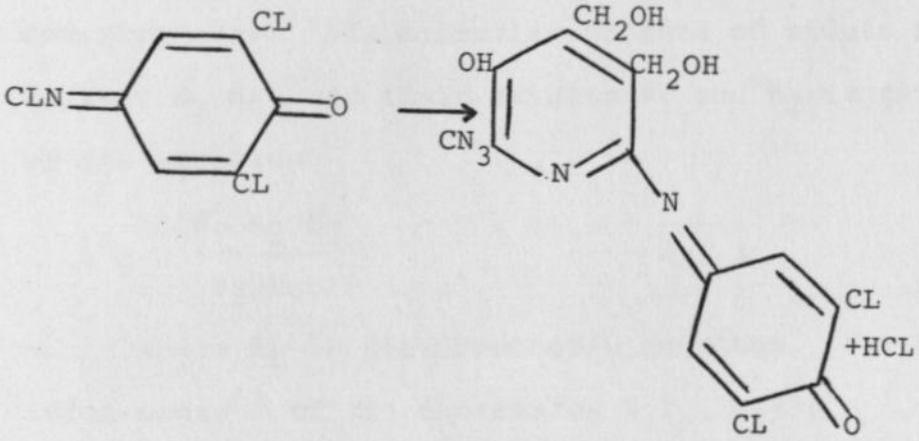
Unlike the other water-soluble Vitamins whose concentration in red blood cells are higher than in

plasma, the concentration of Pyridoxine is higher in plasma than in red cells. In one study of the Vitamin B_6 level for whole blood, red cells and plasma of normal subject were reported to be 37 ±6, 20 ±3 and 58 ±3 $ngml^{-1}$ (± Mean SD).[48]

1.3.5 CHEMICAL ASSAY

The phenolic nature of Pyridoxol has been the basis for its chemical determination through coupling with 2,6-dichloro-p-quinone chlorimide. In the presence of borate, Pyridoxol forms a complex which does not react with chlorimide, allowing the determination of blank.[49,50]

Figure 1.3.1J



The term vitamin B_6 moiety is used to mean a portion of the structure that is typical of the viatmin B_6. In this case anything referred to as vitamin B_6 or its moiety must have the structure shown in figure 1.3.1A as the main component. Vitamin B_6 moiety with a proton attached is shown in structure A in figure 1.3.1C. Pyridoxine hydrochloride therefore dissociates to give the described moiety and the chloride ion.

CHAPTER 2

2.0.0 THEORETICAL

2.1.0 DEPRESSION OF FREEZING POINT

2.1.1 DERIVATION OF EQUATIONS

When a substance is dissolved in a given liquid solvent, the freezing point of the solvent is lowered. This phenomenon constitute a colligative property, the magnitude of which is proportional to the number of moles of the substance that are present in a given amount of solvent. The amount, ΔT_f, by which the freezing point is lowered is called the Freezing Point Depression.

The relationship between the lowering of freezing point ΔT_f, molecular weights of solute and solvent M_1, M_2, and their weights W_1 and W_2 is given by the equation

$$\Delta T_f = \frac{K_f . W_2 . M_1}{M_2 . W_1} \qquad \text{-----2.1.1}$$

where K_f is the Cryoscopic constant. Further refinements[51] of the expression 2.1.1 gives

$$\Delta T_f = \frac{mK_f}{(1 + \varepsilon \Delta T_f)} \qquad \text{-----2.1.2}$$

$$\Delta T_f = \frac{1000 W_2 K_f (1 - \varepsilon \Delta T_f)}{M_2 W_1} \quad \text{----2.1.3}$$

$$M_2 = \frac{1000 W_2 K_f (1 - \varepsilon \Delta T_f)}{W_1 \cdot \Delta T_f} \quad \text{----2.1.4}$$

where ε is a correction factor for ΔT_f and has a value of 0.54×10^{-3}. The errors involved in the experimental determination of ΔT_f is summarized by the equation.

$$T_t = \frac{K(T_c - T_t)}{S} \quad \text{-----2.1.5}$$

where T_t, T, T_c are the recorded, correct and lowest temperatures respectively; and S is the amount of ice separated at equilibrium and K is a constant inversely proportional to the heat of insulation.

2.1.2 DEVIATION FROM DEPRESSION OF FREEZING POINT

Binary solutions are characterised by similar solvent-solvent, solvent-solute and solute-solute interaction. In addition, the molecular volume of each species should be the same and there are no volume and heat changes on mixing the two. In practice most systems encountered are non - ideal. Raoult's Law applies to ideal solutions very well and in the non-ideal solution if the solution is

dilute where the mole fraction approaches unity. However, this applies only if the solute does not change its molecular identity. Within the limitation of dilute solution, deviation from the depression of freezing point law may arise owing to the following:

a) The solute dissociates to form two or more fragments or ions thus increasing the effective number of moles of solute. The depression will be greater since the colligative property is dependent on the number rather than the nature of the solute present. For strong electrolyte such as NaCl[52] where complete dissociation takes place, the depression of freezing point has been observed to be twice of that expected for an ideal case. The phenomenon corresponds to most weak and strong electrolytes in which ionization increases the effective number of solute particles.

b) The solute associates to form dimers or trimers, and hence the effective number of moles of solute is reduced and the observed depression will be less than that expected for the ideal case. Acetic Acid is a case in point. Its lowering of freezing point in Naphthalene is less than that of Benzene or Biphenyl. Both of which show essentially ideal behavior in Naphthalene.[53,54]

c) The solvent A and solute B combine partially in the liquid phase to form a third substance AB. The formation of AB decreases the number of solute molecules and free solvent. Hence the mole fraction of solvent is smaller while for the solute is greater than expected, In this case the depression of freezing point is more than predicted by the law. M-Dinitrobenzene forms such a molecular compound with Naphthalene[55] and Picric Acid in Naphthalene show a similar deviation.[56]

2.1.3 MODIFICATION OF DEPRESSION OF FREEZING POINT EQUATION

The general form of the depression of freezing point expression for ideal binary solution is[57]

$$\Delta T_f = mK_f \qquad \text{------2.1.6}$$

and the more accurate form as[58]

$$\Delta T_f = \frac{mK_f}{(1 + \varepsilon\Delta T_f)} \qquad \text{-----2.1.7}$$

For non-ideal electrolytes, the interaction of the ions results in a change in the number of solute molecules. The depression increases with increase in the number of solute molecules or decreases with decrease in the number of solute. For NaCl, a strong electrolyte, the depression of freezing point has been observed to be twice that

of the expected for an ideal case. To accommodate the deviation from ideality, the effective number of solute species (molecules or ions) present in solution at equilibrium should be introduced in the equation i.e.

$$\Delta T_f = nmK_f \qquad \text{------2.1.8}$$

and

$$\Delta T_f = \frac{nmK_f}{(1 + \varepsilon\Delta T_f)} \qquad \text{-----2.1.9}$$

where n is the number of species produced by a mole of solute. For a 1:1 electrolyte solution, n equals 2. In cases of non-ideal solutes the molality m, is ideally the total effective molality-the number of moles of all solute species present, whether ionic or molecular.

In acidic medium Vitamin B_6 dissociates like a weak electrolyte.

$$VB_6HCl \rightleftharpoons VB_6H^+ + Cl^-$$

and the equilibrium concentrations of VB_6HCl, VB_6H^+ and Cl^- will be $m(1 - \alpha)$, $m\alpha$ and $m\alpha$ respectively, where α is the degree of dissociation and m is the molality. The total molality m′ of all solute species will thus be

$$m' = m(1 + \alpha) \qquad \text{----------2.1.10}$$

$$= mn$$

Hence $n = (1 + \alpha)$

The equilibrium constant K can be calculated from the equation[51]

$$K = \frac{c\alpha f_{\pm}^2}{(1 - \alpha)} \qquad \text{-----2.1.11}$$

where $f_{\pm}^2$ is the mean activity coefficient given by the equation

$$f_{\pm}^2 = \frac{-A|Z^2|(c\alpha)^{0.5}}{1+B_3\mathring{a}(c\alpha)^{0.5}} \qquad \text{-----2.1.12}$$

Where A = 0.5115, B = 0.3248×10^8 and $\mathring{a}$ = 5Å respectively. Equation 2.1.11 yields

$$\alpha = \frac{-K \pm \{K^2 + 4f_{\pm}^2 K\}^{0.5}}{2cf_{\pm}^2} \qquad \text{-----2.1.13}$$

Calculations are initiated by putting K arrived at by conductance measurements and choosing an arbitrary value of α. The iteration is continued till α is constant. The final α is the one used in $(1 + \alpha)$ to find n.

2.2.0 CONDUCTANCE

2.2.1 BASIC CONCEPTS AND DEFINITIONS

Both solids and electrolyte solutions conduct electricity. In solids,it is conducted by electrons whereas in solutions it is conducted by ions. Both obey Ohm's Law i.e.

$$I = \frac{V}{R} \qquad \text{-----2.2.1}$$

where V is the potential difference in volts across the resistance, R,(Ohm) carrying a current, I, (ampere).

As for the solutions, the resistance, offered by any electrolyte solution to the flow of electric current depends upon the effective length, L, and the cross-sectional areas, A, of the liquid column between the electrode.; Thus

$$R \propto \frac{L}{A}$$

or

$$R = \rho\frac{L}{A} \qquad \text{-----2.2.2}$$

ρ is called the resistivity of the solution. Equation 2.2.2 can be written as

$$\frac{1}{\rho} = \frac{(1 \times L)}{(R \times A)}$$

or

$$\kappa = \frac{(1 \times L)}{(R \times A)} \qquad \text{-----2.2.3}$$

the quantity $1/\rho$ is termed as conductivity or specific conductance (κ) and may be defined as the conductance of any solution held between two electrode 1 cm^2 in area and 1 cm apart.

The units of specific conductivity are obviously

$$\kappa = \frac{(1 \times L)}{(R \times A)} \quad \frac{Ohm^{-1} \times cm}{cm^2} = Ohm^{-1}cm^{-1}$$

or Scm^{-1}

In the measurements of conductivities of electrolyte solutions L and A are fixed, hence the quantity L/A is always constant, it is called the cell constant of the conductance cell and it is designated by the letter J so that

$$J = \kappa R$$

$$\kappa = J/R \qquad \text{------ 2.2.4}$$

The equivalent or molar conductance, Λ of a solution is defined by equation

$$\Lambda = \frac{1000\kappa}{[X]} \qquad \text{------ 2.2.5}$$

depending on the choice of concentration units.

2.2.2 KOHLRAUSCH'S LAW AND EQUATION.

Kohlrausch extensively studied equivalent conductance of various electrolytes. As a result he proposed the following:

(a) Law of Independent Migration of Ions

According to this law, the equivalent conductance of an electrolyte solution of moderate concentration is additively composed of the mobilities of the constituent ions.

$$\Lambda = l_- + l_+$$

Stated differently, the equivalent conductance of electrolyte AB at infinite dilution Λ°_{AB} is equal to the sum of the equivalent conductance of ions A^+ and B^- at infinite dilution, $\lambda^\circ_{A^+}$ and $\lambda^\circ_{B^-}$

$$\Lambda^\circ_{AB} = \lambda^\circ_{A^+} + \lambda^\circ_{B^-} \qquad \text{----- 2.2.6}$$

(b) Empirical Conductance Equation

He also proposed an empirical equation connecting observed equivalent conductance to concentration. Thus

$$\Lambda = \Lambda^\circ - Ac^{0.5} \qquad \text{-----2.2.7}$$

where A is a constant to which he could not assign any specific physical meaning and c is concentration in g equivalent per litre. The constant A is characteristic of the salt under investigation.

It is apparent from the equation that Λ should decrease with increase in concentration. Arrhenius attempted to explain this phenomenon. The following section gives an account of his theory.

2.2.3 ARRHENIUS THEORY (1983)

According to this theory[59], the conductance of a solution depends upon:

(a) The total number of ions

(b) The charge on the ions

(c) The speed of the ions

It was assumed that the mobilities of the ions are not affected by concentration. Therefore for certain electrolytes the variation of equivalent conductance with dilution was attributed to the change in the total number of ions resulting from the dissociation of electrolyte. He suggested that the degree of dissociation, α, is given by the ratio

$$\alpha = \frac{\Lambda}{\Lambda^{\circ}} \qquad \text{-------2.2.8}$$

where Λ° is the conductance at infinite dilution and Λ is conductance at finite concentration.

Arrhenius theory did explain very well the concentration dependence of equivalent conductance in weak electrolyte solutions but not in strong electrolytes which ionize completely at all reasonable concentrations. A theory explaining both types of solutions was therefore imperative.

2.2.4 INTERIONIC THEORY

At finite temperatures, the ions in solution tend to have a limited structural arrangement brought about by interionic attraction which causes the mean distance between oppositely charged ions to be smaller than that between ions of like signs. In a time averaged frame of reference therefore, there will be more negative ions than positive ions in the region of positive ions and vise versa. The modified "structure" in ionic solutions which is the net result of electrostatic interaction and thermal motion, leads to the concept of the "ion atmosphere". The electrostatic potential energy of ionic solution thus depends upon the energy of interaction between the ions and their surrounding ionic atmosphere. The ionic atmosphere will maintain the symmetry so long as it is not exposed to applied electric field or to a shearing force.[60]

Debye and Huckel[61,62,63] developed the interionic theory upon the model described above and showed that it led to a quantitatively correct interpretation of the decrease in equivalent conductance with increasing concentration. The two forces that disturb symmetry were thus calculated and explained (see later). Following are the assumptions used in their theory.[64]

(I) Only coulombic forces are important in interionic attraction, any other intermolecular forces are negligible.

(II) The dielectric constant of the solution is not essentially different from that of the solvent.

(III) The ions can be regarded as point charges, unpolarisable and possessing a symmetrical coulombic field.

(IV) The interionic attractive potential energy is small compared with the energy of the thermal motion.

(VI) Strong electrolytes in solution are completely dissociated at all concentration in which the theory is valid.

According to Debye and Huckel the ionic-atmosphere concept gives rise to two effects, "the electrophoretic effect and the time of relaxation effect[65] which are discussed in the following sections.

2.2.5 TIME OF RELAXATION EFFECT (ASYMMETRY EFFECT)

The radius of the ionic atmosphere, is an important factor in the theory of the effect of interionic attraction on the thermodynamic properties of electrolyte solutions. In dynamic

processes like ionic migration under an applied electric field, the ionic atmosphere is continually disturbed by the undirectional movement of the central ion and is therefore continually in the process of building itself up again. Irreversible processes in ionic solution are therefore affected not only by this radius but also by the time necessary to form the ionic atmosphere i.e. the relaxation time. The net result is that the ionic atmosphere becomes unsymmetrical around an ion in motion. This asymmetry means that there is an excess negative charge "behind" a moving positive ion. This excess charge exercises an electrostatic retarding force on the moving ion, consequently the velocity of the central positive ion is reduced.[61,62,63]

2.2.6 ELECTROPHORETIC EFFECT

Another factor which opposes the motion of the ions is the tendency of the applied EMF to move the ionic atmosphere, with its associated molecules of solvent, in a direction opposite to that in which the central ion is moving. Thus the central ion has to move against the direction of the solvent molecules. It is just like swimming upstream. This effect obviously increases with increasing ionic concentration.

Both the electrophoretic and the relaxation effects provide an explanation of the fact that, although the dissociation of strong electrolytes at low concentrations is known to be practically complete, their equivalent conductance decrease with increasing concentration.[61,62,63]

Debye, Huckel and Onsager have given a quantitative treatment in terms of the two effects mentioned above. The asymmetry of the ionic atmosphere during ionic migration introduces an additional field, F_{rel} (which acts against the applied field on a given ion). In the case of binary electrolyte , F_{rel} depends on the reciprocal of the radius of ionic atmosphere, on the viscous flow of both ions and on their ionic mobilities. The conductance decrease due to the relaxation effect clearly depends upon the same factor. The effect caused by the electrophoretic field depends on the reciprocal of the ionic radius and on the viscous drag of the solvent, but is independent of any specific properties of the ions.

By expressing the magnitude of these two effects in terms of the physical properties of the ions and the solvent, the following equations[66] resulted for equivalent conductance on an ion i.

$$\Lambda_i = \lambda^{o}_i - \left[\frac{2.801 \times 10^{6} z_+ z_- q . \lambda^{o}_i}{(DT)^{3/2}\{1 + q^{0.5}\}}\right] + \left[\frac{41.25 z_i I^{0.5}}{\eta (DT)^{0.5}}\right] \quad \text{------2.2.9}$$

where
$$q = \frac{z_+ z_- (\lambda^{o}_+ + \lambda^{o}_-)}{(z_+ + z_-)(z_-\lambda^{o}_+ + z_+\lambda^{o}_-)} \quad \text{------2.2.10}$$

and the ionic strength

$$I = 1/2 \Sigma c_i z_i \quad \text{------2.2.11}$$

in which Λ_i and λ^{o}_i have the meanings defined earlier; z_+ and z_- represent the valence of the positive and negative ions respectively. D and η are the dielectric constant and the viscosity of the solvent respectively at absolute temperature T. The first term in the brackets in equation 2.2.9 accounts for the time of relaxation effect and the second for the electrophoretic effect.

For a uni-univalent electrolyte equation 2.2.9 becomes

$$\Lambda_i = \lambda^{o}_i - (B_1 . \lambda^{o}_i + 1/2\ B_2) c^{0.5} \quad \text{----2.2.12}$$

By adding two such terms-one for anion and another for the cation respectively- we get an equation for the equivalent conductance of completely ionized electrolyte. Thus for a 1:1 electrolyte the equation takes the form

$$\Lambda = \Lambda^{o} - (B_1 . \Lambda^{o} + B_2)\ c^{0.5} \quad \text{-----2.2.13}$$

which is Onsager conductance equation. A comparison

of equation 2.2.13 and 2.2.7 shows that the constant A in Arrhenius equation is equal to $(B_1 \Lambda^o + B_2)$.

For aqueous solution at 25°C, B_1=0.2300 and B_2 =60.65; these are based on the value of η=0.008903 poise and D=78.35. According to equation 2.2.13 a plot of Λ against $c^{0.5}$ should give a straight line of slope $(B_1 \Lambda^o + B_2)$. This has been found to be the case for dilute solutions of strong uni-univalent electrolyte. The applicability of this equation extends to Ionic strength, $I = 5 \times 10^{-3}$. At higher ionic concentration there is positive deviation from experimental values. This is because the derivation of equation 2.2.13 involves a number of simplifying assumptions and mathematical approximations which can only be applied to dilute solutions. Hence the equation is only a limiting equation. When $(\Lambda^o - \Lambda)$ is 10% of Λ^o, Onsager[67] found out that the error introduced in the calculation of Λ from the equation is about 1%. This equation fits very well for experimental data for many 1:1 electrolyte up to 0.002M and also for 1:2 and 1:3 electrolytes at lower concentration.

2.2.7 EXTENSION OF THE LIMITING LAWS FOR MORE CONCENTRATED SOLUTIONS.

Various attempts have been made to extend the limiting law so as to account for the deviation in concentrated solutions.[68] In very concentrated solutions a quasi-crystalline state is assumed.[69] It is often possible to extend the limiting law , equation 2.2.7 by the use of empirically determined parameters up to an ionic strength[70] of about 10^{-1}. This is important in practice because measurements at higher concentration are always simpler and less subject to error, and lead therefore to more accurate extrapolation for Λ°. Furthermore, in solvent media of low concentration constants, the Debye-Huckel limiting range is often at concentrations which are too low for accurate measurements, so that extension of the equations are essential. Shedlovsky's equation

$$\Lambda = \Lambda^{\circ} - (B_1\Lambda^{\circ} + B_2)c^{0.5} + bc(1 - B_1c^{0.5}) \qquad \text{------2.2.14}$$

fits well for many electrolytes in relatively higher concentrations but unfortunately **no simple meaning being attached to b coefficient**. The extra term allows for the fact that experimental equivalent conductances are greater than the values given by the limiting law. If equation 2.2.14

proves inadequate, a further term in $c^{3/2}$ is added and the resultant equation reproduces the measurement with 1:1 salt quite well upto c = 0.1M, assuming that no negative deviations are observed. For salts with higher valency ions, equations with additional term in c and $c^{3/2}$ are still inadequate and a useful empirical equation for such systems is

$$\Lambda = \Lambda^{\circ} - (B_1 \Lambda^{\circ} + B_2)c^{0.5} + bc(1 - B_1 c^{0.5}) + Dc\log c \qquad \text{-----2.2.15}$$

where D is an empirical constant.

Robinson and Stokes[71] have proposed, neglecting the cross-product of the relaxation and electrophoretic terms, the following equation

$$\Lambda = \Lambda^{\circ} - \frac{(B_1 \Lambda^{\circ} + B_2)c^{0.5}}{1 + B_3 \mathring{a} c^{0.5}} \qquad \text{-----2.2.16}$$

where B_3 is a constant having a value of 0.329 x 10^{-8} and å is the distance of closest approach[72] of the ions in Angstron Å. Equation 2.2.16 gives a fairly good account of the conductance of aqueous solutions of strong 1:1 electrolytes up to 0.1M and yields reasonable values of the å parameter. The equation fails to account for the concentration dependence in non-aqueous systems.[73]

2.2.8 CONDUCTANCE EQUATION FOR WEAK ELECTROLYTES

Equation 2.2.13 applies to completely dissociated electrolytes. In case of a weak electrolyte only a fraction of it undergoes dissociation, hence the corresponding equation is

$$\Lambda = \alpha [\Lambda^{o} - \{B_1 \Lambda^{o} + B_2\}(c\alpha)^{0.5} \quad \text{----2.2.17}$$

$$\alpha = \frac{\Lambda}{\Lambda^{o}[1 - \{B_1 \Lambda^{o} + B_2\}(c\alpha)^{0.5}/\Lambda^{o}]} \quad \text{----2.2.18}$$

This provides a method of calculating the degree of dissociation α by successive approximation. In the limit c--->0, the equation reduces to

$$\alpha = \frac{\Lambda}{\Lambda^{o}}$$

which is the Arrhenius equation

Shedlovsky[74] has used the quadratic equation

$$\Lambda = \alpha\Lambda^{o} - \{\Lambda (\Lambda^{o} B_1 + B_2)[c\alpha]^{0.5}\}/\Lambda^{o} \quad \text{-----2.2.19}$$

which is much simpler to solve than equation 2.2.17 in which is cubic in $\alpha^{0.5}$. Equation 2.2.19 gives

$$\alpha = \frac{\Lambda}{\Lambda^{o}} + \frac{(B_1 \Lambda^{o} + B_2).\Lambda.(c\alpha)^{0.5}}{(\Lambda^{o})^{2}} \quad \text{----2.2.20}$$

which together with the law of mass action

$$K = \frac{c\alpha^2 f_{\pm}^2}{1 - \alpha} \qquad \text{------2.2.21}$$

provides a very convenient method for simultaneously calculating Λ^0 and dissociation constant K of weak electrolytes.

$f_{\pm}^2$ in equation 2.2.21, is the mean activity coefficient which can be calculated using Debye-Huckel law in the form

$$\log f_{\pm}^2 = \frac{-1.0230(c\alpha)^{0.5}}{1 + B_3(c\alpha)^{0.5}} \qquad \text{-----2.2.22}$$

Trying to solve equation 2.2.22 for α in terms of a variable Z, defined thus.[75]

$$Z = (B_1 \Lambda^0 + B_2).(c\Lambda)^{0.5}(\Lambda^0)^{-1.5}$$

one obtains

$$\alpha = \frac{\Lambda [Z/2 \{1 + (Z/2)^2\}^{0.5}]^2}{\Lambda^0}$$

$$= \frac{\Lambda S(Z)}{\Lambda^0} \qquad \text{-----2.2.23}$$

The terms in the brackets can be used in expanded form

$$S(Z) = 1 + Z + Z^2/2 + Z^3/8 + \ldots\ldots \qquad \text{-----2.2.24}$$

for small values of Z. Ordinarily it is not necessary to employ terms higher than Z^2 in evaluating S(Z), From equation 2.2.20 and 2.2.23 one obtains

$$\frac{1}{\Lambda S(Z)} = \frac{1}{\Lambda^0} + \frac{c.\Lambda.f_{\pm}^2 S(Z)}{K_a(\Lambda^0)^2} \qquad \text{-----2.2.25}$$

Thus plotting $1/[\Lambda S(Z]$ against $c.\Lambda.f_{\pm}^2$ gives $1/\Lambda^{\circ}$ as the intercept and the slope equal to $1/K_a(\Lambda^{\circ})$. This method has been used to obtain Λ° and K_a values for the Vitamin in aqueous solution. The corresponding Robinson and Stokes equation for weak electrolytes is

$$-\frac{\Lambda}{\alpha} = \Lambda^{\circ} - \frac{(B_1\Lambda^{\circ} + B_2)(c\alpha)^{0.5}}{1 + B_3\mathring{a}(c\alpha)^{0.5}} \quad \text{-----2.2.26}$$

This equation has been used[76] to obtain (fit) Λ° values if the dissociation constant is known. The author has used this equation to cross-check the values obtained by Shedlovsky's method.

2.3.0 REFRACTIVE INDEX

2.3.1 THEORY AND DERIVATION OF EQUATION

When a light beam enters a lighter medium from a more dense one, the angle of incidence is smaller than the angle of refraction. The refractive index n is defined by Snell's Law as

$$n = \frac{\mathrm{Sin}\alpha_2}{\mathrm{Sin}\alpha_1} \qquad \text{-----2.3.1}$$

where α_2 is the angle of refraction and α_1 is the angle of incidence. According to corpuscular theory, the critical condition corresponds to

$$\mathrm{Sin}\alpha_1 = 1/n \qquad \text{-----2.3.2}$$

When $\mathrm{Sin}\alpha_1 < 1/n$ refraction takes place instead of reflection. Experiments show that when α_1, is less than the critical angle, some light is nevertheless reflected, and that when it exceeds that critical angle, some light is still transmitted into the second medium, though with a relatively weak intensity which rapidly diminishes with the depth of penetration.

The wave theory of light explains these facts in terms of velocity. The refractive index becomes

$$n = \frac{\mathrm{Sin}\alpha_2}{\mathrm{Sin}\alpha_1} = \frac{w_2}{w_2} \qquad \text{-----2.3.3}$$

where w is the velocity with which the waves

advance. The refractive index varies considerably with the physical state of a given substance.

It is to be anticipated that the variation of density is the main factor causing variation in refractive indices of a given substance. Many attempts were made to relate the two. The most commonly used refractivity formula was published independently by Lorenz and Lorentz.[77]

$$L = \frac{(n^2 - 1) \times 1}{(n^2 + 2) \times \rho} \qquad \text{-----2.3.4}$$

When the specific refractivity, L, is multiplied by the Molecular Weight of the substance, it gives the molar refractivity, $\bar{R}_D$; therefore equation 2.3.4 becomes

$$\bar{R}_D = \frac{(n^2 - 1)M}{(n^2 + 2)\rho} \qquad \text{-----2.3.5}$$

This is a constant for a particular substance determined for a given wavelength. In the above equation, M/ρ gives the apparent molar volume of the substance.

2.3.2 EVALUATION OF LORENZ-LORENTZ MOLAR REFRACTIVITY

The Lorenz-Lorentz Molar Refraction has been used as an additive property. In many cases, even a

small deviation of $\overline{R}_D$ from additivity allows one to draw important conclusion concerning intermolecular and intramolecular forces and the electronic structure of molecules.

The precision technique for measuring the Apparent Molar Refraction of solutes, $\overline{R}_2$ has been developed by Kohner, Gefficken and Kruis[78], for aqueous solution. They emphasized the importance of differential measurement and of a temperature control commensurate with the improved precision. The reliability of the result depends on the following:-

(i) Solution and solvent should be compared under identical conditions for example temperature and instrument.

(ii) Measurement of the difference between refractive index of solution and solvent $(n-n_o)$ should be made on a sample identical with that used for the determination of the density difference.

(iii) Special devices should be employed for transferring and storing liquid to prevent change of concentration in the solution by evaporation.

(iv) The solvent used in preparing the reference liquid and solution should come from same place.

(v) If the change of $\overline{R}_2$ with concentration is required, various concentrations should be made up

by diluting a single carefully analysed stock solution, using weighed burettes.

The Apparent Molar Refraction, $\bar{R}_2$, of a solute of molecular weight M_2 is defined by the condition that the total refraction $\bar{R}_D$ of a solution containing one Mole of solute is the sum of $\bar{R}_2$ and the refraction which the amount (w_1 grams) of solvent present in this solution would have in pure state. If $\bar{R}_1$ is refractivity of the solvent we have

$$\bar{R}_D = \bar{R}_2 + r_1 w_1 \qquad \text{------2.3.6}$$

and

$$\bar{R}_2 = \frac{(n^2 - 1)(M_2 + w_1)}{(n^2 + 2)\ d} - \frac{(n_1^2 - 1)w_1}{(n_1^2 + 2)d_1} \qquad \text{-----2.3.7}$$

where r_1 is the specific refraction of the solvent in pure state, $\bar{R}_2$ is not identical with the molar refraction of the pure solute, $\bar{R}_{crst}$. The difference, $\bar{R}_2 - \bar{R}_{crst}$, represents the deviation from exact additivity in the mixture due to all possible causes; it includes any refractometric effect of a change in the state of the solute and the solvent which ought to arise as a result of mixing. The value of $\bar{R}_2$ should be equal to $\bar{R}_{crst}$ within a few hundredth of a centimeter.[79] $\bar{R}_2 - \bar{R}_{crst}$ will be small for solution in which the component have similar physical properties or do not exert

strong forces on each other. When concentrations are expressed in terms of mole fraction X we use the formula,

$$X_2\bar{R}_2 = \frac{(n^2-1)}{(n^2+2)}\cdot\frac{(X_2M_2 + X_1M_1)}{d} - \frac{(n_1^2-1)(X_1M_1)}{(n_1^2+2)\,d_1}$$

Or

$$\bar{R}_D = \bar{R}_1X_1 + \bar{R}_2X_2$$

$$\bar{R}_D = \bar{R}_1 + (\bar{R}_2 - \bar{R}_1)X_1 \qquad \text{-----2.3.8}$$

But the molar volume of a binary solution i.e the volume of the solution containing in all one gram-mole of substance is defined in terms of the molar refraction, and the molar weight of the components, and the density of the solution.

$$\bar{V} = \frac{X_1M_1 + X_2M_2}{d}$$

$$= \frac{M_1 + (M_2 - M_1)X_2}{d} \qquad \text{------2.3.9}$$

Therefore

$$\bar{R}_D = \frac{\bar{V}(n^2-1)}{(n^2+2)} = \bar{R}_1 + (\bar{R}_2 - \bar{R}_1)X_2 \qquad \text{-----2.3.10}$$

The accuracy in $\bar{R}_2$ depends primarily on the accuracy of the differences Δn and $(d - d_1)$ and of concentration.[80,81]

2.3.3 EFFECT OF CONCENTRATION ON REFRACTIVE INDEX OF SOLUTION

If the refractive index is to be used as a measure of concentration, or vice versa, the most reliable method is interpolation from an empirical curve. In certain cases there exists a linear relationship between the two quantities but more generally the data are represented by a curve, the shape of which is influenced by the way in which the concentration is expressed. Molarity, c, is more likely to give a nearly linear relationship than weight percentage, p.

Arshid, Giles et al[82] have shown that plots of n^2 versus mole fractions often fit straight lines. In cases of strong interaction between two components in a solution, the plots of n^2 against the mole fraction consist of two straight lines which intersect at the mole ratio corresponding to the interaction complex.

2.3.4 APPLICATION OF MOLAR REFRACTION

(a) Measurements of molar refraction have been used in several cases, to provide information concerning molecular structure.[83] In fact molar dispersion, $R_{\lambda_1} - R_{\lambda_2}$, i.e the difference in molar refraction between two wavelength

λ_1 and λ_2 is a characteristic property of molecules.

(b) It is also used as a criterion for purity.

(c) It is also used in quantitative analysis of a solution.

2.4.0 DENSITY AND PARTIAL VOLUME

2.4.1 DENSITY

Density is defined as mass per unit volume and for liquids it can be determined by any of the three methods[84,85]:-

(i) Pyknometric

(ii) Displacement

(iii) Harnes method and its modifications

The author used the first method in determining the density of aqueous Vitamin solution. Earlier workers on density measurements advanced three main objections to Pyknometry namely:

(a) The difficulty of keeping a large volume of unstirred water at a constant and fixed temperature.

(b) The reduction in sensitivity of the balance when weighing a relatively heavy load.

(c) The difficulty in removing or correctly allowing the effects of humidity in weighing the apparatus.

The author has been able to eliminate these effects. The first two by the type and the set up of the apparatus, and the use of a light load on the balance.

2.4.2 DENSITY OF SOLID BY DENSITY INCREMENT

The approximate density of the powdered Vitamin B_6 Hydrochloride can be approximated using density increment.[86] This method is described fully below.

The density increment, symbolized K_d^o of a compound is defined as the difference between the density of the solution and that of the solvent, divided by the concentration in gml^{-1}.

Under certain conditions, the influence of the solute is so small that the density increments are constant. The apparent density of a solute is determined when the density of the solution is equal to that of the solvent i.e $K_d^o=0$. If the density increment be a constant or a known function of the solution concentration, it is possible to compute the solution concentration from measurements of density. If the solution concentration is known, it is possible to compute the density.

$$K_d^o = \frac{\rho' - \rho_o}{c} \qquad \text{-----2.4.1}$$

where ρ' = density of solution

ρ_o = density of solvent

c = concentration in gml^{-1}

A constant K_d^o indicates a linear relationship between density and concentration.

2.4.3 PARTIAL VOLUME

Physically, Partial Molar Volume may be regarded as the increase or decrease in volume arising from the addition of a mole of the component to an infinite amount of the solution at constant temperature and pressure.[87]

For a system at constant temperature and pressure it can be written as

$$\overline{V} = n_1\overline{V}_1 + n_2\overline{V}_2 + \ldots\ldots \quad \text{-----2.4.2}$$

where $\overline{V}$ is the contribution per mole of each particular constituent to the total volume in the system under consideration. The magnitude of $\overline{V}$ may of course, vary with concentration of the particular constituent in the system. It can therefore be expressed on any concentration scale except in thermodynamics where molarity is most convenient.[88]

2.4.4 DETERMINATION OF PARTIAL VOLUME

Density, ρ of any substance is defined by the equation

$$\rho = \frac{\text{mass}}{\text{volume}} \qquad \text{------2.4.3}$$

This can be used to calculate the partial molar volume, $\overline{V}$ of a solute in solution by the method described below.

2.4.5 APPARENT MOLAR VOLUME

Partial Molal and Apparent Molal Volumes are derived from densities using equation described below.[89]

The Apparent Molal Volume is given by the expression,

$$\phi_v = \frac{V - n_1\bar{V}_1^o}{n_2} \qquad \text{-----2.4.4}$$

where V is the total volume of the solution, $\bar{V}_1^o$ the partial molal volume of solvent in standard state and n_1 and n_2 the number of moles of solvent and solute respectively. Equation 2.4.4 becomes

$$\phi_v = \frac{1000(d_o - d)}{cd} + \frac{M_2}{d_o} \qquad \text{------2.4.5}$$

If $n_2 = c$, the number of moles of solute in V = 1000 c.c of solution. In terms of molality, m, it is

$$\phi_v = \frac{1000(d_o - d)}{mdd_o} + \frac{M_2}{d} \qquad \text{------2.4.6}$$

or

$$\phi_v = \frac{V - V_o}{m} \qquad \text{------2.4.7}$$

where V is defined as above and V_o the corresponding volume of pure solvent. Equation 2.4.5 shows that the accuracy of the determination of ϕ_v depends largely on the density difference, $\Delta d = d_o - d$. Therefore the solution and solvent

should be measured under identical condition.

It was found by Masson that ϕ_v varies linearly with $c^{0.5}$ in dilute solution, thus

$$\phi_v = \phi_v^\circ + S_v c^{0.5} \quad \text{------2.4.8}$$

S_v, a constant, is the experimental slope for apparent molal volume, ϕ_v° is the standard apparent molal volume at infinite dilution. The equation applies in many cases in the range 1 - 10M with a slope S_v that may differ considerably from that for dilute solution.

ϕ_v is not seriously influenced by ordinary experimental errors in the concentration, but it is very sensitive to experimental uncertainties in the density at high dilutions.[90] Deviations from the Massons equation have been attributed to change in co-ordination of water molecules.[91]

ϕ_v for non-electrolyte varies linearly with c rather than $c^{0.5}$ in dilute solution[92] but Bhagwat found otherwise[93] i.e. ϕ_v not to vary much with concentration for non-electrolyte.

Because of the linear relationship of equation 2.4.6, the most convenient calculations of Partial Molal Volumes make use of the c-scale.[94] Instead of equation 2.4.6, a power series of the third order in molality,m, was used by some investigators, and it appeared that this treatment tend to exaggerate

the deviations.[95] The equation used is[96]

$$\overline{V}_2 = \overline{V}_2^{\circ} + 1.5(S_v c^{0.5}) \qquad \text{-----2.4.9}$$

or more exact

$$\overline{V}_2 = \frac{\overline{V}_2^{\circ} + 1.5 S_v c^{0.5}}{[1 + (S_v c^{1.5})/2000]} \qquad \text{-----2.4.10}$$

Partial Molal Volume, $\overline{V}_2$ and Apparent Molal Volume $\emptyset_v$, converge at infinite dilution. Therefore

$$\emptyset_v^{\circ} = \overline{V}_2^{\circ} \qquad \text{-----2.4.11}$$

This is a consequence of a well known thermodynamic relation.[97]

$$\overline{V}_2 = \emptyset_v + n_2(\partial \emptyset_v / \partial n_2)n_1 \qquad \text{----2.4.12}$$

in which the requirement is that $\emptyset_v$ must approach a characteristic value at infinite dilution.

Substituting $\emptyset^{\circ}_v$ for $\overline{V}_2^{\circ}$ in 2.4.9 and 2.4.10 we have

$$\overline{V}_2 = \emptyset_v^{\circ} + 1.5(S_v c^{0.5}) \qquad \text{-----2.4.13}$$

and

$$\overline{V}_2 = \frac{\emptyset_v^{\circ} + 1.5(S_v c^{0.5})}{[1 + (S_v c^{1.5})/2000]} \qquad \text{-----2.4.14}$$

CHAPTER 3

3.1.0 EXPERIMENTAL

3.1.1 CONDUCTANCE MEASUREMENTS

There are three very important requirements that must be met to obtain accurate and precise conductance data. These are, an oil bath thermostated at least within ±0.01°C, a precision conductance bridge and a properly designed conductance cell. These items are discussed in the following lines:

3.1.2 OIL BATH

The density, refractive index and conductance are both concentration and temperature dependent. The density decreases[98] by 0.03%, most electrolyte solutions show a 2% change in equivalent conductance when the temperature changes by one degree. The refractive index of most liquids change by about one or two units. It is, therefore, desirable to maintain a constant temperature[99] controlled to within ±0.02°C.

Two different types of thermostats were used to maintain a constant temperature of 25°C ±0.02°C. For the density and refractive index measurements, a thermostated water-bath made in the University of Nairobi, Faculty of Science Workshop was used. The dimensions of the tank were 37 cm wide, 34 cm high and 69 cm long. All the five sides were made of

glass which enabled easy inspection of any apparatus immersed into it. The temperatures of the solution placed in the refractometer was controlled by circulating water from this tank. The temperature was controlled using THERMOMIX 1420 from Braun Company Limited. The temperature variations were monitored using a Beckmann Thermometer which could be read within ±0.01°C. The water level in the tank was maintained by adding some water at regular intervals.

For the conductance measurements, a second tank having dimensions 29.5cm x 29.5cm x 50cm filled with Diala BX oil was used. The temperature was controlled by a relay model NGW - LRVI type 5347006 using a 45 Watts light bulb as a heating element. The temperature of the bath was controlled within ±0.02°C.

In both two cases, the tanks were placed in convenient working positions that gave maximum accessibility to the equipment and ease in observations.

3.1.3 CONDUCTANCE BRIDGE

A conductance bridge was assembled by using the following components:

Measuring Circuit:	A.C Wheatstone bridge with Wagner ground and capacitive balance controls
Range :	0 - 999,999 Ohms measured resistance
Resistance:	Resistance was measured using Decade Resistance Heathkit Model IN - 11
Condenser:	Decade condenser Heathkit IN - 21
Amplifier:	The amplifier used was made at the University of Nairobi Science Workshop. It had a Voltage of 9V
Generator:	An A.F signal generator. Type HTD with a voltage of 4V and frequency range of 1000Hz to 3000Hz was used
Detector:	A Central 272E general purpose oscilloscope was used for detection

The circuit for conductance bridge is shown in Figure 3.1.2

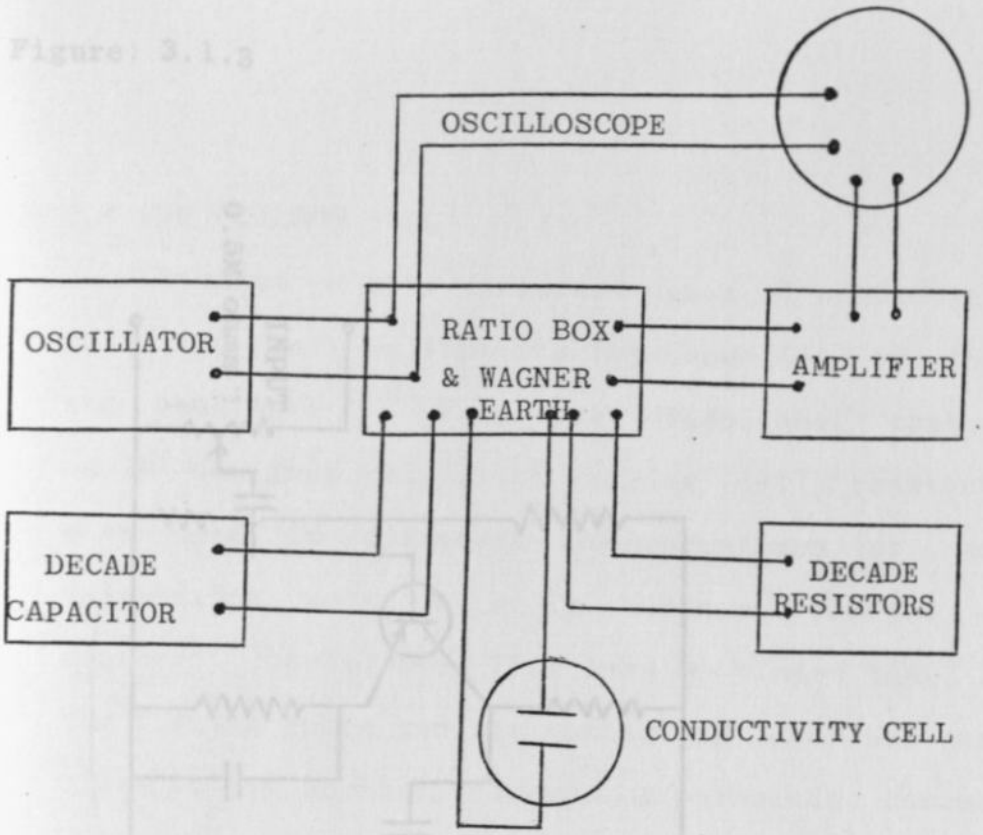


Figure 3:1:1 COMPONENTS OF THE CIRCUIT

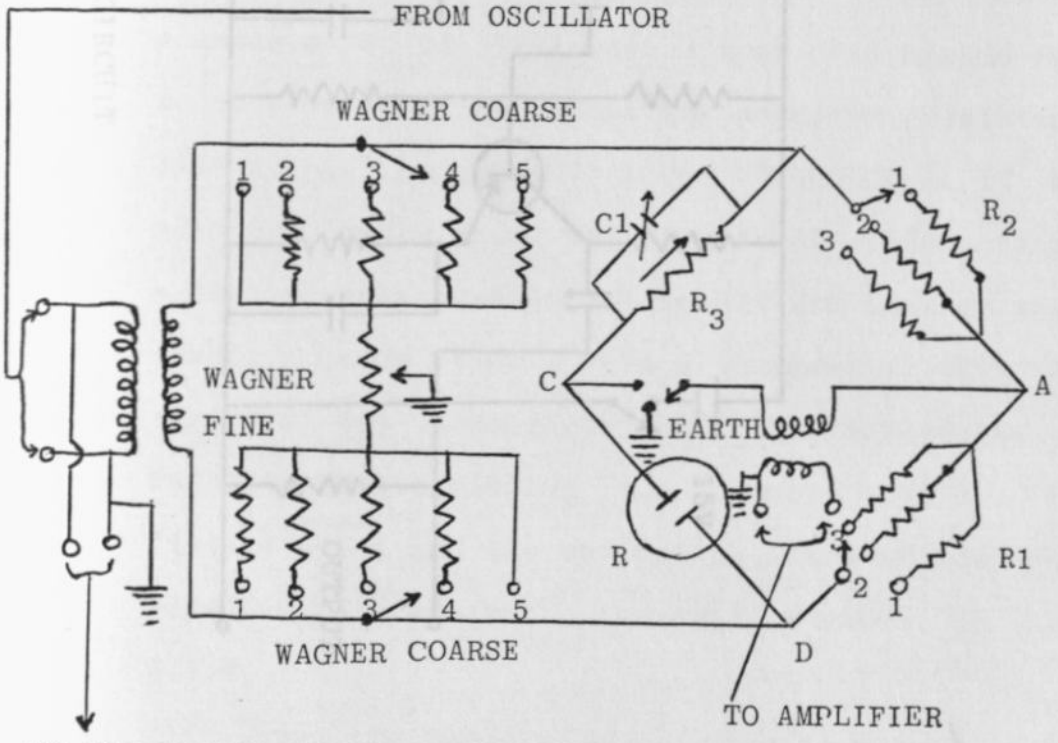
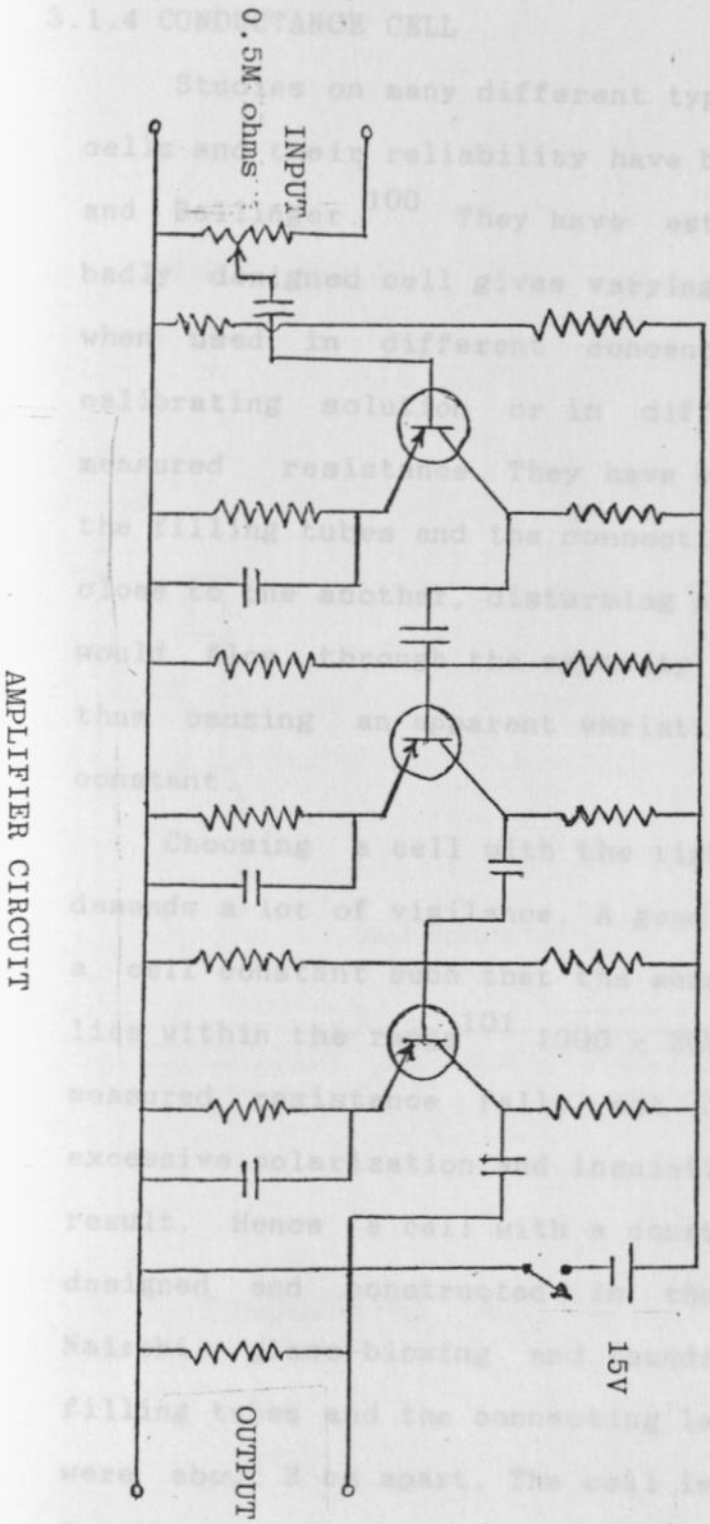


Figure 3.1.2 COMPLETE CIRCUIT DIAGRAM

Figure: 3.1.3



AMPLIFIER CIRCUIT

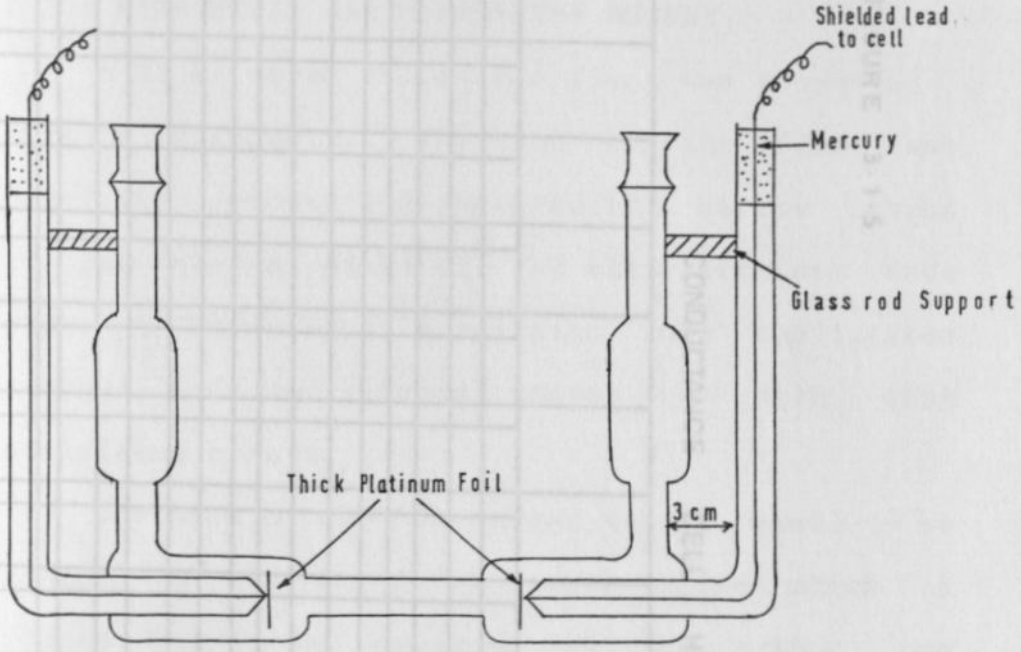
3.1.4 CONDUCTANCE CELL

Studies on many different types of conductance cells and their reliability have been done by Jone and Bollinger.[100] They have established that a badly designed cell gives varying cell constants when used in different concentrations of the calibrating solution or in different ranges of measured resistance. They have concluded that if the filling tubes and the connecting leads are very close to one another, disturbing parasitic current would flow through the capacity resistance path thus causing an apparent variation in the cell constant.

Choosing a cell with the right cell constant demands a lot of vigilance. A good cell should have a cell constant such that the measured resistance lies within the range[101] 1000 - 30000 Ohm . If the measured resistance falls out of this range, excessive polarization and insulation leakage would result. Hence a cell with a constant 5.4212 was designed and constructed in the University of Nairobi, glass-blowing and foundry section. The filling tubes and the connecting leads of the cell were about 3 cm apart. The cell is shown in Fig. 3.1.4.

Given the small size and fragile nature of the conductance cell, clamping it on a stand and then

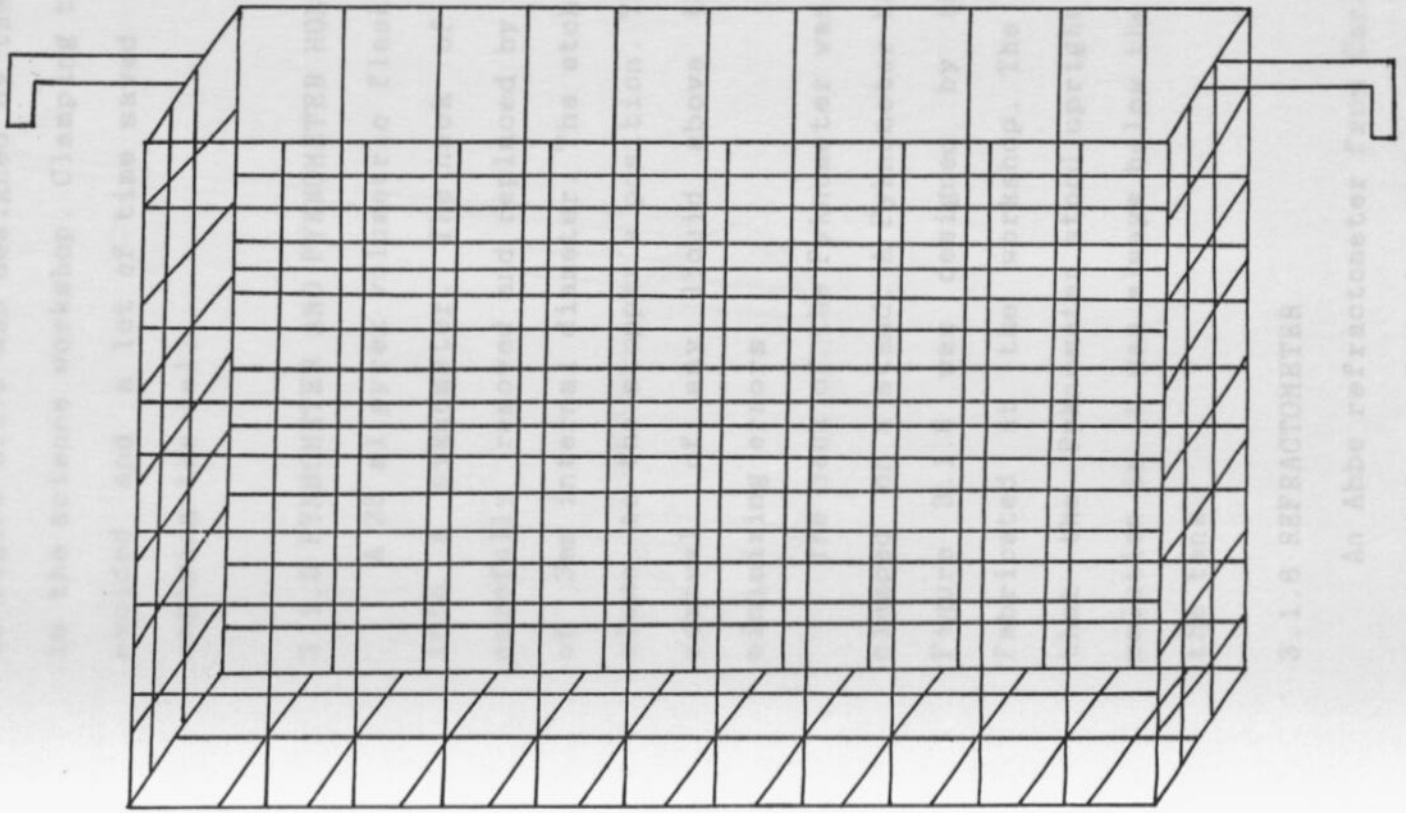
FIGURE 3·1·4



CONDUCTANCE CELL

FIGURE 3·1·5

CONDUCTANCE CELL HOLDER



Length = 13.8 Cm.
Width = 2.2 Cm.

immersing in the oil bath proved very inconvenient and time consuming. A conductance cell holder shown in Figure 3.1.5 was designed by the author and made in the science workshop. Clamping the cell was thus avoided and a lot of time saved in filling and emptying the cell.

3.1.5 PYKNOMETER AND PYKNOMETER HOLDER

A 25 ml pyrex volumetric flask was converted into a pyknometer. The neck of the flask was carefully removed and replaced by a narrow tubing of 3mm internal diameter. The etch-mark was made close to the stopper's position. This facilitated removal of any liquid above the mark thus minimizing errors.

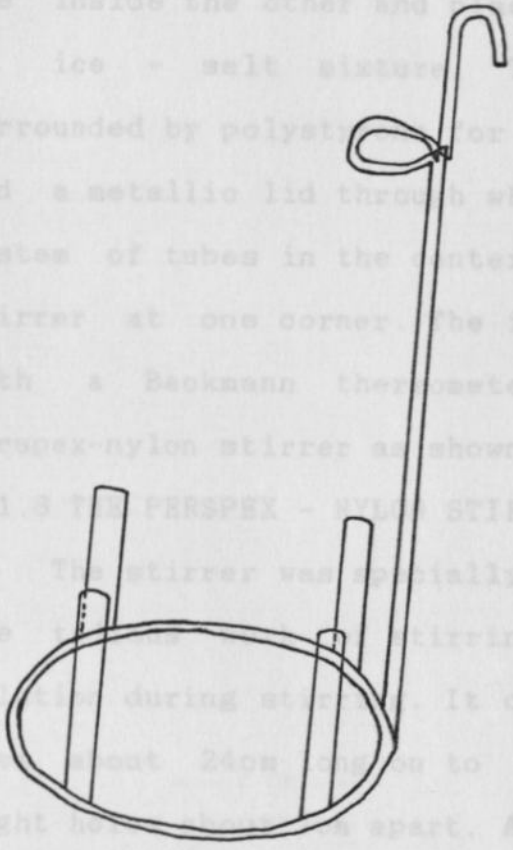
The neck of the Pyknometer was too small to be clamped on a stand. A Pyknometer holder shown in Figure 3.1.6 was designed by the author and fabricated at the workshop. The holder ensured that the Pyknometer stood upright and that the solution in it was always below the water level of the tank.

3.1.6 REFRACTOMETER

An Abbe refractometer from Carl Zeiss Limited was used for refractive index measurements.

FIGURE 3.1.6

PYKNOMETER HOLDER



3.1.7 DEPRESSION OF FREEZING POINT APPARATUS

The apparatus consisted of two test tubes held one inside the other and placed in a cooling bath of ice - salt mixture. The glass bath was surrounded by polystyrene for insulation. The bath had a metallic lid through which were passed the system of tubes in the center and a metallic bath stirrer at one corner. The inner tube is fitted with a Beckmann thermometer and a seven-ring perspex-nylon stirrer as shown in Figure 3.1.7.

3.1.8 THE PERSPEX - NYLON STIRRER

The stirrer was specially improvised to reduce the tedious work of stirring and splashing of solution during stirring. It consisted of a perspex stem about 24cm long on to which were drilled eight holes about 1cm apart. A tennis racket string of about 1mm diameter was then passed through the hole to form a spiral shaped stirrer having seven rings each of about 2cm diameter. Finally to hold the rings in place, the string was glued into the stem at the holes. Figure 3.1.8 shows the three main steps in making such a stirrer.

To facilitate stirring, a groove was made near the upper end of the perspex stem and a rubber band used to connect the groove to the body of thermometer shown in Figure 3.1.7. Such a stirrer has been used previously.[102,103]

FIGURE 3·1·8

THE PERSPEX-NYLON STIRRER

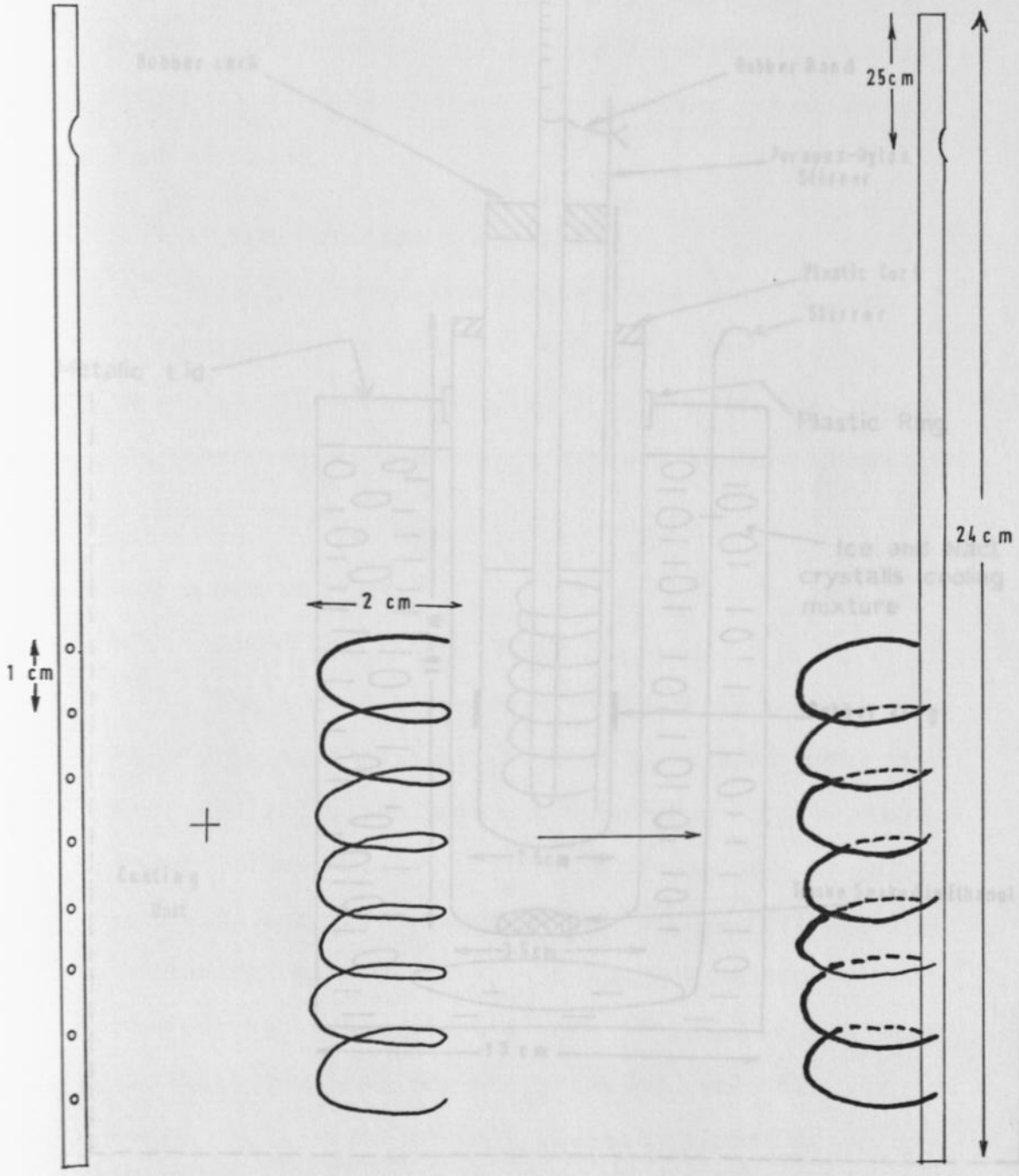
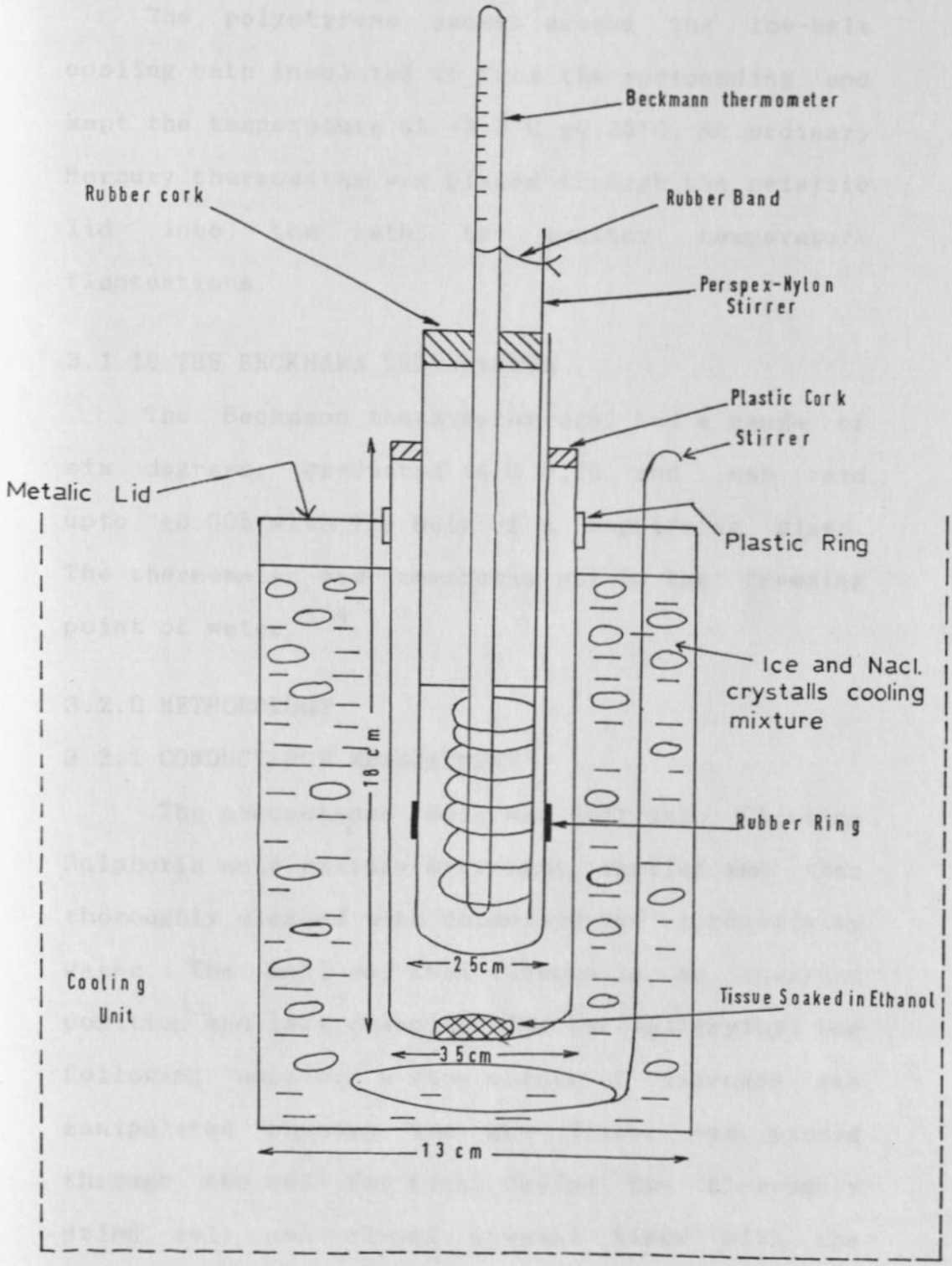


FIGURE 3·1·7

THE FREEZING POINT APPARATUS



3.1.9 THE COOLING BATH

The polystyrene jacket around the ice-salt cooling bath insulated it from the surrounding and kept the temperature at -3.0°C ±0.25°C. An ordinary Mercury thermometer was placed through the metallic lid into the bath to monitor temperature fluctuations.

3.1.10 THE BECKMANN THERMOMETER

The Beckmann thermometer used had a range of six degrees, graduated in 0.01°C, and was read upto ±0.005 with the help of a magnifying glass. The thermometer was previously set to the freezing point of water.[104]

3.2.0 METHODOLOGY

3.2.1 CONDUCTANCE MEASUREMENT

The conductance cell was left into Chromic-Sulphuric acid mixture overnight, emptied and then thoroughly cleaned with deionized and conductivity water. The cell was then clamped in an inverted position and left overnight for partial drying. The following morning a slow stream of nitrogen gas manipulated through the gas filter was passed through the cell for final drying. The thoroughly dried cell was rinsed several times with the solution whose resistance was to be determined and finally filled with the same and left for about

half an hour for thermal equilibrium to take place into the oil bath.

The cell was then removed from the oil bath and its outer surface washed free of oil using Carbon tetrachloride. It was then emptied, washed, dried and filled with a different concentration of the same solution. As before, half an hour was allowed for thermal equilibrium before any reading could be taken at a frequency of 3000Hz. The readings were taken in triplicate and resistance results agreed within $\pm$0.5 Ohms.

3.2.2 DETERMINATION OF CELL CONSTANT

The resistance R of a KCL solution of known specific conductivity L was measured and the cell constant J, calculated from the equation

$$J = LR$$

Potassium Chloride solution was used because its specific conductivities at various concentration at 25°C are available.[105,106] The Bradshow demal solutions are most commonly used.[107]

The preparation of demal solution is very tedious and instead the author used an alternative method which allowed the use of KCl solution of any moderate concentration. The conductance of the aqueous Potassium Chloride solution was calculated

from the equations[108,109]

$$\Lambda = 149.93 - 94.65c^{0.5} + 58.74c\log c + 198.4c \qquad \text{---------3.2.2A}$$

and $$\Lambda = (\Lambda^{o} + Bc + Dc\log c)(1 - c^{0.5}) - 2\sigma c^{0.5} \qquad \text{---------3.2.2B}$$

Where c is concentration expressed in gram equivalent^{-1}.

The specific conductance of KCl solution at 25°C was calculated using equation

$$\kappa = \frac{c\,\Lambda}{1000}$$

and the cell constant from the equation J=LR.

The measured resistances of various KCL solutions at 3000Hz and the resulting cell constants are shown in Table 3.2.2.

TABLE 3.2.2: CELL CONSTANT OF CONDUCTANCE CELL USING DIFFERENT EQUATIONS

c	Λ	R*	J(1)	J(2)
0.00839	141.901	4573.0	5.4431	5.4468
0.01014	141.222	3768.9	5.3981	5.3993
0.01046	141.106	3679.9	5.4340	5.4351
0.01537	139.609	2529.6	5.4271	5.4265
0.02123	138.256	1837.0	5.4029	5.3989
0.02944	136.883	1354.2	5.4579	5.4475
0.03495	136.179	1133.8	5.3967	5.3814
0.03988	135.663	1001.0	5.4161	5.3956
0.04960	134.890	809.1	5.4132	5.3815

Units: c, $moll^{-1}$, Λ ; Scm^2equiv^{-1};

R,Ohm; J,cm^{-1}

J(1) Using equation No 3.2.2A

J(2) Using equation No 3.2.2B

* An average of four readings

STATISTICAL ANALYSIS OF J(1)

Mean cell constant = 5.4212 cm^{-1}

Mean Deviation = 0.0184939

Standard Deviation = 2.1455×10^{-2}

Standard Error of mean = 7.1517×10^{-3}

STATISTICAL ANALYSIS OF J(2)

Mean cell constant = 5.4125 cm^{-1}

Mean Deviation = 0.023165

Standard Deviation = 2.6645×10^{-2}

Standard Error of Mean = 8.8817×10^{-3}

It is clear that the cell showed no trend in the cell constant. The mean value of 5.4212 cm^{-1} was taken as the cell constant for subsequent calculations.

3.2.3 PLATINIZATION OF ELECTRODES

Kohlrausch[110] found out that platinizing the electrode greatly increases the surface area and considerably reduces polarization.

To platinize the electrode of the cell it was first thoroughly cleaned with Chromic-Sulphuric acid mixture then washed several times with deionized and conductivity water. After filling the cell with a solution containing 0.3% Chloroplatinic acid and 0.025% lead acetate[111], a DC current of about 15mA/cm^2 was passed. The current was adjusted so as to cause moderate gassing and polarity was reversed every half minute until the electrodes were coated with a deposit of platinum black. Addition of lead acetate improved the adherence of deposited platinum.[112] The cell was then cleaned with the Chromic-Sulphuric acid mixture followed by washing with deionized and conductivity water. Whenever not in use, the cell was always filled with conductivity water and covered with tin-foil.

3.2.4 DENSITY MEASUREMENTS

The pyknometer was soaked overnight in Chromic-Nitric acids mixture and thoroughly cleaned with distilled water. It was calibrated using double distilled water at 25°C ±0.02°C. After cleaning and drying with acetone the weight of the empty pyknometer was taken five times and the average taken to represent the actual weight. The process was repeated with the pyknometer filled with double-distilled water. The water was introduced into the pyknometer by a fine tubing attached to a hypodermic syringe. It was usually filled to just above the etch-mark and left in the water bath for half an hour for thermal equilibrium. The level of the water was adjusted accordingly either by soaking out water with tissue or adding drops using a syringe. The pyknometer was then removed and its outer surface thoroughly dried and the weight taken.

Using density of water as 0.99707 gcm^{-3} at 25°C and the observed weight, the volume of the pyknometer was calculated.

For the Vitamin solution, the pyknometer was first rinsed with the solution under investigation and finally filled with the same.

An average of four readings was taken agreeing within ±0.0005g. The density was calculated from the volume and the weights obtained. Table 3.2.6 shows the calibration values.

TABLE 3.2.6: CALIBRATION OF THE PYKNOMETER

Weight of water (g)	Volume (ml)
24.395	24.467
24.398	24.470
24.398	24.470
24.398	24.469
24.398	24.470

Mean Volume of pyknometer = 24.470 cm^3

Mean Deviation = 7.018×10^{-4}

Standard Deviation = 1.3083×10^{-3}

Coefficient of variation = 0.0%

3.2.5 REFRACTIVE INDEX MEASUREMENT

A drop of the liquid was placed on the lower surface of the prism. It was then closed, locked and left for about ten minutes for thermal equilibrium to be achieved.

The refractometer was first calibrated with the double distilled water used in preparing the solutions.[113,114] A refractive index of 1.3325 for the water at 25°C was in agreement with the literature value.[115] Various concentrations of Vitamin B_6 solutions were prepared and their refractive indices recorded. The recorded values were averages of four readings. Two readings, by approaching the mark from the bottom and the other from the top. Fresh solution for each concentration was used. Between readings of each concentration, the surface of the refractometer prism was cleaned with ethanol using particle-free tissue paper.

The values for the refractive indices were measured within an accuracy of ± 0.002. The last place in refractive index i.e. 0.001 corresponds to 2.29×10^{-3} $moll^{-1}$. The concentration derived from equation 4.3.1 was within 1.18×10^{-4} $moll^{-1}$

3.2.6 DEPRESSION OF FREEZING POINT RUN

The solutions were prepared in calibrated volumetric flasks and transferred into the inner tube enough to cover the bulb of the thermometer. The tube was then fitted with a plastic cork and put into the tube containing a piece of tissue paper soaked in ethanol. The air space between the two tubes ensured a slow and uniform rate of cooling. Condensation of water vapour resulted in ice formation on the outer surface of the inner tube and inner surface of the outer one and caused insulation thus retarding the cooling process. This situation was overcome by using ethanol-soaked tissue paper as alcohol-waters vapours required a much lower temperature to form ice film on the surface of the tubes.

A continuous stirring of the solution was maintained at a rate of 40 taps per minute and the temperature reading taken at one minute interval. The solution cooled till a stage of nucleation was achieved. At this stage the latent heat of crystallization caused a sudden and sharp increase in temperature. After this point, the temperature readings were taken for another 10 minutes then after 5 minutes intervals on a Beckmann thermometer adjusted at 0° C.[116,117,118]

The inner tube was taken out and the frozen solution remelted by holding the tube in the palm and stirring of the solution. For each concentration three to four sets of readings were taken.

The temperature was plotted against time and the depression of freezing point determined from the difference between the freezing points of solvent and solution. If the readings were not constant after the temperature rise, an extrapolation of the curve was done to get the correct freezing point. Such a cooling curve for water and a solution is shown in Figure 3.1.9.

3.2.7 SUPERCOOLING

Supercooling is an important source of error[119] in cryoscopic experiments. Supercooling should not exceed[120] 0.3 - 0.5°C and is best if kept below 1°C.[121] If there is too much of supercooling, a large quantity of ice would separate out at the freezing point of solution, thus changing the concentration of the solution appreciably and hence the freezing point will not be that of the solution as prepared. The reliability of the data was assessed on the following grounds:-

i) The freezing point of solution remained constant for over ten minutes after the sudden rise.

ii) Similar cooling pattern was observed for both solvent and solution (Figure 3.2.9).

iii) The effect of supercooling is explained by the equation.[122]

$$T_t - T = K(T_c - T_t)/S \qquad \text{------3.2.8}$$

where

T_t = freezing point on the thermometer

T = the correct value

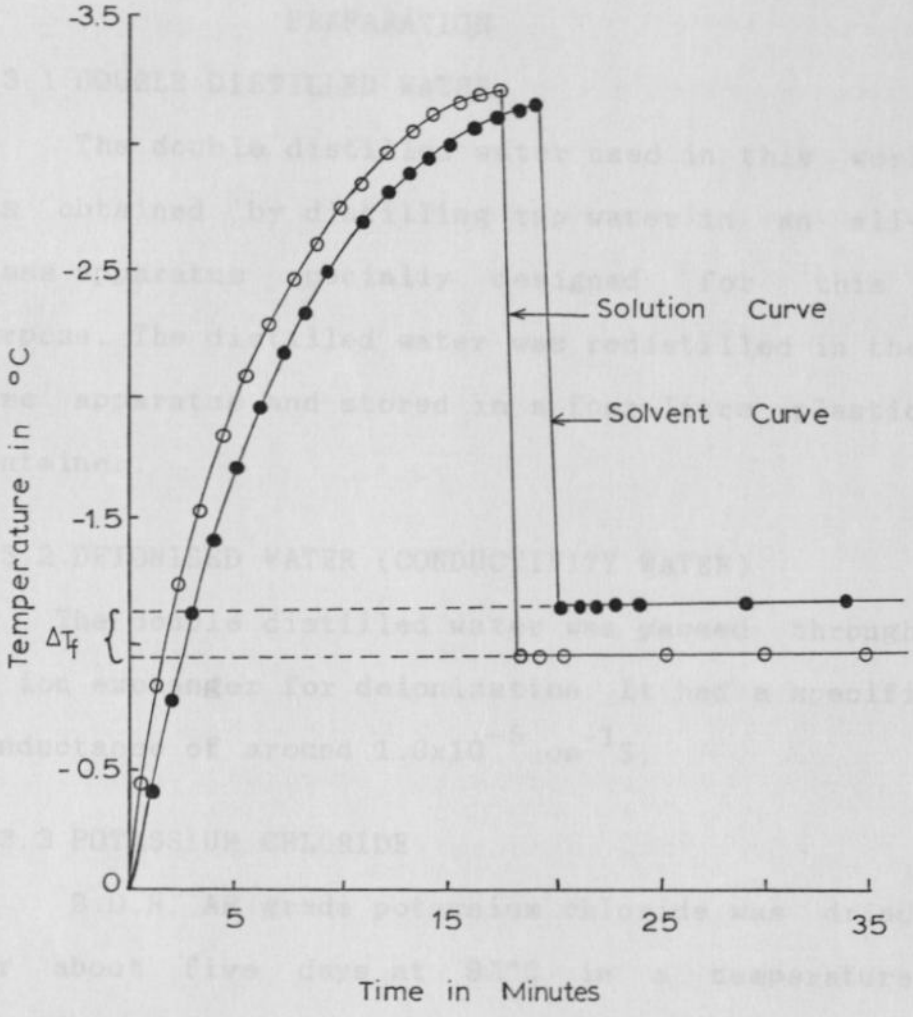
T_c = the lowest temperature attainable by the inner tube

K = a constant

S = a measure of the amount of ice separated at equilibrium which is approximated to be equal to the extent of supercooling. Accordingly if S is large the temperature recorded represent a more accurate value of freezing point of solution.

FIGURE 3·1·9

COOLING CURVES FOR SOLVENT AND SOLUTION.



3.3.0 CHEMICALS: PURIFICATION AND SOLUTION PREPARATION

3.3.1 DOUBLE DISTILLED WATER

The double distilled water used in this work was obtained by distilling tap water in an all-glass apparatus specially designed for this purpose. The distilled water was redistilled in the same apparatus and stored in a four-litre plastic container.

3.3.2 DEIONISED WATER (CONDUCTIVITY WATER)

The double distilled water was passed through an ion exchanger for deionization. It had a specific conductance of around 1.0×10^{-5} $cm^{-1}S$.

3.3.3 POTASSIUM CHLORIDE

B.D.H. AR grade potassium chloride was dried for about five days at 90°C in a temperature controlled oven. The bottle was removed and shaken regularly to remove any trapped water-vapours. A weighed amount of KCl was transferred into calibrated volumetric flasks and the solutions made up to the mark at 25°C as described earlier.

3.3.4 ETHANOL

AR grade ethanol was used, (without any purification) for cleaning of the prism of the refractometer and in cryoscopic measurements.

3.3.5 VITAMIN B_6.HCL

The Vitamin was transferred into a dark glass bottle and dried for about thirty minutes in an oven at a temperature of 195°C. It was then removed and shaken and put in a desiccator. Weighed amounts were transferred into the calibrated flasks that had been painted black. The solution were made up to the mark at 25°C in the water bath. Degassed water was used in preparing all solutions.

3.4.0 APPARATUS

3.4.1 CALIBRATION OF VOLUMETRIC FLASKS

The outer surface of the commercially available flasks were painted black except at the bottom and a very small area above and below the etch-mark. The weighed flasks were then filled with air-free distilled water (degassed) to just above the mark. They were left in the water bath at 25°C for about 30 minutes for thermal equilibrium. The levels were adjusted accordingly. Any drop of water sticking to the neck above the calibration mark was removed with the help of a tissue paper. The flasks were then weighed and the exact volume calculated by dividing the weight of water in the flask by the density of water at 25°C- 0.99707 $gcm.^{-3}$

3.4.2 WASHING OF GLASSWARE

All glasswares used in this work were thoroughly cleaned by soaking them in Chromic-Nitric acids mixture overnight. They were then cleaned with ordinary tap water followed by several rinsing with distilled water.

CHAPTER 4

RESULTS AND DISCUSSION

4.1.0 INTRODUCTORY REMARKS

The density, refractive index, conductance and cryoscopic results will be discussed in the following sections.

A lot of work has been done on the determination of pK_a values of the Phenolic and Pyridine hydrogens in changing from one structure to the other. Unfortunately no work has been reported on the dissociation constant of Vitamin B_6 for the cationic structure that exists in acidic medium. Conductance method has not been employed for the purpose, however spectrophotometric methods like UV and IR have been used[123] and the reported pK values from these methods are 5.0 and 8.9, for the phenolic and pyridine hydrogen respectively.

4.2.0 DENSITY RESULTS

4.2.1 MOLARITY SCALE

The density results of aqueous Vitamin B_6.HCl are shown in Table 4.2.1 and its concentration dependence in Figure 4.2.1(A). The density in the entire concentration range is given by the empirical equation

$$\rho = 6.1489 \times 10^{-2} c + 0.99709 \quad \text{-------4.2.1}$$

where c stands for concentration expressed in $moll.^{-1}$

Statistical analysis of the data was done by an INSTAT package on a BBC Personal Computer. The statistical results are shown on Figure 4.2.1(A). The equation gave the density of water as 0.99709, which is in good agreement with 0.99707 reported in the literature. The small difference of 0.00002 may well be due to neglecting the buoyancy correction which could not be applied as density of solid Vitamin B_6 is not available in the literature.

4.2.2 MOLE FRACTION SCALE

The molarity unit of concentration was converted to mole fraction to see the relationship between density and the later unit. Table 4.2.1 gives the corresponding mole fraction of the solute and Figure 4.2.2 shows dependence of density on

mole fraction. Although the linear relationship still prevails, the later unit seems to introduce a relatively bigger percentage error of 0.06% in the density of water vis a vis the molarity unit. The linear equation for this scale has been found to be

$$\rho = 3.3283X + 0.99713 \quad \text{-------4.2.2}$$

where X stands for mole fraction of Vitamin B_6.

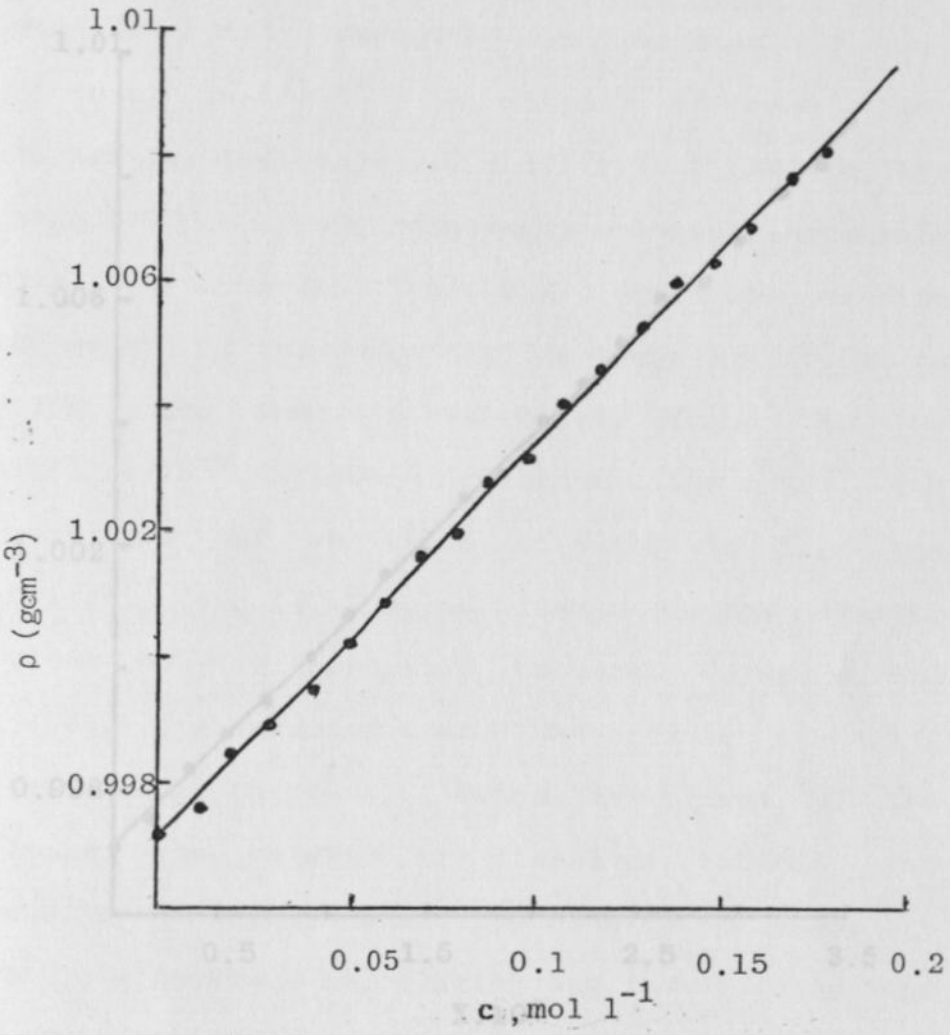
TABLE 4.2.1: DEPENDENCE OF DENSITY ON MOLAR CONCENTRATION AND MOLE FRACTION OF VITAMIN B_6

c	ρ	X . 10^3
0.01000	0.99759	0.1808
0.02000	0.99844	0.3619
0.03000	0.99889	0.5441
0.04002	0.99945	0.7262
0.05000	1.00021	0.9283
0.06000	1.00085	1.0914
0.07000	1.00152	1.2748
0.08000	1.00189	1.4591
0.09000	1.00269	1.6434
0.10000	1.00309	1.8287
0.11000	1.00393	2.0136
0.12000	1.00457	2.1993
0.13000	1.00517	2.3859
0.14000	1.00589	2.5724
0.15000	1.00625	2.7605
0.16000	1.00681	2.9484
0.17000	1.00756	3.1363
0.18000	1.00805	3.3256

Units: c, $moll^{-1}$; ρ, gcm^{-3}; X, mole fraction

Figure: 4.2.1A

DEPENDENCE OF DENSITY ON CONCENTRATION



Coefficient of correlation = 0.9996

R-squared = 0.9991

Standard Deviation = 7.9415×10^{-4}

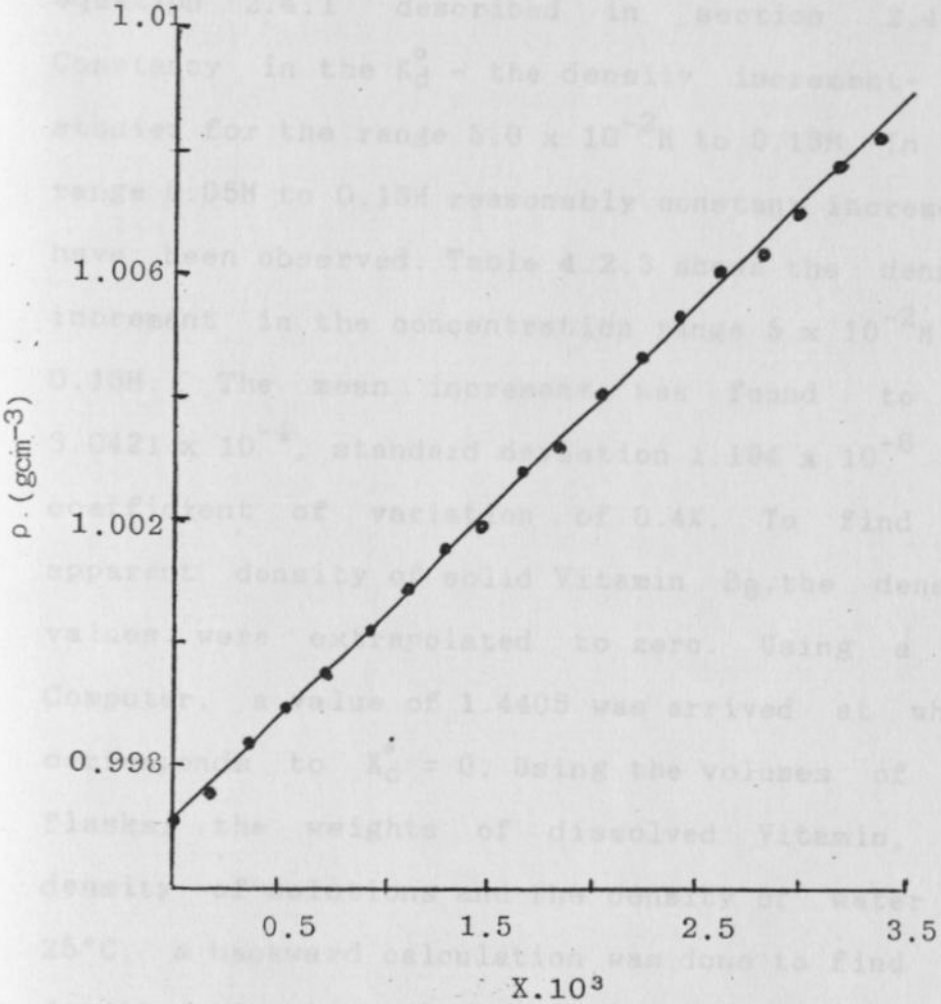
Average Deviation = $\pm 9.149 \times 10^{-5}$

Intercept = 0.99709

Gradient = 6.148×10^{-2}

Figure: 4.2.2.

DEPENDENCE OF DENSITY ON MOLE FRACTION OF VITAMIN B_6 AT 25°C



Coefficient of correlation = 0.9995

R-squared = 0.9989

Intercept = 0.99713

Gradient = 6.0176×10^{-3}

4.2.3 DENSITY INCREMENT

Calculation of density increment was based on equation 2.4.1 described in section 2.4.2. Constancy in the K_d^o - the density increment- was studied for the range 5.0×10^{-2}M to 0.13M. In the range 0.05M to 0.13M reasonably constant increments have been observed. Table 4.2.3 shows the density increment in the concentration range 5×10^{-2}M to 0.13M. The mean increment was found to be 3.0421×10^{-4}, standard deviation 1.194×10^{-6} and coefficient of variation of 0.4%. To find the apparent density of solid Vitamin B_6,the density values were extrapolated to zero. Using a BBC Computer, a value of 1.4405 was arrived at which corresponds to $K_d^o = 0$. Using the volumes of the flasks, the weights of dissolved Vitamin, the density of solutions and the density of water at 25°C, a backward calculation was done to find the density of the solid Vitamin B_6. The seven values recorded in Table 4.2.3 gave an average of 1.4330 gcm^{-3}. For the ranges where the increment is not constant, it obviously implies that K_d^o is independent of concentration.[124]

Ideally equation 4.2.2 should give the density of pure Vitamin B_6 by putting X = 1 if the nature of the solute and solvent remain unaffected by the

composition. Since density increment and partial molar volume V show a different trend, the equation can not be used with confidence. Nevertheless it can be concluded that the density of solid Vitamin is 1.5 $\pm$0.1 gcm^{-3}.

TABLE 4.2.3: DEPENDENCE OF DENSITY INCREMENT ON CONCENTRATION

$c \times 10^2$	ρ	$K_d^o \times 10^4$
5.0000	1.0002	3.0566
6.0000	1.0008	3.0605
8.0000	1.0020	3.0406
9.0000	1.0027	3.0383
11.000	1.0039	3.0333
12.000	1 0046	3.0373
13.000	1.0052	3.0283

Units c, $moll^{-1}$; ρ, gcm^{-3}

4.2.4 APPARENT VOLUME IN THE CONCENTRATION RANGE 0.0025M TO 0.4M

From the density measurements, apparent volumes were calculated using equation 2.4.5 and 2.4.6. Extrapolations were done to find the apparent volumes at infinite dilution $\emptyset^{\circ}_{v(c)}$ and $\emptyset^{\circ}_{v(m)}$. The intercepts and gradients so obtained in the given range were incorporated into equation 2.4.10 to find partial volumes. Table 4.2.4 shows the apparent volumes based on the two units. Figure 4.2.4 shows plots of apparent molar volumes against square roots of molarity and molality.

The units tend to exaggerate the apparent molal volume in the high concentration range. Such exaggerations have been reported earlier by work on Strontium Chloride and other weak electrolytes.[125,126,127,128,129] Values derived from the molarity unit are taken to be more reliable. It appears that the first significant departure from Masson's equation takes place at low concentration. At moderate dilution the S-shape is more pronounced and in concentrated solutions $\emptyset_v$ becomes linear again.

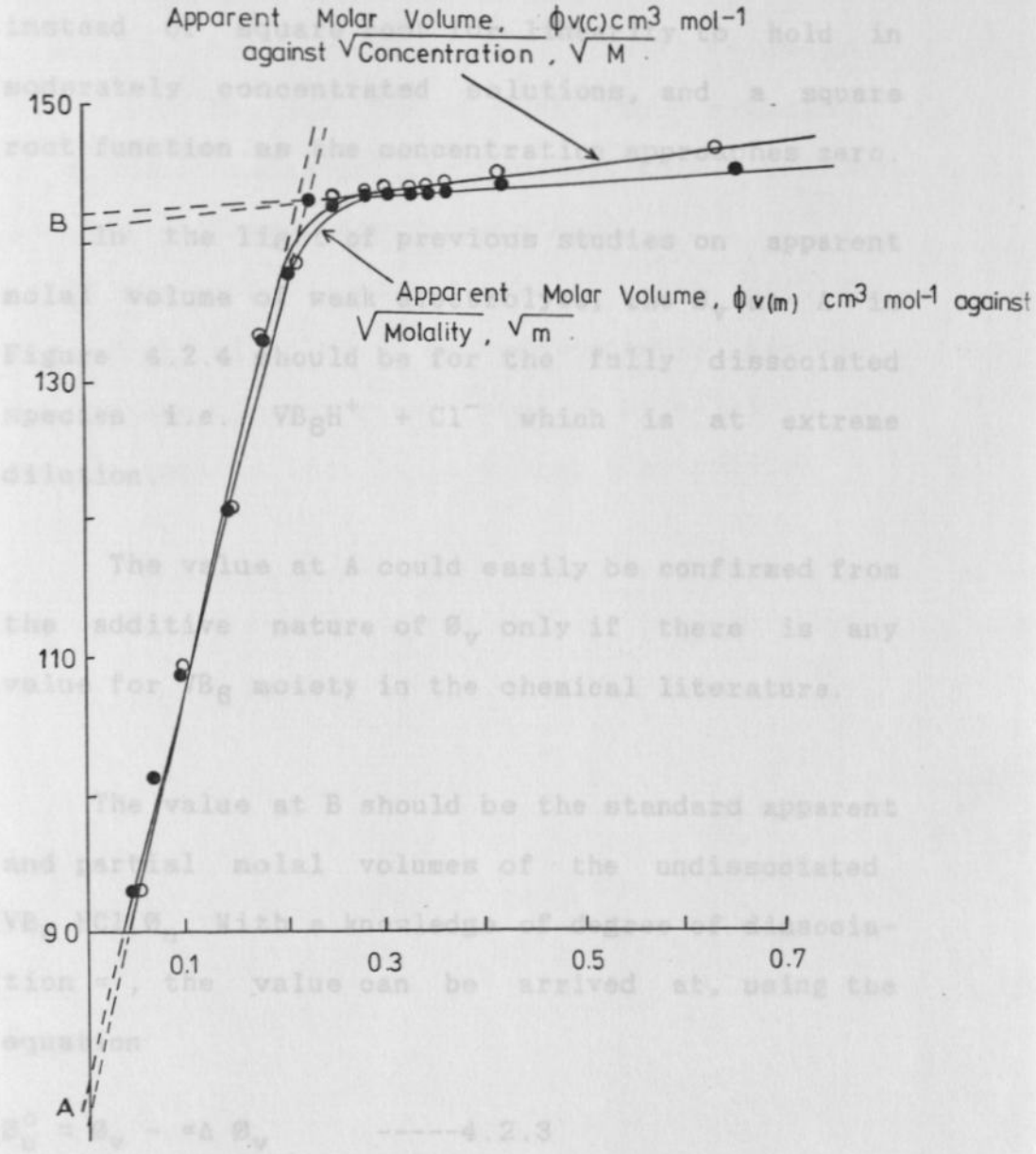
Charkravarti and Prasad[130] found that $\emptyset_v$ of single electrolyte in aqueous solution obeys

TABLE 4.2.4: APPARENT VOLUME OF VITAMIN B_6

c	m	ρ	$\emptyset_{v(c)}$	$\emptyset_{v(m)}$
0.00250	0.00251	0.99735	93.167	93.135
0.00500	0.00502	0.99759	101.36	101.30
0.01000	0.01004	0.99803	109.21	109.12
0.02003	0.02014	0.99877	121.08	120.94
0.03000	0.03020	0.99926	133.31	133.15
0.04000	0.04034	0.99980	138.08	137.89
0.05000	0.05051	1.00021	143.40	143.20
0.06000	0.06070	1.00085	143.36	143.12
0.08000	0.08117	1.00207	143.85	143.53
0.09000	0.09145	1.00269	144.93	143.58
0.11000	0.11210	1.00393	144.11	143.68
0.12000	0.12246	1.00457	144.07	143.60
0.13000	0.13287	1.00517	144.29	143.79
0.17000	0.17479	1.00756	144.97	144.33
0.40034	0.42634	1.02134	146.88	145.43

Units: c, $moll^{-1}$; m, $molkg^{-1}$; ρ , gcm^{-3}; $\emptyset_v$, cm^3mol^{-1}

FIGURE 4-2-4
COMPARISON OF APPARENT MOLAR VOLUMES ON THE SQUARE ROOTS OF MOLARITY OF VITAMIN B_6 IN THE CONCENTRATION 0·0025M—0·4M



Masson's equation for concentration above 0.03M. Singh[131] suggested a cube root of concentration instead of square root for linearity to hold in moderately concentrated solutions, and a square root function as the concentration approaches zero.

In the light of previous studies on apparent molal volume of weak electrolyte, the $\emptyset_v$ at A in Figure 4.2.4 should be for the fully dissociated species i.e. $VB_6H^+ + Cl^-$ which is at extreme dilution.

The value at A could easily be confirmed from the additive nature of $\emptyset_v$ only if there is any value for VB_6 moiety in the chemical literature.

The value at B should be the standard apparent and partial molal volumes of the undissociated $VB_6.HCl, \emptyset_u$. With a knowledge of degree of dissociation α, the value can be arrived at, using the equation

$$\emptyset_u^o = \emptyset_v - \alpha\Delta\emptyset_v \qquad \text{-----4.2.3}$$

where $\emptyset_v$ is the correction factor for hydrolysis given by the equation

$$\Delta\emptyset_v = \Sigma\emptyset_i - \emptyset_u \qquad \text{-----4.2.4}$$

The ionic apparent molal volume $\emptyset_i$, involves linear combination of $\emptyset_v$ values for compounds with VB_6 moeity and $\emptyset_u$ is the apparent molal volume of undissociated VB_6.HCl. The straight line emanating from B should represent the apparent molal volume of partially dissociated Vitamin B_6HCl.

If the plot for apparent molal volume against square roots of molality and consequently partial molal volume against square root of molality is a curve, it implies that it is a weak electrolyte.

4.2.5 PARTIAL VOLUME IN THE CONCENTRATION RANGES 0.0025M - 0.04M AND 0.05M - 0.4M

Plots of Apparent Volumes against the square roots of Molality and Molarity were done for the two concentration ranges as shown in Figures 4.2.5(A), 4.2.5(B), 4.2.5(C) and 4.2.5(D).

Inserting intercepts and gradients in equation 2.4.10 the Standard Partial Volume of undissociated VB_6HCL was calculated to be 141.89 cm^3mol^{-1} or 141.27. Since the partial molal volume of Chloride[132] ion is 19.6, the partial volume of the VB_6H^+ ion works out to be 59.491 or 59.403.

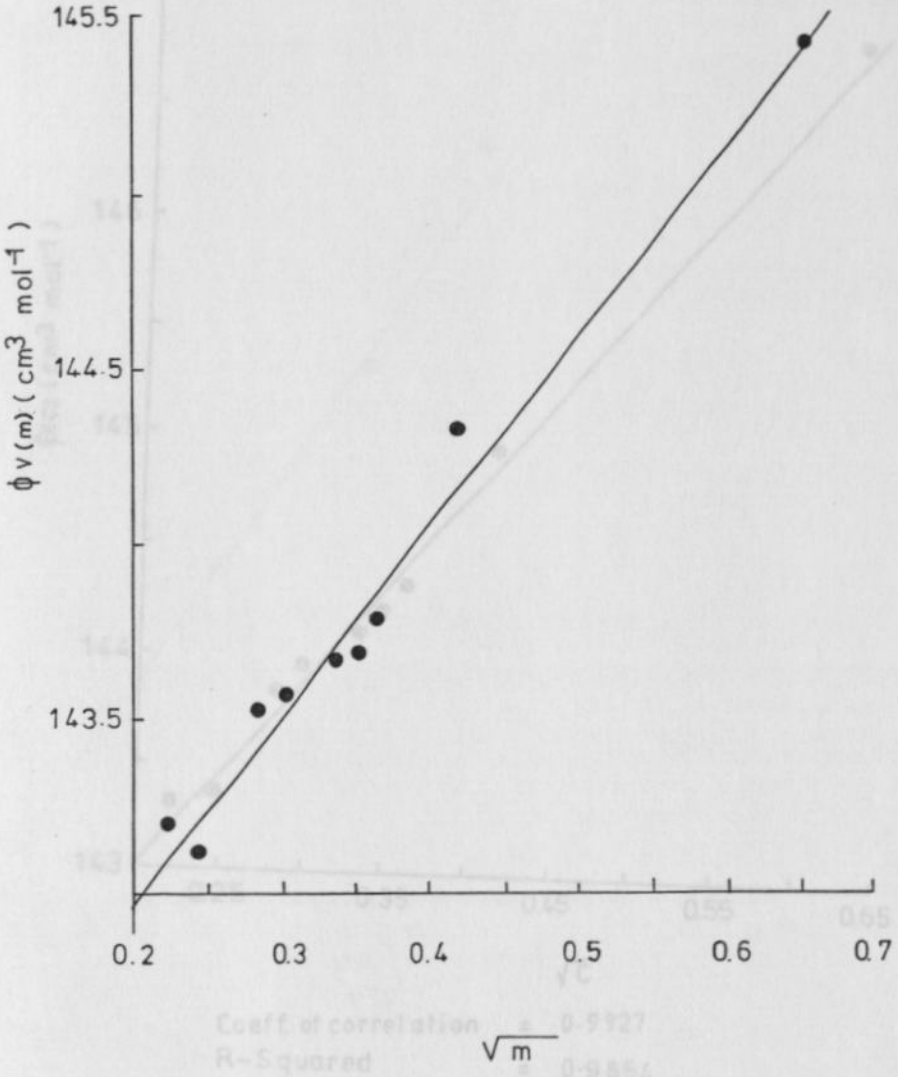
It is evident from the figures that the molarity scale is superior to molality in this respect. Comparison of columns in Table 4.2.5 indicate that the apparent and partial volume show similar trends. Deviation is more pronounced in the first range than in the second one. All of them show an increase up to a point then these become averagely constant. The difference between the two in the lower concentration range becomes bigger as the concentrations ascend, and in the higher range the difference between the two becomes very small.

TABLE 4.2.5: PARTIAL VOLUME OF VITAMIN B_6

ρ	$\emptyset_{v(c)}$	$\overline{V}_{(c)}$	$\emptyset_{v(m)}$	$\overline{V}_{(m)}$
0.99735	93.17	101.66	93.14	102.41
0.99759	101.36	111.04	101.30	111.73
0.99804	109.21	124.30	109.12	124.92
0.99877	121.08	143.09	120.94	143.62
0.99926	133.31	157.37	133.15	157.89
0.99980	138.08	169.43	137.89	169.96
⋮	⋮	⋮	⋮	⋮
1.0002	143.40	144.20	143.20	143.71
1.0008	143.36	144.48	143.12	143.89
1.0021	143.85	144.97	143.53	144.20
1.0027	143.93	145.19	143.58	144.34
1.0039	144.11	145.60	143.68	144.60
1.0046	144.07	145.79	143.60	144.72
1.0052	144.29	145.98	143.79	144.84
1.0076	144.97	146.64	144.33	145.27
1.0213	146.88	149.42	145.43	147.10

Units: ρ, gcm^3, $\emptyset v$, $cm^3 mol^{-1}$; $\overline{V}$, $cm^3 mol^{-1}$

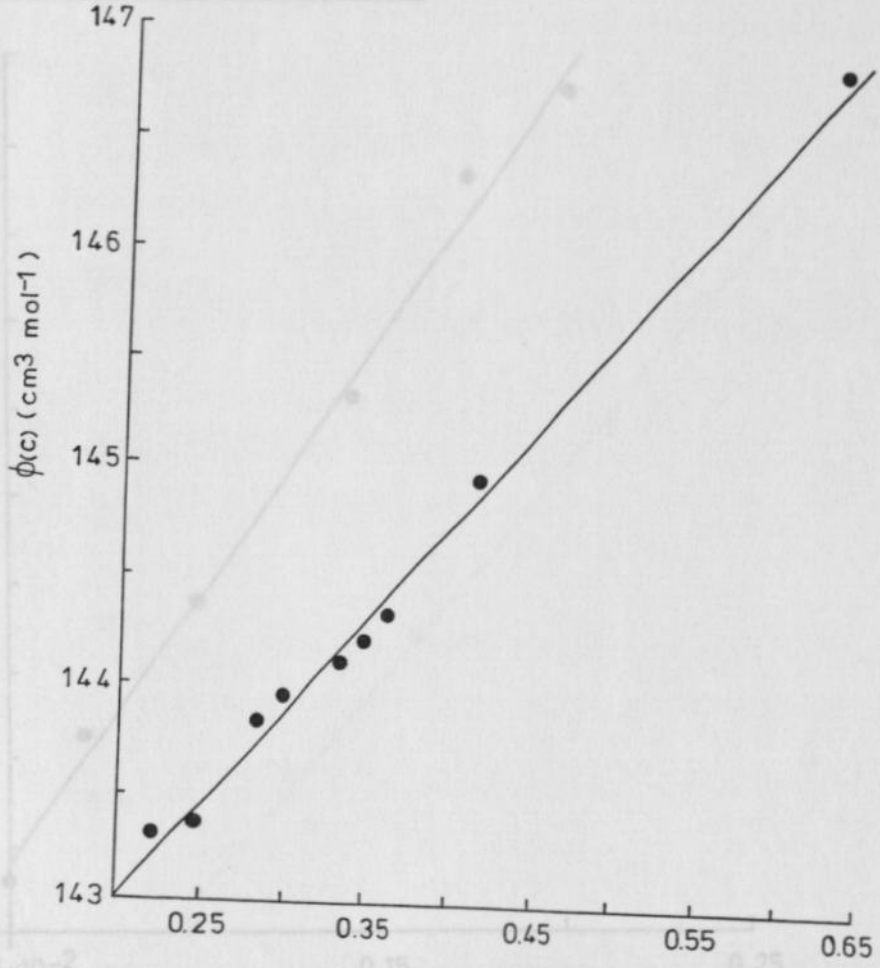
FIGURE: 4·2·5(A)
DEPENDENCE OF APPARENT MOLAR VOLUME ON SQUARE—ROOT OF MOLALITY OF VITAMIN B_6 IN THE CONCENTRATION RANGE 0·05M—0·4M.



Coefficient of Correlation = 0·9869
R—Squared = 0.9739
Equation : $\phi v(m)$ = $5.4293\sqrt{m}+141.89$

FIGURE: 4·2·5(B)

DEPENDENCE OF APPARENT MOLAR VOLUME ON SQUARE ROOT OF CONCENTRATION OF VITAMIN B_6 IN THE CONCENTRATION RANGE 0·05M——0·4M.

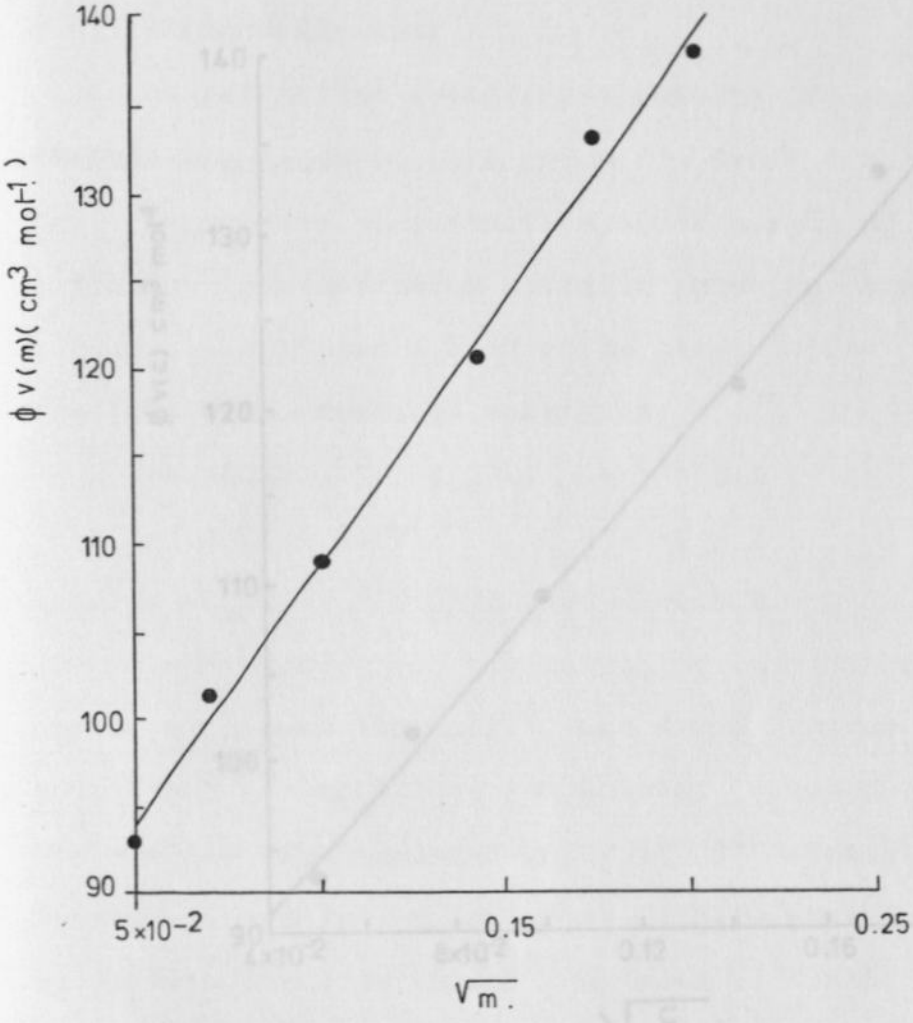


Coeff. of correlation = 0·9927

R–Squared = 0·9854

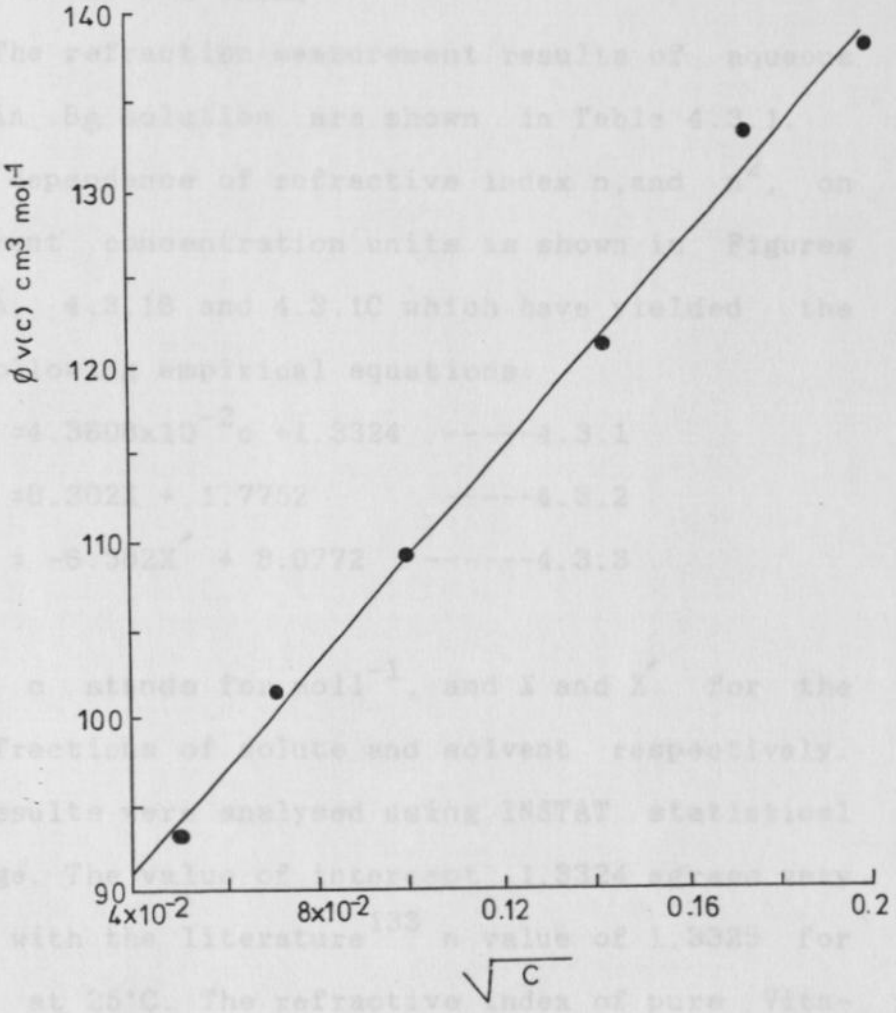
Equation: $\phi v(c) = 8{\cdot}7585\sqrt{c} + 141{\cdot}27$

FIGURE: 4·2·5(C)
DEPENDENCE OF APPARENT MOLAR VOLUME ON SQUARE—ROOT OF MOLALITY OF VITAMIN B_6 IN THE CONCENTRATION RANGE 0·0025 M — 0·04 M.



Coefficient of Correlation = 0·9914
R—Squared = 0·9948
Equation : $\phi v(m)$ = $299.58\sqrt{m} + 79.091$

FIGURE: 4·2·5(D)
DEPENDENCE OF APPARENT MOLAR VOLUME ON SQUARE-ROOT OF CONCENTRATION OF VITAMIN B_6 IN THE CONCENTRATION RANGE 0.0025M —— 0.04M.



Coefficient of Correlation = 0.9975

R-Squared = 0·9949

Equation : $\phi v(c)$ = $302{\cdot}12\sqrt{c} + 79{\cdot}003$

4.3.0 REFRACTION MEASUREMENTS

4.3.1 REFRACTIVE INDEX

The refraction measurement results of aqueous Vitamin B_6 solution are shown in Table 4.3.1. The dependence of refractive index n, and n^2, on different concentration units is shown in Figures 4.3.1A, 4.3.1B and 4.3.1C which have yielded the the following empirical equations:

$$n = 4.3608 \times 10^{-2} c + 1.3324 \quad \text{-----4.3.1}$$

$$n^2 = 6.302X + 1.7752 \quad \text{-----4.3.2}$$

$$n^2 = -6.302X' + 8.0772 \quad \text{------4.3.3}$$

where c stands for $moll^{-1}$, and X and X' for the mole fractions of solute and solvent respectively. The results were analysed using INSTAT statistical package. The value of intercept ,1.3324 agrees very well with the literature[133] n value of 1.3325 for water at 25°C. The refractive index of pure Vitamin B_6 was thus found to be 2.8421. As mentioned in section 2.3.4 the last two plots indicate no interaction[134] between the Vitamin and water;and that n^2 versus mole fraction gave a better straight line. The refractive index measurements in aqueous Vitamin B_6 solutions have not been reported in the chemical literature up to date.

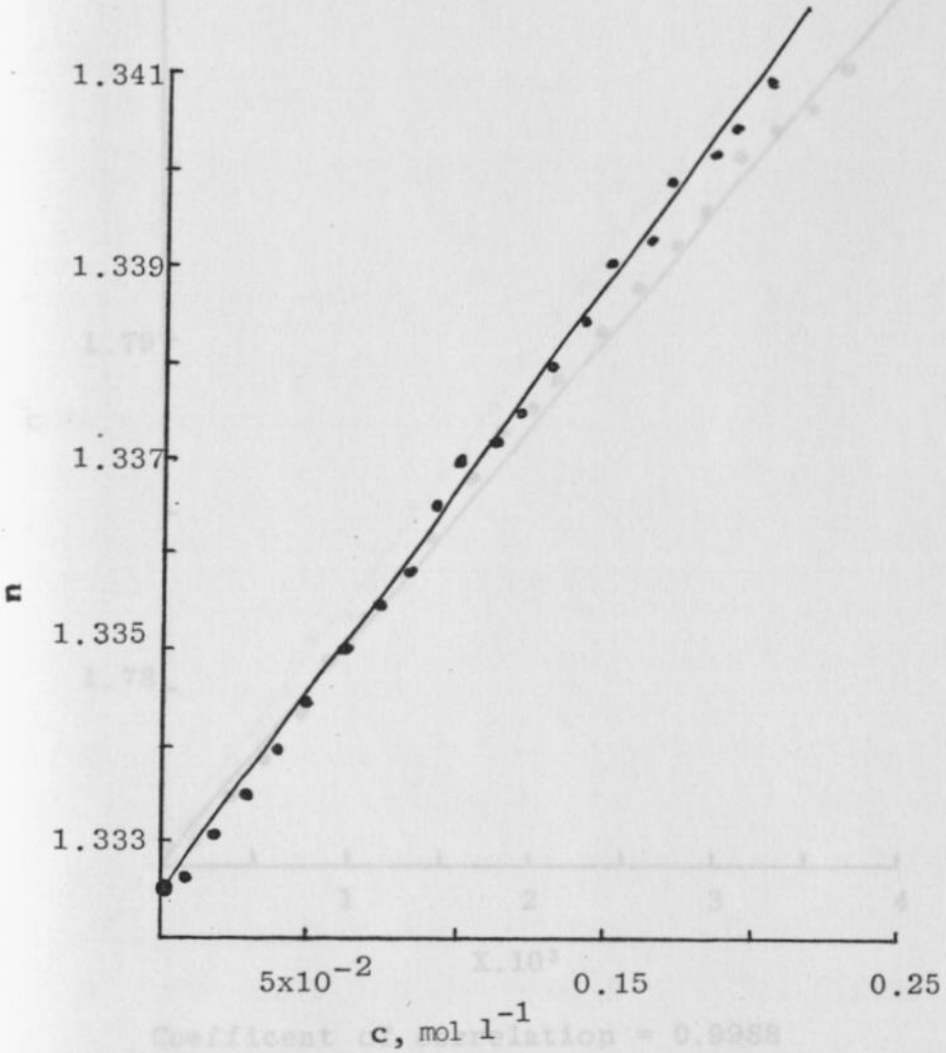
TABLE 4.3.1: REFRACTIVE INDEX OF VITAMIN B_6

$c \cdot 10^2$	$X \times 10^3$	n_{obs}	n_{Ftd}
0	0	1.3325	1.33235
0.24994	0.0451	1.3325	1.33246
1.0009	0.1809	1.3326	1.33279
1.9942	0.3607	1.3331	1.33322
3.0025	0.5441	1.3335	1.33366
4.0019	0.7262	1.3340	1.33410
5.0044	0.9092	1.3345	1.33453
6.0047	1.0923	1.3350	1.33497
7.0063	1.2761	1.3355	1.33540
8.0068	1.4602	1.3358	1.33584
9.0075	1.6448	1.3365	1.33628
10.009	1.8300	1.3370	1.33671
11.010	2.0156	1.3372	1.33715
12.011	2.1575	1.3375	1.33759
13.011	2.3883	1.3380	1.33803
14.000	2.5724	1.3385	1.33846
15.013	2.7627	1.3390	1.33890
16.000	2.9484	1.3393	1.33933
17.015	3.1391	1.3399	1.33977
18.062	3.3368	1.3402	1.34023
19.017	3.5176	1.3405	1.34064
20.017	3.7074	1.3409	1.34108

Units: c, $moll^{-1}$;X,mole fraction.

Figure: 4.3.1A

DEPENDENCE OF REFRACTIVE INDEX ON CONCENTRATION OF VITAMIN B_6.



Coefficient of correlation = 0.9989

R-squared = 0.9979

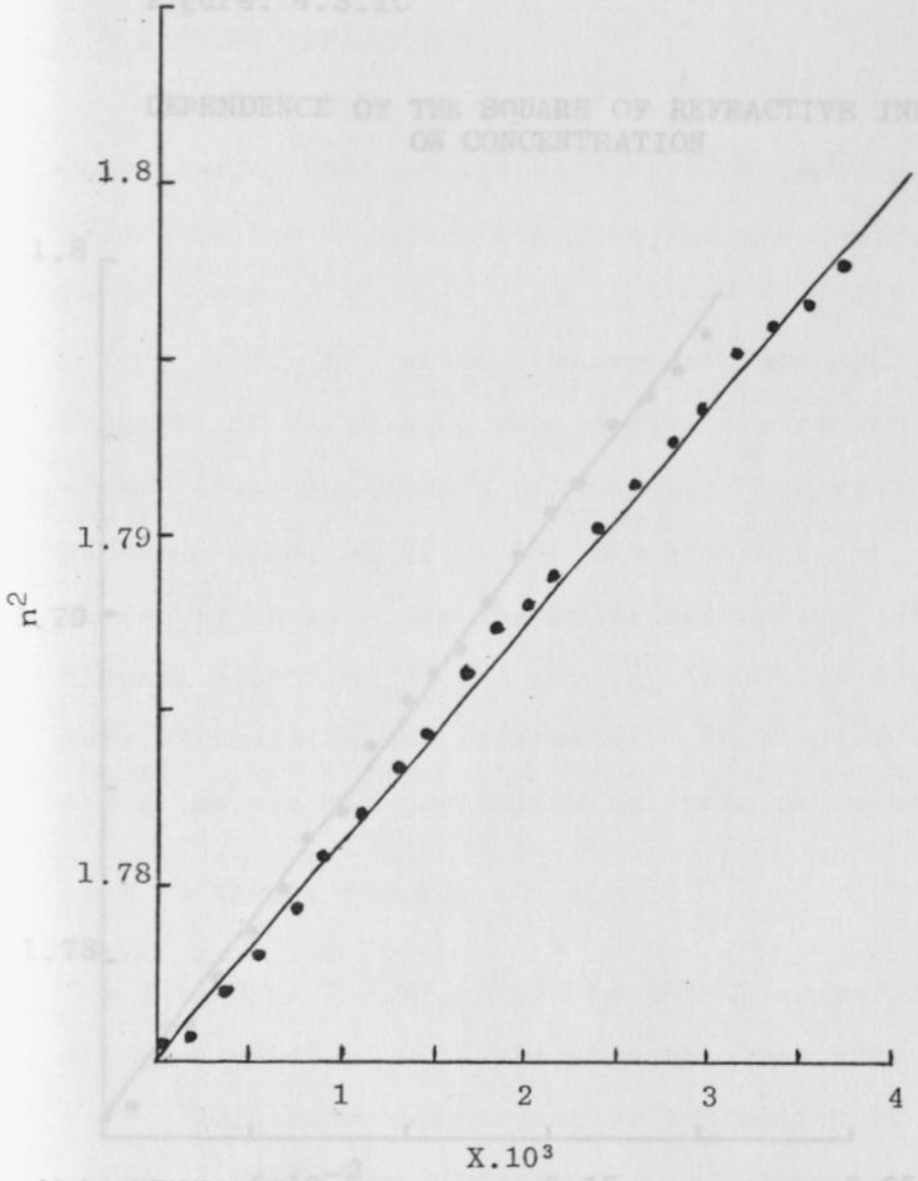
Standard Deviation = 2.7983×10^{-3}

Intercept = 1.3324

Gradient = 4.3608×10^{-2}

Figure: 4.3.1B

DEFENCE OF THE SQUARE OF REFRACTIVE INDEX ON MOLE FRACTION OF VITAMIN B_6



Coefficent of correlation = 0.9988

Standard Deviation = 7.4799×10^{-3}

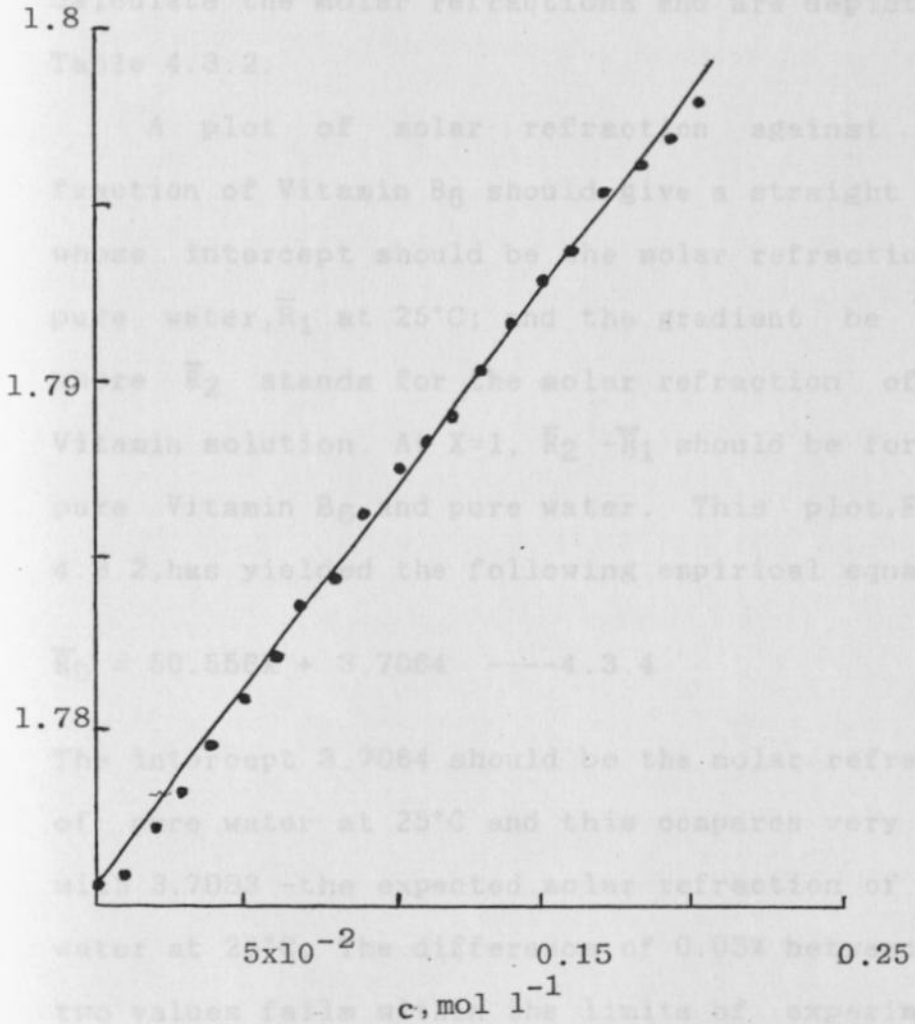
R-squared = 0.9977

Intercept = 1.7752

Gradient = 6.302

Figure: 4.3.1C

DEPENDENCE OF THE SQUARE OF REFRACTIVE INDEX ON CONCENTRATION



Coefficient of correlation = 0.9990

R-squared = 0.9979

Standard Deviation = 7.4799×10^{-3}

Intercept = 1.7752

Gradient = 0.11657

4.3.2 MOLAR REFRACTION

Results shown in Table 4.3.1 together with the densities of the aqueous of Vitamin B_6 were used to calculate the molar refractions and are depicted in Table 4.3.2.

A plot of molar refraction against mole fraction of Vitamin B_6 should give a straight line whose intercept should be the molar refraction of pure water, $\overline{R}_1$ at 25°C; and the gradient be $\overline{R}_2-\overline{R}_1$ where $\overline{R}_2$ stands for the molar refraction of the Vitamin solution. At X=1, $\overline{R}_2 - \overline{R}_1$ should be for the pure Vitamin B_6 and pure water. This plot,Figure 4.3.2,has yielded the following empirical equation:

$$\overline{R}_D = 50.556X + 3.7064 \quad \text{----4.3.4}$$

The intercept 3.7064 should be the molar refraction of pure water at 25°C and this compares very well with 3.7083 -the expected molar refraction of pure water at 25°C. The difference of 0.05% between the two values falls within the limits of experimental errors. The gradient, 50.556, gave the molar refraction of pure Vitamin B_6 obtained by adding either the experimental or theoretical value of $\overline{R}_D$ for water. Using both, we get $\overline{R}_D$ for pure Vitamin B_6 to be 54.2624 and 54.2743 respectively. The difference between the two values is only 0.022%. Workers on Lithium Chloride[135] have reported a

difference of 0.6370% between such two values. The average of the two values is 54.2684 which can safely be taken as $\overline{R}_D$ value for the Vitamin.

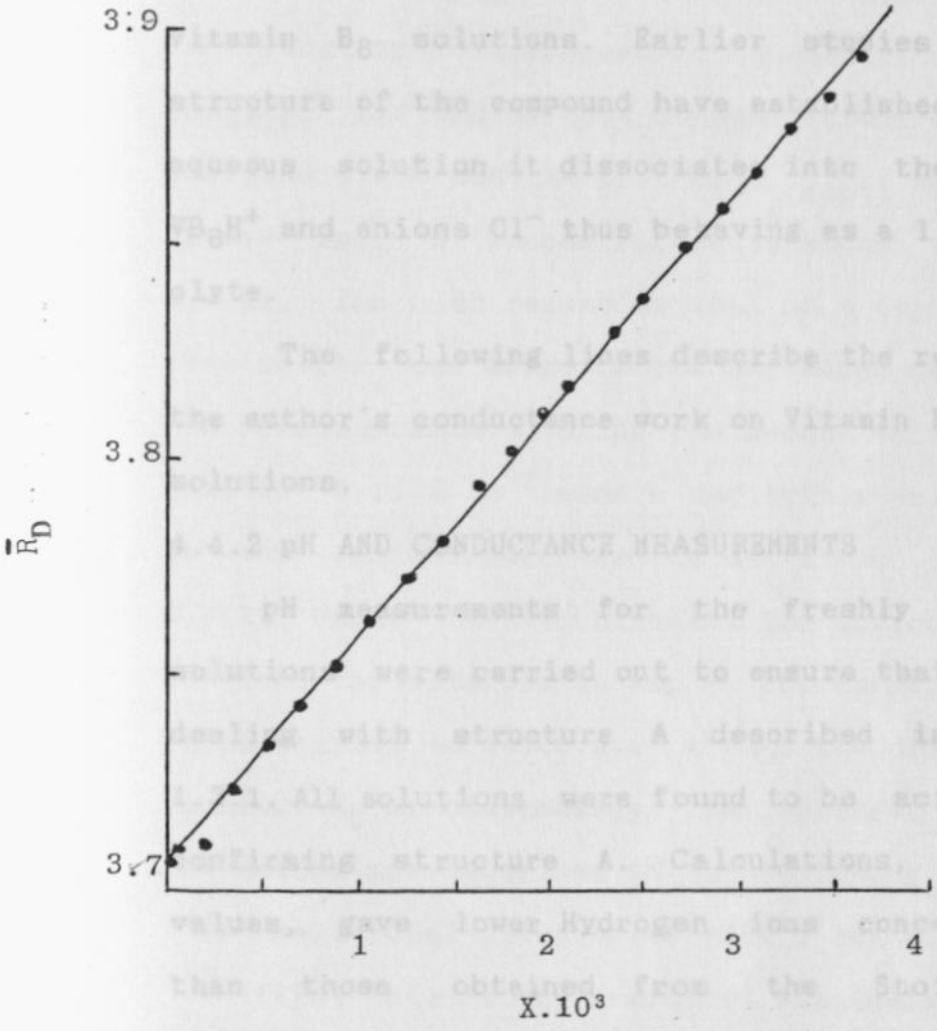
TABLE 4.3.2: MOLAR REFRACTION OF VITAMIN B_6

$X \cdot 10^3$	ρ	n	$\bar{V}$	$\bar{R}_D$
0	0.99707	1.3325	18.053	3.7083
0.04513	0.99735	1.3325	18.056	3.7090
0.18091	0.99839	1.3326	18.063	3.7114
0.36068	0.99843	1.3331	18.096	3.7233
0.54412	0.99889	1.3335	18.122	3.7327
0.72620	0.99945	1.3340	18.146	3.7428
0.90918	1.0004	1.3345	18.163	3.7513
1.0923	1.0006	1.3350	18.195	3.7630
1.2761	1.0015	1.3355	18.212	3.7716
1.4602	1.0019	1.3358	18.240	3.7804
1.6448	1.0022	1.3365	18.269	3.7936
1.8300	1.0035	1.3370	18.279	3.8009
2.0156	1.0039	1.3372	18.307	3.8088
2.1575	1.0046	1.3375	18.321	3.8147
2.3883	1.0052	1.3380	18.353	3.8266
2.5724	1.0059	1.3385	18.375	3.8361
2.7627	1.0062	1.3390	18.403	3.8473
2.9484	1.0068	1.3393	18.428	3.8555
3.1391	1.0076	1.3399	18.449	3.8662
3.3368	1.0081	1.3402	18.477	3.8751
3.5176	1.0088	1.3405	18.498	3.8825
3.7074	1.0094	1.3409	18.522	3.8916

Units: ρ, gcm^{-3}; $\bar{V}$, cm^3mol^{-1}; $\bar{R}_D$ = cm^3mol^{-1}

Figure: 4.3.2.

DEPENDENCE OF MOLAR REFRACTION ON MOLE FRACTION OF VITAMIN B_6



Coefficient of correlation = 0.9996

R-squared = 0.9992

Intercept = 3.7064

Gradient = 50.556

Standard Deviation = 5.9959×10^{-2}

4.4.0 CONDUCTANCE RESULTS

4.4.1 RAW DATA AND CALCULATION OF Λ^{o} AND K_D

Up to-date literature survey indicates that no conductance work has been done so far on aqueous Vitamin B_6 solutions. Earlier studies on the structure of the compound have established that in aqueous solution it dissociates into the cations VB_6H^+ and anions Cl^- thus behaving as a 1:1 electrolyte.

The following lines describe the results of the author's conductance work on Vitamin B_6 aqueous solutions.

4.4.2 pH AND CONDUCTANCE MEASUREMENTS

pH measurements for the freshly prepared solutions were carried out to ensure that we were dealing with structure A described in section 1.3.1. All solutions were found to be acidic thus confirming structure A. Calculations, using pH values, gave lower Hydrogen ions concentrations than those obtained from the Stoichometric considerations. This suggested that the compound was undergoing incomplete dissociation otherwise the calculated and the observed H^+ ion concentration would have been the same. The observation provided a premise to treat the Vitamin in aqueous solution as a 1:1 weak electrolyte and hence to derive Λ^{o} and pK values.

From the measured resistance, R, of an aqueous Vitamin solution, equivalent conductance , $\Lambda_{(expt)}$, was calculated from the definition of the quantity using 5.4212 cm^{-1} as the cell constant and incorporating stoichometric concentration. The $\Lambda_{(expt)}$ for the various concentrations are shown in Table 4.4.2 and the plot of $\Lambda_{(expt)}$ against the square root of concentration is shown in Figure 4.4.2A. The plot resembles that of a typical weak electrolyte and supports the inference drawn from the pH measurements. Using the second value of cell constant 5.1425 cm^{-1},made a negligibly small effect on the conductance results as shown in Figure 4.4.2A. Under the circumstances the obvious course to treat the data was to employ Shedlovsky's method which enables to calculate Λ^{o} and the dissociation constant K_D simultaneously. The important steps of the method are described in the following lines[136]:

(a) An initial value of Λ^{o}=165.5 $Scm^{2}equiv^{-1}$ was first obtained by graphical extrapolation (see Figure 4.4.2(A) to calculate S(Z) function defined by the expression:

$$S(Z) = 1 + Z + \frac{Z^2}{2} + ...$$

where

$$Z = \{ B_1\Lambda^{o} + B_2 \}.(c\ \Lambda)^{0.5}.(\Lambda^{o})^{-1.5}$$

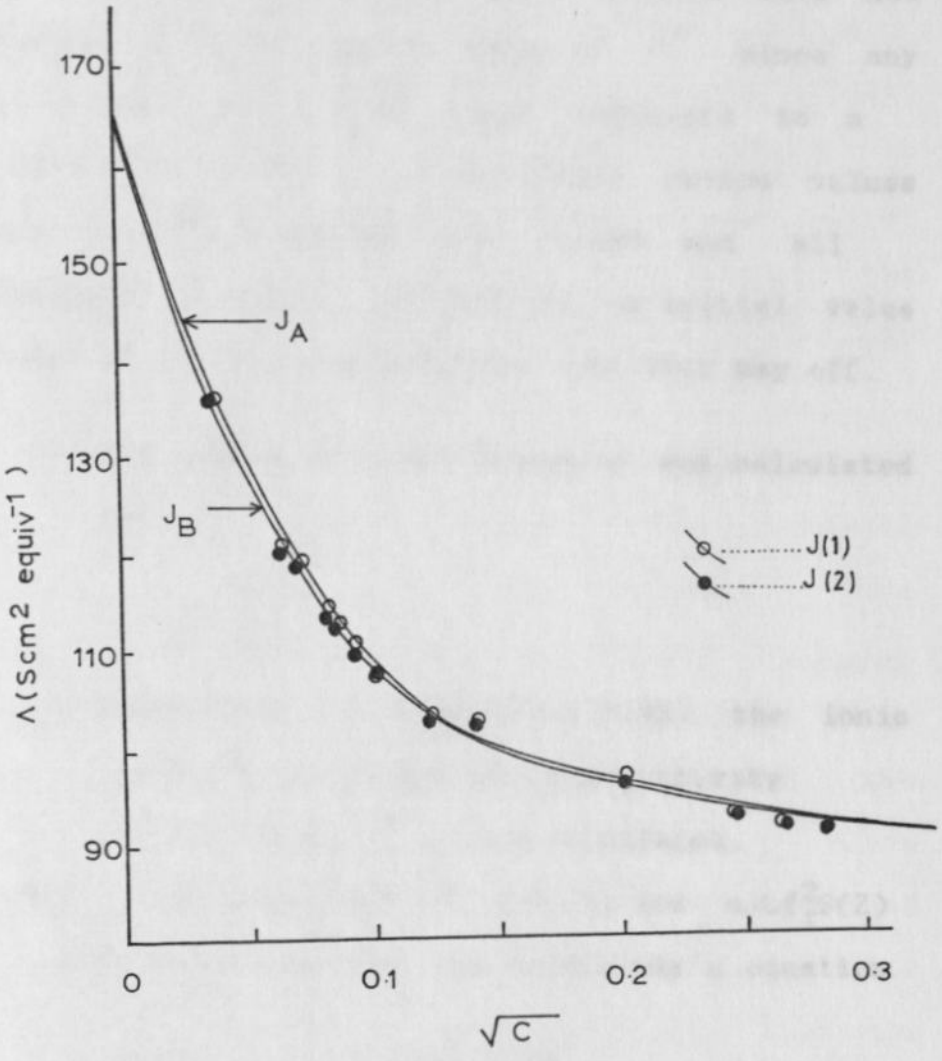
TABLE 4.4.2: CONDUCTANCE MEASUREMENTS IN AQUEOUS VITAMIN B_6 SOLUTION AT 25°C

c . 10^2	R*	Λ_{obs}	Λ_{Ftd}	$\Lambda_{Ftd} - \Lambda_{obs}$
0.15090	26346.1	136.36	135.41	-0.9513
0.40004	11224.0	120.74	122.13	1.3909
0.49994	9079.5	119.43	118.82	-0.6151
0.67278	7053.5	114.24	114.39	0.1489
0.73525	6530.2	112.91	113.08	0.1714
0.86523	5669.0	110.53	110.73	0.2017
1.0008	5032.4	107.64	108.69	1.0551
1.5356	3422.8	103.14	103.31	0.1709
2.0007	2637.8	102.72	100.62	-2.1035
4.0019	1404.4	96.46	96.10	-0.3584
6.0000	985.3	91.71	93.20	1.4976
7.0001	854.3	90.65	90.95	0.3019
7.9998	763.1	88.81	87.90	-0.9100

Units, c, $moll^{-1}$; R,Ohm; Λ , Scm^2equiv^{-1}

* An average of 5 readings

FIGURE: 4·4·2 A
EQUIVALENT CONDUCTANCE AGAINST SQUARE ROOT OF CONCENTRATIO OF AQUEOUS VITAMIN B HYDROCHLORIDE AT 25°C USING TWO EQUATIONS FOR CELL CONSTANT.



$J(1) = 5{\cdot}4212$, using $\Lambda^{25}_{kcl} = 149{\cdot}93 - 94{\cdot}65\sqrt{C} + 58{\cdot}74C\log C + 198{\cdot}4C$

$J(2) = 5{\cdot}4125$, using $\Lambda^{25}_{kcl} = (\Lambda^{o} + BC + DC\log C)(1-\gamma\sqrt{C}) - 2\sigma\sqrt{C}$

where: $B = 153{\cdot}70$, $D = 32{\cdot}10$, $\sigma = 30{\cdot}09$
$\gamma = 0{\cdot}2289$, $\Lambda = 149{\cdot}88$, C = Concentratio

R–Squared for both graphs = 0·9947

In fact the mathematics involved does not require a very accurate value of Λ^o since any value used for a given range converges to a single value for Λ^o. Accordingly random values like 100,200,1000,5000 were tried and all converged to yield Λ^o= 138.29. An initial value closer to 138 converges faster than that way off.

(b) The degree of dissociation α was calculated using

$$\alpha = \frac{S(Z)}{\Lambda^o}$$

(c) Inserting α in equation 2.2.22, the ionic strength and hence the mean activity coefficient, $f_{\pm}^2$ was calculated.

(d) The quantities $1/[\Lambda S(Z)]$ and $c.\Lambda.f_{\pm}^2 S(Z)$ were calculated for the Shedlovsky's equation

$$\frac{1}{\Lambda S(Z)} = \frac{1}{\Lambda^o} + \frac{c.\Lambda.f_{\pm}^2 S(Z)}{K_D(\Lambda^o)^2}$$

(e) A better estimate of Λ^o was made by plotting $1/[\Lambda S(Z)]$ against $c.\Lambda.f_{\pm}^2 S(Z)$

Since the numerical values of the quantities $1/[\Lambda S(Z)]$ and $c.\Lambda.f_{\pm}^2 S(Z)$ are very small, it was extremely difficult to get accurate values from a manual plot. Accordingly a computer programme, given in Appendix I , was written to continue the

iteration and to read the intercept and the gradient by the least square method. It also included instructions to calculate K_D, pKa values and the correlation coefficient and to transfer the data for $1/[\ \Lambda S(Z)]$ and $c.\Lambda.f_{\pm}^{2}S(Z)$ values to an INSTAT statistical package to recheck the intercept, gradient and correlation coefficient and finally to plot the values.

When all the values recorded in Table 4.4.2 were fed into the programme, the print-out indicated Λ^{o} value of 124.35 $Scm^{2}equiv^{-1}$. This value is smaller than even the highest $\Lambda_{(expt)}$ recorded. Considering lower concentration range improved the correlation coefficient and gave higher Λ^{o} value [Appendix II -III shows different Λ^{o} s obtained in different concentration range]. It was therefore inferred that some data points were not falling on the straight line and the program must have ignored these values.

The linear nature of the plot for the entire concentration range is greatly marred in the high concentration range due to ion association and hydration effects.

In the concentration range below 0.0001M, it is very difficult to make satisfactory measurements due to the errors involved in making such dilute solutions and measuring rather very high resistance

accurately. The two factors thus introduce large errors.[137] Hence points 2,3, 4, 5, 6, 7 were taken. These points greatly improved correlation coefficient giving, as expected, Λ^{o} higher than the highest value in the concentration range studied.

The resistance of the six solutions which fall within the permissible limits (700 - 70,000 Ohm) produced Λ^{o} =138.29 and pK_a = 1.447 with a correlation coefficient 0.9929.

The data for these six concentration were rigorously treated and various initial value of Λ^{o} i.e.300, 500, 600 and 1000 Scm^2 $equiv^{-1}$ were tried. Each value,however,converged to 138.29 $Scm^2 equiv^{-1}$. Table 4.4.2B shows important steps in the calculations and displays $\frac{1}{\Lambda S(Z)}$ and $c.\Lambda f_{\pm}^2 S(Z)$ values. These being small quantities, a small error in $\frac{1}{\Lambda S(Z)}$ would give a big change in Λ^{o} and K_a value.[138]

It is to be noted that the Λ^{o} value so obtained is smaller than for the proton[139] i.e $\lambda^{o}_{H^+}$ = 349.8. This is not surprising as the proton remains attached to the pyridine ring to give the cationic structure described in section 1.3.1. The Λ^{o} value thus lends support to the structure and gives $\lambda^{o}B_6H^{+}$ = to 61.94.(Since[140] $\lambda^{o}_{Cl^-}$ = 76.35). The Λ^{o} is smaller than $\lambda^{o}_{H^+}$ because VB_6H^+ is a

bigger entity than H^+ and can not conduct electricity by the electron transfer process as free H^+ ions do.[141]

TABLE 4.4.2B: CALCULATION OF VARIOUS QUANTITIES FOR SHEDLOVSKY CONDUCTANCE EQUATION FOR SELECTED RANGE

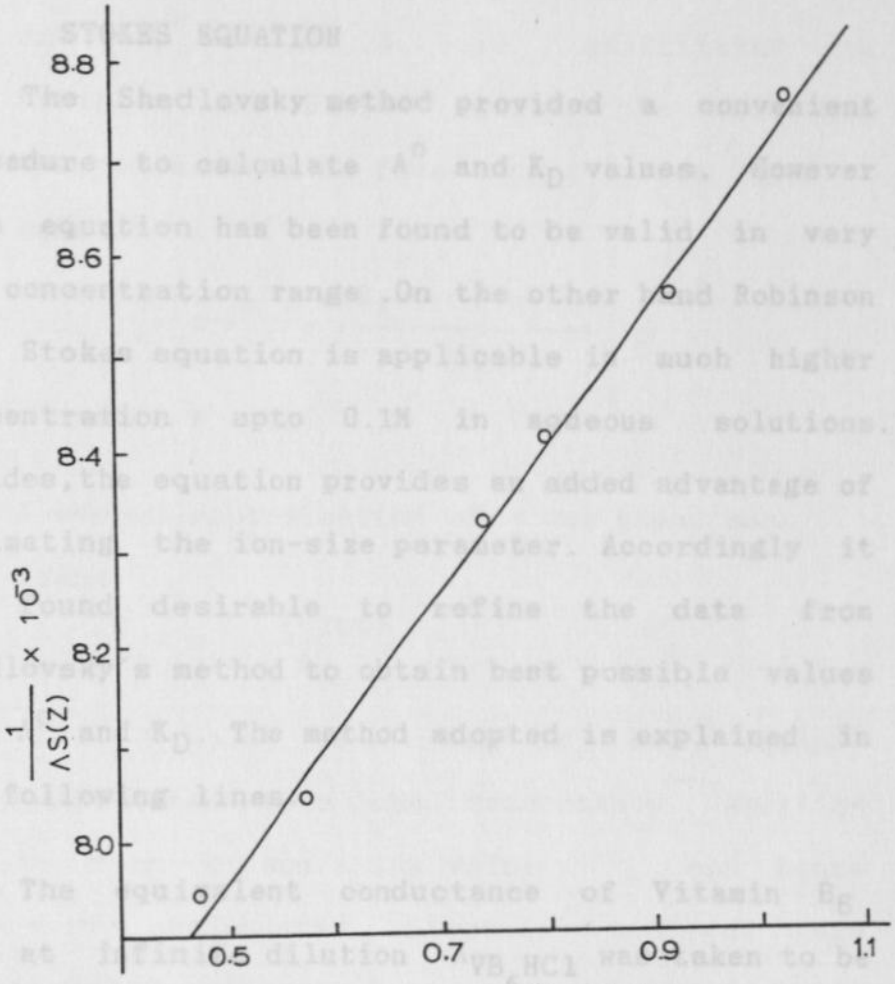
Λ	$S(Z)$	α	$f_{\pm}^2$	$\frac{1 \times 10^3}{\Lambda S(Z)}$	$c\Lambda f_{\pm}^2 S(Z)$
120.74	1.0403	0.90829	0.93273	7.9616	0.46866
119.43	1.0449	0.90242	0.92607	8.0133	0.57776
114.24	1.0511	0.86831	0.91740	8.3281	0.74112
112.91	1.0532	0.85988	0.91458	8.4097	0.79961
110.53	1.0571	0.84492	0.90921	8.5586	0.91916
107.64	1.0608	0.82564	0.90446	8.7585	1.03354

Units: Λ, Scm^2equiv^{-1}

INTERCEPT	$= 7.2313 \times 10^{-3}$
GRADIENT	$= 1.430 \times 10^{-3}$
CORRELATION COEFFICIENT	= 0.99290
Λ^o	= 138.29
DISSOCIATION CONSTANT	$= 3.5744 \times 10^{-2}$
$-\log K_D$	= 1.4468

FIGURE: 4·4·2 B

PLOT OF $[\Lambda S(Z)]^{-1}$ AGAINST $C\Lambda f^2 \pm S(Z)$.



Intercept	$= 7.2313 \times 10^{3}$
Gradient	$= 1.4630 \times 10^{3}$
Correlation Coefficient	= 0·99290
R — Squared	= 0·9858
Λ^{o}	= 138·29
Dissociation	$= 3.5744 \times 10^{-2}$
pKa Value	= 1·4468

4.4.3 REFINEMENT OF Λ^{o} AND K_D USING ROBINSON AND STOKES EQUATION

The Shedlovsky method provided a convenient procedure to calculate Λ^{o} and K_D values. However this equation has been found to be valid in very low concentration range .On the other hand Robinson and Stokes equation is applicable in much higher concentration upto 0.1M in aqueous solutions. Besides,the equation provides an added advantage of estimating the ion-size parameter. Accordingly it was found desirable to refine the data from Shedlovsky's method to obtain best possible values for Λ^{o} and K_D. The method adopted is explained in the following lines:

(a) The equivalent conductance of Vitamin B_6 at infinite dilution Λ_{VB_6HCl} was taken to be 138.29 Scm^2equiv^{-1} as calculated from Shedlvosky's method.

(b) An α value for the degree of dissociation was obtained from the equation

$$\alpha = \frac{\Lambda_{(Expt)}}{138.29}$$

(c) The degree of dissociation α from step (b) and Λ^{o} = 138.29 were substituted in Robinson and Stokes equation to calculate the conductance of free ions Λ , from their equation

$$\frac{\Lambda}{\alpha} = \Lambda^{o} - \frac{(B_1 \Lambda^{o} + B_2)(c\alpha)^{0.5}}{1 + B_3 \mathring{a}(c\alpha)^{0.5}}$$

with $\mathring{a}$ = 5Å

(d) A second approximation of α was then made using

$$\alpha = \frac{\Lambda(expt)}{\Lambda}$$

(e) The α value from step (d) was again substituted into the conductance equation in step (c) and a new value of Λ and hence α was calculated. Steps (c) and (d) were iterated using a Computer program, given in appendix IV, till α was constant.

Table 4.4.3A shows the experimental conductance and the calculated conductances for the entire range studied. Robinson's method gave Λ^{o}=133.05 if the whole range is taken into account. The value 133.05 is still lower than the highest value in the table. Appendix V shows a print out of the results.

TABLE 4.4.3A : Λ BY USING ROBINSON & STOKES EQUATION FOR THE ENTIRE RANGE

$c \cdot 10^2$	Λ_{expt}	Λ_{Cal}
0.15090	136.36	135.63
0.40004	120.74	126.77
0.49994	119.43	125.83
0.67278	114.24	122.71
0.73525	112.91	121.88
0.86523	110.53	120.37
1.0004	107.64	115.44
1.5356	103.14	114.73
2.0007	102.72	109.83
4.0019	96.460	106.20
6.0000	91.706	105.21
7.0001	90.650	103.82
7.9998	88.810	122.33

Units: c, $moll^{-1}$; Λ , Scm^2equiv^{-1}

Analysis of the six points gave a refined value of Λ^{o} = 142.58 which is still close to the initial value. All the six points lie on a straight line as shown in Figure 4.4.3B. K_D values for different concentrations were calculated from α values obtained by Robinson and Stokes method and converted to the molal scale using the equation,[142]

$$pK_{(m)} = pK_{(c)} + \log d_o$$

where d_o is the density of the solvent.

Appendix VI shows a Computer printout of the results. Table 4.4.3C gives a summary of the results.

A mean value of 7.2867×10^{-3} was found for K_D. The conductance value of 142.58 and K_D can confidently be assigned to the Vitamin, since the K_D so obtained gave a theoretical Λ^{o} close to the observed.

TABLE 4.4.3B : Λ BY ROBINSON AND STOKES EQUATION FOR SELECTED RANGE

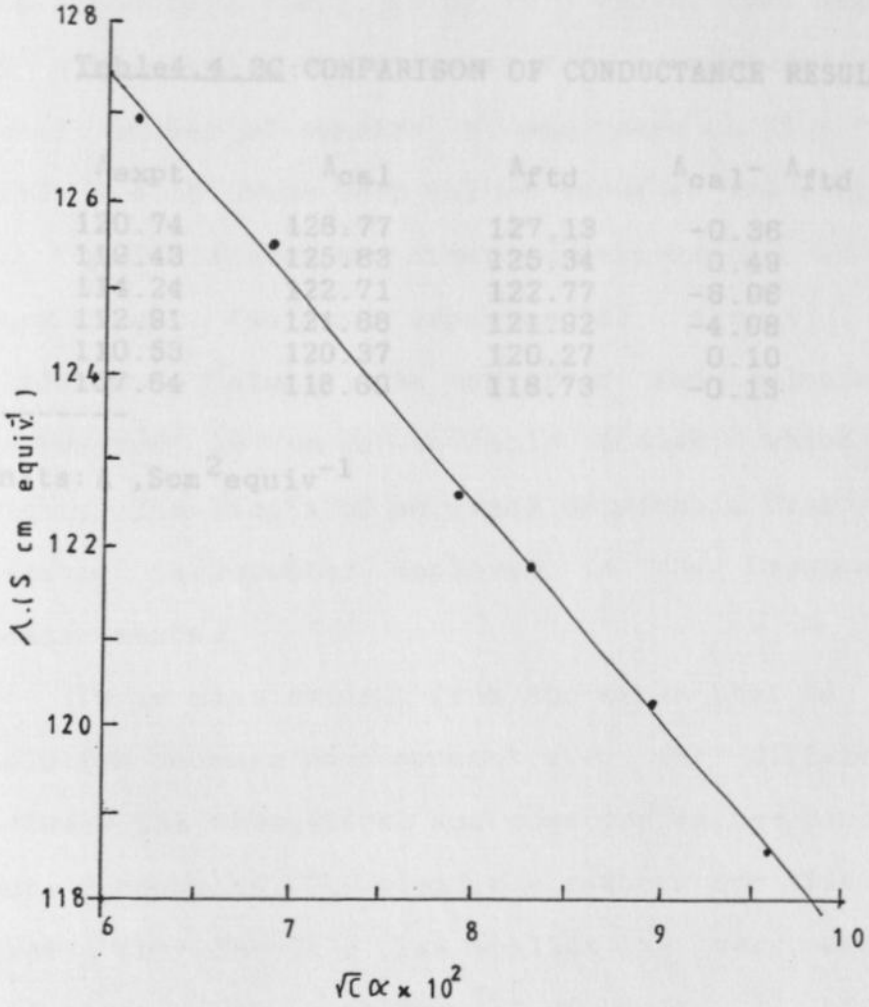
$c \cdot 10^3$	Λ_{expt}	Λ_{Cal}	α	I x 10
4.0004	120.73	126.77	0.95240	6.1725
4.9994	119.43	125.83	0.94917	6.8886
6.7278	114.24	122.71	0.93096	7.9141
7.3525	112.91	121.88	0.92638	8.2530
8.6523	110.53	120.37	0.91818	8.9131
10.0008	107.64	118.60	0.90759	9.5307

Units : Λ Scm^2equiv^{-1}

Intercept	=142.576
Gradient	=-5.08533
Correlation Coefficient	=-0.9959
Λ^o	=142.576

FIGURE 4.4.3 B.

DEPENDENCE OF REFINED CONDUTANCE ON THE SQUARE ROOT OF IONIC STRENGTH FOR 4.000×10^{-3}M TO 1.0008×10^{-3}M



Correlation coefficient	=	-0.9959
R-Squared	=	0.9919
Standard deviation	=	3.1393
Intercept	=	142.58
Gradient	=	-250
Λ°	=	142.58

Table4.4.3C:COMPARISON OF CONDUCTANCE RESULTS

Λ_{expt}	Λ_{cal}	Λ_{ftd}	$\Lambda_{cal} - \Lambda_{ftd}$
120.74	126.77	127.13	-0.36
119.43	125.83	125.34	0.49
114.24	122.71	122.77	-6.06
112.91	121.88	121.92	-4.08
110.53	120.37	120.27	0.10
107.64	118.60	118.73	-0.13

Units: Λ , $Scm^2 equiv^{-1}$

4.4.4 CRYOSCOPIC RESULTS

The ionic model described in section 2.1.3 was tried on the basis that it is a weak electrolyte. The K_D values obtained by Shedlovsky's method and its refined version were each tried in the cryoscopic study. Using this value, the degree of dissociation was calculated by iteration. The total number of species ,n, was taken as $(1 + \alpha)$. Table 4.4.4A shows such values based on the refined K_D. The calculated and observed depressions agreed very well within experimental errors. The difference between the observed and calculated depression is shown in Table 4.4.4B. which is within the limits of accuracy obtainable from the Beckman thermometer employed in the Cryoscopic measurements.

It is also evident from the table that as the solution becomes more concentrated, the difference between the theoretical and observed values becomes more pronounced. The plausible reasons for this are that, the Raoult's law applies to very dilute solutions upto a maximum[143] of 0.1m. At higher concentration and at sub-zero temperatures formation of other associated species come into being.

Table 4.4.4C indicates the calculated molecular mass using $\varepsilon = 0.54 \times 10^{-3}$ and $K_f = 1.86$. Molecular masses calculated using the two K_D values are also compared. The refined value of 7.2867×10^{-3} gave a mean equal to 206.70g which is 0.52% more than the actual value. The K_D value from Shedlovsky's method gave a mean of 210.68 which is 2.5% higher than the actual value. The refined K_D value thus claims its weightage. The difference can be attributed to three main factors. Firstly the weights were not corrected for the air buoyancy, secondly, the K_D was not corrected for the temperature, and thirdly the third decimal places in the readings were estimates, as the thermometer could only be read upto the second decimal place. The error introduced by a difference of 0.001 in the freezing point shows a marked effect as the concentration decreases. This is expected since depression is very small in very dilute solutions and a small change can result in a bigger percentage error.

TABLE 4.4.4A: CALCULATION OF n,THE NUMBER OF MOLES

m	α	$n = (1+\alpha)$
0.01015	0.99734	1.99734
0.02010	0.99070	1.99070
0.02020	0.99071	1.99071
0.04040	0.96945	1.96945
0.04039	0.96943	1.96943
0.06076	0.94246	1.94246
0.07098	0.92798	1.92798
0.09153	0.89845	1.89845
0.11200	0.86928	1.86928

Units: m, $molKg^{-1}$;n,total number of species

TABLE 4.4.4B: CALCULATION OF THEORETICAL DEPRESSION OF FREEZING POINT.

m	ΔT_f*	ΔT_f	n	ΔT_f* − ΔT_f
0.01015	0.037391	0.0370	1.99734	−0.0004
0.02010	0.074535	0.0729	1.99070	−0.0016
0.02020	0.074593	0.0742	1.99071	−0.0004
0.04040	0.14968	0.1450	1.96945	−0.0047
0.04039	0.14973	0.1450	1.96943	−0.0047
0.06076	0.22449	0.2175	1.94246	−0.0070
0.07098	0.26176	0.2550	1.92798	−0.0068
0.09153	0.33606	0.3275	1.89845	−0.0086
0.11200	0.40999	0.3965	1.86928	0.0135

Units: ΔT_f, ΔT_f*; °C, m, molKg^{-1}

* Theoretical value.

TABLE 4.4.4C: MOLECULAR MASS FROM DEPRESSION OF FREEZING POINT

m	ΔT_f	$M_{(S)}$	$M_{(R)}$
0.01015	0.0370	207.81	207.59
0.02010	0.0729	210.25	209.48
0.02020	0.0742	206.45	205.69
0.04040	0.1450	212.28	209.73
0.04039	0.1450	212.36	209.81
0.06076	0.2175	212.25	207.52
0.07098	0.2550	211.09	205.24
0.09153	0.3275	211.02	202.91
0.11200	0.3965	212.63	202.29

Units: m, $molKg^{-1}$; R.F.M, g; ΔT_f, °C

Average $M_{(s)}$=210.68 Mol Wt. using K_D from Shedlovsky equation

Average $M_{(R)}$=206.70 Mol Wt using K_D from Robinson and Stoke equation.

CONCLUSION

The presence of HCL in the Vitamin B_6 deceivingly suggested that the Vitamin would behave as a strong electrolyte. However, the independent measurements undertaken in this work have shown that it behaves as a 1:1 weak electrolyte and that there is little solvent-solute interaction. Partial Molal Volume, $\overline{V}$, studies show a steep increase with increase in concentration to a point, then it levels off. The two portions of the curve clearly explain the ionic species expected in the Acidic aqueous medium.

The study has produced the following new Physicochemical data at 25°C.

(a) Density of solid Vitamin B_6 = 1.44 gml^{-1}

(b) Equation for density in the range (0.01M - 0.18M)

$$\rho = 6.1489 \times 10^{-2}C + 0.99709$$

(c) Average Partial Molal Volume, $\overline{V}$, for fully dissociated Vitamin B_6 Hydrochloride and Vitamin B_6 with attached proton as 79 and 59.49 dm^3Mol^{-1} respectively.

(d) Limiting Equivalent Conductance

Λ^o= 142.58 and $\lambda^o_{VB_6H^+}$ = 66.23 Scm^2equiv^{-1}

(e) Dissociation constant K_D = 7.2867×10^{-3}

(f) Refractive Index, n = 2.8421

(g) Molar Refraction, $\bar{R}_D$ = 54.2624

(h) Equation for Refractive Index in the range (0.01M - 0.20M)

$n = 4.3608 \times 10^{-2} c + 1.3324$

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```
BL.
   10 MODE0
   20 REM FILE "SHEDOVL"
   30 DIM L(100),C(100),Z(100),X(100),Y(100),A(100),F2(100),SZ(100)
   40 B1=0.23:B2=60.65:B3=0.3291E8:A0=5E-8:POWER=-1.5
   50 DATA READ FROM CONDUCT,A DATA FILE OF PREVIOUSLY STORED DATA
   60 INPUT "TYPE NAME OF DATA FILE ",NAME$
   70 PRINT TAB(2);"ROW";TAB(10);"CONC";TAB(25);"LAMDA"
   80 QQ=OPENIN NAME$
   90 N=0
  100 REPEAT
  110 INPUT £QQ,C(N+1),L(N+1)
  120 PRINT TAB(3);N+1;TAB(10);C(N+1);TAB(25);L(N+1)
  130 N=N+1
  140 UNTIL EOF£QQ
  150 CLOSE£QQ
  160 INPUT "DO YOU WANT TO ANALYSE ALL THE DATA Y/N  " ANS$
  170 PRINT
  180 IF ANS$ =  "Y" THEN 300
  190 PRINT"TYPE 1ST AND LAST ROW NUMBERS OF THE RANGE YOU WANT TO ANALYSE"
  200 INPUT "PLEASE  SEPARATE VALUES WITH COMMAS.  "ST1, EN2
  210 PRINT
  220 PRINT "CONCENTRATION RANGE FOR ANALYSIS: ",C(ST1);"       TO  ";C(EN2)
  230 REM CONVERT RANGE FROM  ST1 TO EN2 INTO 1 TO N
  240 N=0
  250 FOR I = ST1 TO EN2
  260 C(N+1) = C(I): L(N+1) = L(I)
  270 N=N+1
  280 NEXT I
  290 PRINT
  300 INPUT"ENTER THE VALUE OF LAMDA-O";LO
  310 REM PROCESSING STAGE
  320 PRINT
  330 PRINT "WAIT UNTIL OPTIMIMATION IS COMPLETE"
  340 FOR H=1 TO 10
  350 FOR I=1 TO N
  360 Z(I)=((B1*LO)+B2)*SQR(C(I)*L(I))
  370 Z(I)=Z(I)*(LO^POWER)
  380 SZ(I)=1+Z(I)+(Z(I)^2)/2
  390 A(I)=(L(I)*SZ(I))/LO
  400 F1=-0.5515*SQR(C(I)*A(I))
  410 F3=1+B3*A0*SQR(C(I)*A(I))
  420 F4=(F1/F3):F2(I)=10^F4
  430 NEXT I
  440 REM METHOD OF LEAST SQUARES
  450 SX=0:SY=0
  460 SX2=0:SY2=0:SXY=0
  470 FOR J=1 TO N
  480 Y(J)=1/(L(J)*SZ(J))
  490 X(J)=(C(J))*L(J)*F2(J)*(SZ(J))
  500 SX=SX+X(J):SX2=SX2+X(J)^2:SY=SY+Y(J):SY2=SY2+Y(J)^2:SXY=SXY+(X(J)*Y(J))
  510 NEXT J
  520 S=S+1
  530 INCPT=(SY*SX2-SX*SXY)/(N*SX2-(SX^2))
  540 LO =1/INCPT
  550 NEXT H
```

```
560 REM RWSULT
570 GRAD=(N*SXY-SX*SY)/(N*SX2-(SX^2))
580 SR=SQR(((N*SX2-(SX^2))*(N*SY2-(SY^2))))
590 COEFF=(N*SXY-SX*SY)/SR
600 PRINT TAB(1);"LAMDA";TAB(15);"SZ";TAB(29);"ALFHA";TAB(43);"ACT.CO";TAB(57)
;"Y-AXS";TAB(71);"X-AXS"
610 FOR I=1 TO N
620 PRINT TAB(0);L(I);TAB(12);SZ(I);TAB(24);A(I);TAB(37);F2(I);TAB(51);Y(I);TA
B(66);X(I)
630 NEXT I
640 K=1/((LO ^2)*GRAD)
650 K1=-LOG K
660 PRINT
670 PRINT
680 PRINT "INTERCEPT=    ";TAB(26);INCIT
690 PRINT "GRADIENT=     ";TAB(26);GRAD
700 PRINT"CORRELATION COEFFICIENT = ";COEFF
710 PRINT "LAMDA-0   =    ";TAB(26);LO
720 PRINT "DISSOCIATION CONSTANT =    ";TAB(26);K
730 PRINT "PK VALUE =     ";TAB(26);K1
740 PRINT
750 INPUT "DO YOU WANT TO SELECT ANOTHER RANGE Y/N ";ANS2$
760 IF ANS2$ = "Y" THEN 80
770 INPUT "DO YOU WANT TO CHANGE THE INITIAL LAMDA0 VALUE Y/N ";ANS$
780 IF ANS$="Y" THEN 300
790 INPUT"TYPE Y TO QUIT OR R TO LOAD ANOTHER DATA FILE ";ANS3$
800 IF ANS3$ = "R" THEN 60
810 CLOSE£0
820 INPUT" DO YOU WANT TO ANALYSE DATA IN INSTAT Y/N "; ANS4$
830 IF ANS4$ <> "Y" THEN 930
840 INPUT "INSERT INSTAT DISKETTE INTO DRIVE AND TYPE Y TO CONTINUE "ANS5$
860 *SPOOL STAT
865 PRINT"REM X1 X2"
870 PRINT "REA X1 X2"
880 FOR I = 1 TO N
890 PRINT TAB(10);X(I);TAB(30);Y(I)
900 NEXT I
910 PRINT"EOD"
920  *SPOOL
```

```
RUN
TYPE NAME OF DATA FILE ?COND5
  ROW       CONC            LAMDA
    1       1.508979E-3     136.3628
    2       4.0004E-3       120.7381
    3       4.9993784E-3    119.431115
    4       6.7278E-3       114.239987
    5       7.3525E-3       112.909938
    6       8.6523E-3       110.525841
    7       1.00084E-2      107.636234
    8       1.5356E-2       103.139461
    9       2.0007E-2       102.72263
   10       4.00194E-2      96.460498
   11       5.99999E-2      91.70615
   12       7.00012E-2      90.649796
   13       7.99982E-2      88.810071
DO YOU WANT TO ANALYSE ALL THE DATA Y/N  N

TYPE 1ST AND LAST ROW NUMBERS OF THE RANGE YOU WANT TO ANALYSE
PLEASE  SEPARATE VALUES WITH COMMAS.  2,7

CONCENTRATION RANGE FOR ANALYSIS:        4.0004E-3       TO  1.00084E-2

ENTER THE VALUE OF LAMDA-O?500

WAIT UNTIL OPTIMIMATION IS COMPLETE
 LAMDA          SZ              ALPHA          ACT.CO          Y-AXS          X-AXS
120.7381    1.04029339  0.908278652  0.93273078    7.96159031E-3  0.468662172
119.431115  1.04489693  0.90242241   0.926069391   8.0132568E-3   0.577764001
114.239987  1.05108575  0.868310846  0.917396348   8.32805734E-3  0.741116313
112.909938  1.05314379  0.85988183   0.914581287   8.40969336E-3  0.799608098
110.525841  1.05714384  0.844922436  0.909205081   8.5585874E-3   0.919160458
107.636234  1.060751    0.825640291  0.904461799   8.75846612E-3  1.0335389


INTERCEPT=                  7.23134252E-3
GRADIENT=                   1.46295498E-3
CORRELATION COEFFICIENT = 0.992898308
LAMDA-O  =                  138.286908
DISSOCIATION CONSTANT =     3.57443087E-2
PK VALUE =                  1.4467931

DO YOU WANT TO SELECT ANOTHER RANGE Y/N ?N
DO YOU WANT TO CHANGE THE INITIAL LAMDA0 VALUE Y/N ?N
TYPE Y TO QUIT OR R TO LOAD ANOTHER DATA FILE ?Y
 DO YOU WANT TO ANALYSE DATA IN INSTAT Y/N ?N
```

```
RUN
TYPE NAME OF DATA FILE ?COND5
  ROW      CONC            LAMDA
   1     1.508979E-3      136.3628
   2     4.0004E-3        120.7381
   3     4.9993784E-3     119.431115
   4     6.7278E-3        114.239987
   5     7.3525E-3        112.909938
   6     8.6523E-3        110.525841
   7     1.00084E-2       107.636234
   8     1.5356E-2        103.139461
   9     2.0007E-2        102.72263
   10    4.00194E-2       96.460498
   11    5.99999E-2       91.70615
   12    7.00012E-2       90.649796
   13    7.99982E-2       88.810071
DO YOU WANT TO ANALYSE ALL THE DATA Y/N  N

TYPE 1ST AND LAST ROW NUMBERS OF THE RANGE YOU WANT TO ANALYSE
PLEASE  SEPARATE VALUES WITH COMMAS.  2,9

CONCENTRATION RANGE FOR ANALYSIS:        4.0004E-3        TO  2.0007E-2

ENTER THE VALUE OF LAMDA-O?6500

WAIT UNTIL OPTIMIMATION IS COMPLETE
 LAMDA          SZ             ALPHA          ACT.CO         Y-AXS          X-AXS
120.7381     1.04377066   0.977119106   0.930542215   7.93506662E-3   0.469125372
119.431115   1.04878021   0.971180724   0.923686096   7.98358639E-3   0.578418782
114.239987   1.05551754   0.934935568   0.914765614   8.29309042E-3   0.742106957
112.909938   1.05775865   0.926012478   0.911871732   8.37300295E-3   0.800732658
110.525841   1.06211548   0.910193335   0.906347036   8.51852558E-3   0.920580256
107.636234   1.06604546   0.889676877   0.901474986   8.71496766E-3   1.03526744
103.139461   1.08060934   0.864155017   0.884286991   8.97235456E-3   1.51343897
102.72263    1.09230152   0.869974956   0.871401911   8.9123315E-3    1.9561815


INTERCEPT=                  7.75350521E-3
GRADIENT=                   7.0795769E-4
CORRELATION COEFFICIENT =   0.904248433
LAMDA-O  =                  128.973925
DISSOCIATION CONSTANT =     8.49158698E-2
PK VALUE =                  1.07101114

DO YOU WANT TO SELECT ANOTHER RANGE Y/N ?N
DO YOU WANT TO CHANGE THE INITIAL LAMDAO VALUE Y/N ?N
TYPE Y TO QUIT OR R TO LOAD ANOTHER DATA FILE ?Y
 DO YOU WANT TO ANALYSE DATA IN INSTAT Y/N ?N
```

```
BLI.
   10  REM ROBINS
   20 MODE 0
   30 DIM L(100),L2(100),M(100),D(100),X(100),Y(100)
   40 B1=0.23:B2=60.65:B3=0.3291E8:A0=5E-8:K1=138.286908
   50 DATA ENTRY
   60 PRINT TAB(0,4)"PLEASE ENTER CONC AND LAMDA"
   70 PRINT"IN PAIRS SEPER BY COMMAS"
   80 PRINT
   90 PRINT"ENTER 7.99982E-2,88.810071"
  100 PRINT"AS LAST VALUES"
  110 PRINT
  120 PRINT
  130 N=0
  140INPUT M(N+1),L(N+1)
  150 N=N+1
  160 IF M(N)<> 7.99982E-2 THEN 140
  170 IF L(N)<> 88.810071  THEN 140
  180 REM CAL STAGE
  190 FOR I=1 TO N
  200 D(I)=L(I)/K1
  210 TEST1=L(I)/K1
  230 B4=B3*A0
  240 B5=SQR(M(I)*D(I))
  250 B6=1+(B4*B5)
  260 B7=(B1*K1+B2)
  270 B8=B7*B5
  280 L2(I)=(K1-((B8/B6)))*D(I)
  290 D(I)=L(I)/L2(I)
  300 IF ABS(TEST1-D(I))<0.00001 THEN 330
  320 TEST1=D(I)
  325 GOTO 240
  330 NEXT I
  340 REM
  350 REM LEAST SQUARES
  360 SX=0:SY=0
  370 SX2=0:SY2=0:SXY=0
  380 FOR I=1 TO N
  390 X(I)=SQR(M(I)*D(I))
  400 Y(I)=L2(I)
  410 SX=SX+X(I)
  420 SX2=SX2+X(I)^2
  430 SY=SY+Y(I)
  440 SY2=SY2+Y(I)^2
  450 SXY=SXY+X(I)*Y(I)
  460 NEXT I
  470 REM RESULT
  480 S=S+1
  490 INCEPT=(SY*SX2-SX*SXY)/(N*SX2-(SX^2))
  500 GRAD=(N*SXY-SY*SX)/(N*SX2-(SX2^2))
  510 SR=SQR(((N*SX2-(SX^2))*(N*SY2-(SY^2))))
  520 COEFF=(N*SXY-SX*SY)/SR
  530 LO=INCEPT
  535 PRINT TAB(1);"CONC";TAB(15);"EXLAM";TAB(29);"CALLAM";TAB(43);"ALPHA";TAB(6
```

```
0)"STRNTH"
  540 PRINT
  550 FOR I=1 TO N
  555 PRINT TAB(0);M(I);TAB(15);L(I);TAB(29);L2(I);TAB(43);D(I);TAB(60);X(I)
  560 MODE 0
  580 NEXT I
  590 PRINT"INTERCEPT= ";INCEPT
  600 PRINT"GRADIENT = ";GRAD
  610 PRINT"CORR COEFF = "COEFF
  620 PRINT"LAMD-O     = "LO
  630 CLOSE£0
  640 INPUT"DO YOU WANT TO ANALSE DATA IN INSTAT Y/N";ANS$
  650 IF ANS$ <> "Y" THEN 740
  655 INPUT"INSERT DISKETTE INTO DRIVE AND TYPE Y TO CONTINUE "ANS2$
  660 *SPOOL ROB
  670 PRINT "REM X1 X2"
  680 PRINT "REA X1 X2"
  690 FOR I=1 TO N
  695 PRINT TAB(10);L2(I);TAB(30);X(I)
  710 NEXT I
  720 PRINT"EOD"
  725 *COPY 2 0 ROB
  730 *SPOOL
  740 END
>
```

```
RUN
PLEASE ENTER CONC AND LAMDA
IN PAIRS SEPER BY COMMAS

ENTER 7.99982E-2,88.810071
AS LAST VALUES


?1.508979E-3,136.3628
?4.0004E-3,120.7381
?4.9993784E-3,119.431115
?6.7278E-3,114.239987
?7.3525E-3,112.909938
?8.6523E-3,110.525841
?1.00084E-2,107.636234
?1.53567E-2,103.139461
?2.0007E-2,102.72263
?4.00194E-2,96.4604898
?5.99999E-2,91.70615
?7.00012E-2,90.649796
?7.99982E-2,88.810071
 CONC           EXLAM          CALLAM         ALPHA          STRNTH

1.508979E-3     136.3628       135.631419     1.00539242     3.89501738E-2
4.0004E-3       120.7381       126.772076     0.952402957    6.17251391E-2
4.9993784E-3    119.431115     125.826835     0.949170464    6.88858644E-2
6.7278E-3       114.239987     122.711656     0.930962804    7.91412127E-2
7.3525E-3       112.909938     121.883489     0.926375991    8.25298702E-2
8.6523E-3       110.525841     120.374901     0.918180118    8.91311945E-2
1.00084E-2      107.636234     118.595738     0.907589392    9.53074901E-2
1.5356E-2       103.139461     115.440575     0.893442025    0.117131105
2.0007E-2       102.72263      114.73168      0.895329261    0.133838905
4.00194E-2      96.460498      109.825004     0.878310899    0.187481933
5.99999E-2      91.70615       106.19587      0.863556653    0.227625378
7.00012E-2      90.649796      105.213632     0.86157843     0.245584047
7.99982E-2      88.810071      103.824743     0.855384449    0.261589786
INTERCEPT= 133.051936
GRADIENT = -29.0589594
CORR COEFF = -0.954292089
LAMD-O     = 133.051936
DO YOU WANT TO ANALSE DATA IN INSTAT Y/N?N
>
```

```
BRUN
PLEASE ENTER CONC AND LAMDA
IN PAIRS SEPER BY COMMAS

ENTER 1.00084E-2,107.636234
AS LAST VALUES


?4.0004E-3,120.7381
?4.9993784E-3,119.431115
?6.7278E-3,114.239987
?7.3525E-3,112.909938
?8.6523E-3,110.525841
?1.00084E-2,107.636234
 CONC          EXLAM          CALLAM         ALPHA          STRNTH

4.0004E-3      120.7381       126.772076     0.952402957    6.17251391E-2
4.9993784E-3   119.431115     125.626835     0.949170464    6.88058644E-2
6.7278E-3      114.239987     122.711656     0.930962804    7.91412127E-2
7.3525E-3      112.909938     121.883489     0.926375991    8.25298702E-2
8.6523E-3      110.525841     120.374901     0.918180118    8.91311945E-2
1.00084E-2     107.636234     118.595738     0.907589392    9.53074901E-2
INTERCEPT= 142.576416
GRADIENT = -5.08533571
CORR COEFF = -0.995933809
LAMD-O     = 142.576416
DO YOU WANT TO ANALSE DATA IN INSTAT Y/N?N
->
```