ABSTRACT

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The development of a thiamine (vitamin B_1) ion-selective electrode has been investigated. The particular type of electrode employed was a liquid membrane electrode composed of an ion-exchanger solution consisting of thiamine-bromothymol blue acid dye salt in a water immiscible solvent one-half octanol-1, one-half chloroform. Several properties of the thiamine liquid membrane electrode were studied: response, reproducibility, and pH dependence. The primary purpose of the thiamine electrode was to determine the concentration of thiamine commonly found in commercially available multi-vitamin preparations. The thiamine electrode was tested in the presence of diverse substances normally found in these vitamin preparations. These interference substances were vitamin B_6 (pyridoxine monohydrochloride), vitamin B_2 (riboflavin), vitamin C (ascorbic acid), vitamin B_3 (nicotinamide), and vitamin B_5 (d-calcium pantothenate).

Because of the large number of standard routine samples needed to be analyzed when determining the potential response of ion-selective electrodes, a hard-wire automatic solution addition instrument has been developed which handles addition of a stock solution to desired volumes either by manual control or by an automatic control. In conjunction with this unit, a data recording system was devised to store the electrical potential readings of the electrode cell for the different concentration ranges studied.

APPLICATIONS OF ION-SELECTIVE ELECTRODES

by

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PART I

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CHAPTER ONE

INTRODUCTION

During the past years, a considerable amount of progress has been achieved in analytical potentiometry through the development and introduction of ion-selective electrodes. These electrodes are not only employed in routine chemical analysis but, due to imaginative development, now have applicability to the analysis of biological and clinical samples.

Some advantages of ion-selective electrodes over alternate conventional analytical techniques are the selective rapidity of response towards the ion or constituent of interest, its non-destructive character towards the sample being analyzed, the small quantity of sample needed for analysis, and, in most cases, the analysis of a sample without pretreatment. This last factor eliminates time consuming separations and chemical preparations. A very important aspect of the employment of ion-selective electrodes is the low initial cost and the easy availability of the equipment required for electrode systems.

Some disadvantages of ion-selective electrodes are the frequency of the calibrations of electrodes and the temperature dependence. Also, in the Nernst equation there is the increasing uncertainty of the activity of an ion as the charge on the ion increases and the uncertainty of the activity term due to the presence of foreign complexes and ion-pairs.

Ion-selective electrodes have proved recently that they can be useful as probes for clinical analysis, biomedical monitoring, and

micro-biological processes.[1] These electrodes, in combination with biological materials, can form new sensors that selectively measure non-ionic, biological species in body fluids or living tissue. Miniaturized electrodes are highly useful for constant monitoring of biological fluids in vivo making them an important clinical tool, especially in emergency situations.

CHAPTER TWO

THEORY OF ION-SELECTIVE ELECTRODES

Basic Equipment

As previously stated, the equipment needed for ion-selective electrode analysis is relatively inexpensive, easily available in chemical laboratories, and simple to assemble. The apparatus used for electrode measurement consists of a voltmeter, an external reference electrode, and the ion-selective electrode. Figure 1 depicts such an apparatus. The potential of the ion-selective electrode cell is measured by making electrical contact to the ion-selective elctrode and to the reference electrode which is in contact with the sample solution via a salt bridge. The voltmeter connected across the two electrodes measures the potential of the entire cell.

A simple voltmeter cannot be used because it draws a small current from the cell which changes the cell potential being measured. A potentiometer must be utilized to detect the cell potential under zero current conditions. Alternately, commercial high-input-impedance voltmeters using operational amplifiers which draw negligible current from the cell are capable of measuring cell potential accurately.

The reference electrode is assumed to be of fixed potential with no change of liquid junction potential during the course of an analysis. The reference electrode is a very important part of the complete cell and requires special care. In most cases, the ion-selective electrode cells consist of two reference electrodes: an internal one within the ion-selective electrode that is in contact with a solution containing the ion to be detected and a salt suitable for the particular reference

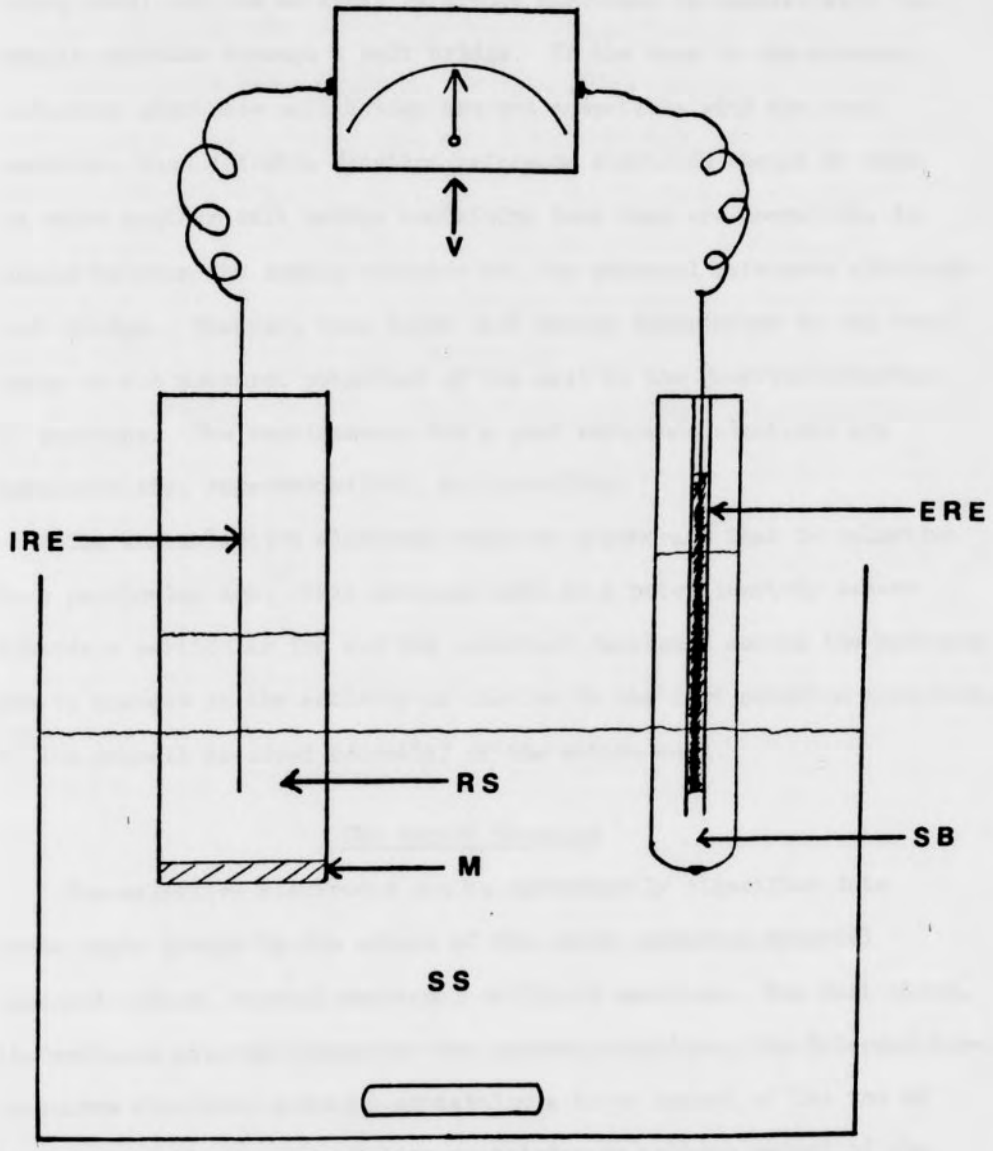


Figure 1. Schematic Diagram of an Ion-Selective Electrode Cell

IRE, internal reference electrode; ERE, external reference electrode; RS, internal reference solution; M, membrane sensor; SB, salt bridge; SS, sample solution; V, voltmeter.

being used; and one external reference electrode in contact with the sample solution through a salt bridge. If the ions in the external reference electrode salt bridge are not compatible with the test solution, then a double junction reference electrode should be used in which another salt bridge containing ions that are compatible is placed between the sample solution and the external reference electrode salt bridge. However, this added salt bridge contributes to the total value of the measured potential of the cell by the junction potential it produces. The requirements for a good reference electrode are reversibility, reproducibility, and stability.

The ion-selective electrode contains a membrane that is selective to a particular ion. This membrane acts as a potentiometric sensor towards a particular ion and the potential developed across the membrane due to changes in the activity of the ion in the test solution contributes to the overall measured potential of the entire cell.

The Nernst Equation

Ion-selective electrodes can be conveniently classified into three basic groups by the nature of the active membrane material employed: glass, crystal membrane, or liquid membrane. For most cases, the membrane material separates two aqueous solutions, the internal ion-selective electrode solution containing a known amount of the ion of interest and the sample solution containing an unknown amount of the ion to be detected. The membrane is constructed to be as selective as possible to one particular ion. All three types of membrane electrodes measure the activity of the desired ions in various sample solutions. When a membrane electrode is immersed in a sample solution, there is a

momentary movement of ions across the membrane towards the solution containing the lower activity of the mobile ion. Because the ions have a charge, there is a point across this membrane where the ions cease to migrate due to an electrical potential which forms due to the transfer of the mobile ions and, eventually, an equilibrium results in which the potential across the membrane now contributes to the total potential of the entire cell.

When operating properly, the membrane electrodes obey the modified Nernst equation:

$$E = \text{CONSTANT} \pm \frac{2.303\ RT}{nF}\left[\log_{10}\left(a_M + \sum k_i^{pot}\ a_{B_i}^{Z_{B_i}/Z_M}\right)\right] \quad \text{(equation 1)}$$

where:

E= measured potential in millivolts

R= gas constant

T= absolute temperature

F= Faraday

n= ionic charge of the ion to be measured

a_M= activity of the ion to be measured in the sample solution

a_{B_i} = activity of the interfering ions in the sample solution

Z_M and Z_{B_i} = ionic charges of the ion M and interfering ions B_i, respectively

k_i^{pot}= selectivity constant

CONSTANT= potential of the reference electrodes and all junctions in the cell

Before the second term of the right side of the Nernst equation the plus sign is used when considering the detection of cations and the minus sign is used when considering the detection of anions. Assuming

there is an internal reference electrode in the membrane electrode, the total measured potential can also be represented by:

$$\Delta E_{measured} = \Delta E_{membrane} + \Sigma E_j + E(\text{int. ref.}) - E(\text{ext. ref.}) \quad \text{(equation 2)}$$

where $\Delta E_{membrane}$ is the potential across the membrane due to the migration of the specific ion towards the solution of lower activity. $\Delta E_{membrane}$ is related to the last term on the right hand side of equation 1. ΣE_j, E(int. ref.), and E(ext. ref.) refer to the potential due to all the junctions in the cell, the internal reference electrode within the membrane electrode, and the external reference electrode immersed in the sample solution, respectively. All three of these terms are assumed to remain constant during the analysis of samples and, therefore, are represented as the "CONSTANT" in equation 1. $\frac{2.303\ RT}{F}$ is the Nernst factor and depends on the temperature. The concentration of the ions is in terms of activity, which contains corrections for the interionic forces of all the ions in the sample solution.

The activity is determined by the equation:

$$a_M = \gamma (C_M) \quad \text{(equation 3)}$$

where:

a_M= activity of the ion M being detected in the sample

γ = activity coefficient of the ion M in the sample

C_M= concentration of the ion M

The activity and concentrations are in terms of moles per liter.

To determine the activity coefficient, the best and the simplest equation derived was from C. W. Davies[2]:

$$-\log_{10}\gamma = Az_M^2 \left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.3\ I \right) \qquad \text{(equation 4)}$$

where:

A= constant depending on temperature and solvent (A= 0.5115 for water at 25°C)

z= ionic charge of the ion M in the sample

I= ionic strength of the solution represented by the term:

$$I = \frac{1}{2}\sum_{1}^{i} C_i \quad Z_i^2 \qquad \text{(equation 5)}$$

where:

C_i= concentration of every ion in the solution

Z_i= ionic charge of every ion in the solution

There is another more simple approach to determine I using a table derived from equation 5. The method uses factors multiplied to the salt concentration of the solution for each ionized salt in the solution to obtain the ionic strength value. For example, use salt $A(\alpha)\ B(\beta)$ where A and B are the ionic parts of the salt with their ionic charge represented by α and β. The results are quite simple as shown in Table 1.

Table 1

Determining Factors to Calculate Ionic Strength for Salt $A(\alpha)\ B(\beta)$

α	β	Factor
1	1	1
1	2	3
2	1	3
2	2	4
etc.	etc.	etc.

Once the factor is determined for each soluble salt in the sample solution, the ionic strength for the particular salt is then determined by multiplying its factor by the concentration (in molarity) of the salt. The total ionic strength of a sample solution is then calculated by

summing all of the individual ionic strengths of the constituent salts.

The selectivity term in the Nernst equation, k^{pot}, is a weighting factor related to the selectivity of the membrane electrode towards the ion of interest and an interfering ion. k^{pot} is a combination of parameters: the ion-exhange equilibrium constant between the aqueous solutions and membrane, the mobilities of the ions, and the activity coefficients within the membrane phase. The selectivity constant indicates the extent to which an interfering ion will alter the response of the membrane electrode. The lower the value of k^{pot}, the better the response of the electrode towards the ion of interest. The selectivity constant is only an approximate value, but it allows some prediction of the kind and the amount of interference that can be tolerated. The mathematical method for determining k^{pot} will be discussed later.

Methods of Analysis

When using an ion-selective electrode cell, it is necessary to calibrate the electrode system with standard solutions of known ion activity. A plot of measured potential versus $\log_{10}$ of the activity of the specific ion for a range of different activities can then be used as a working curve to determine the unknown ion activity in sample solutions. If the membrane electrode is operating in an ideal Nernstian manner, then the working curve should have an array of data points forming a straight line with a slope value equal to $\frac{2.303\ RT}{nF}$ and an intercept value equal to the "CONSTANT" of equation 1. The slope and intercept values are calculated using a linear regression approach where it is assumed that all error is in the Y values, in this case the

potential readings. Each Y value error is weighted the same and it is assumed there is no error in the X values, in this case $\log_{10}$ activity of the specific ion. Depending upon the charge of the ion being detected by the membrane electrode, the slope of the curve will be positive if the ion is a cation or negative if the ion is an anion.

There are two basic approaches to determine the selectivity constant used in the Nernst equation, the separate solution method and the mixed solution method.[3] For the separate solution method, the potential (millivolts) is plotted versus the $\log_{10}$ of the activity of the ion of interest M, sometimes called the primary ion, and then the potential is separately plotted versus the $\log_{10}$ of the activity of the interfering ion B. A plot for divalent cations is given in Figure 2.[4] There are two ways to use this method. The first is to choose the same activity values for both the M^{+2} and B^{+2} ions and determine the selectivity constant k_{MB}^{pot} by the equation[5]

$$\frac{E_2 - E_1}{2.303\ RT/2F} = \log_{10} k_{MB}^{pot} \qquad \text{(equation 6)}$$

where E_1 and E_2 are the potentials due to the primary divalent ion M^{+2} and the interfering ion B^{+2}, respectively, at the log activity value chosen for both. The second way is to choose the same potential for both M^{+2} and B^{+2} ions, locate their log activities at this potential, and determine the selectivity constant by the equation[5]

$$\frac{a_M}{a_B} = k_{MB}^{pot} \qquad \text{(equation 7)}$$

If the interfering ion has a charge n+, then equation 7 becomes

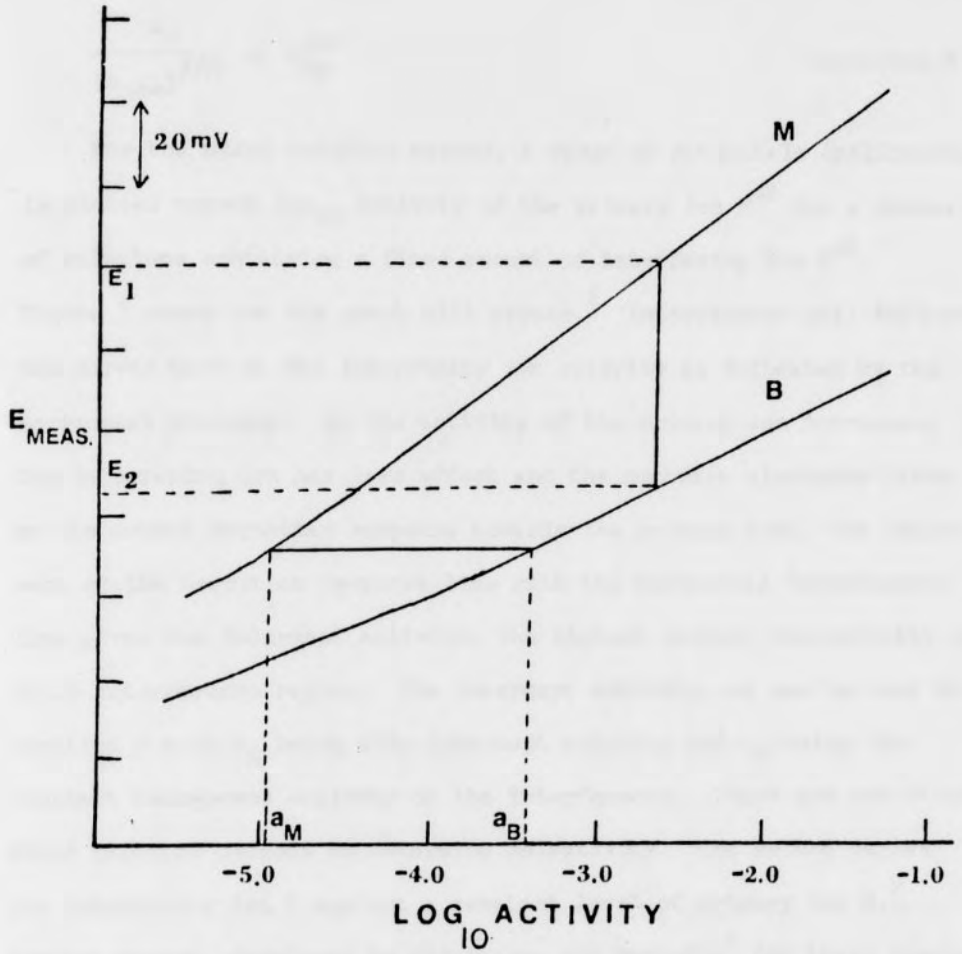


Figure 2. Illustration of Electrode Response Towards Primary Ion M^{+2} and Interferent Ion B^{+2} Using Separate Solution Method

$E_{MEAS.}$, measured potential in millivolts: M, primary ion M^{+2} response curve; B, interferent ion B^{+2} response curve.

$$\frac{a_M}{(a_{Bn+})^{2/n}} = k_{MB}^{pot} \qquad \text{(equation 8)}$$

For the mixed solution method, a range of potentials (millivolts) is plotted versus $\log_{10}$ activity of the primary ion M^{+2} for a number of solutions containing a fixed amount of interfering ion B^{+2}. Figure 3 shows how the graph will appear.[6] Interference will influence the curves more at the low primary ion activity as indicated by the horizontal plateaus. As the activity of the primary ion increases, the interfering ion has less effect and the membrane electrode takes on its normal Nernstian response towards the primary ion. The intercept of the Nernstian response line with the horizontal interference line gives the intercept activity, the highest primary ion activity at which interference begins. The intercept activity now can be used in equation 7 with a_M being this intercept activity and a_B being the constant background activity of the interference. There are two other mixed solution methods to determine selectivity. One method varies the interfering ion B against a constant level of primary ion M.[7] Another method, developed by Srinivasan and Rechnitz[8] for their treatment of mixed solution potential data using an anion liquid membrane electrode, included two equations derived for high and low selectivities.

When comparing the separate solution method to the mixed solution method for determining selectivity of a membrane electrode toward a primary ion over an interfering one, the latter is preferred since it represents a more realistic approach in which the primary ion is in the same solution as the interfering ion.

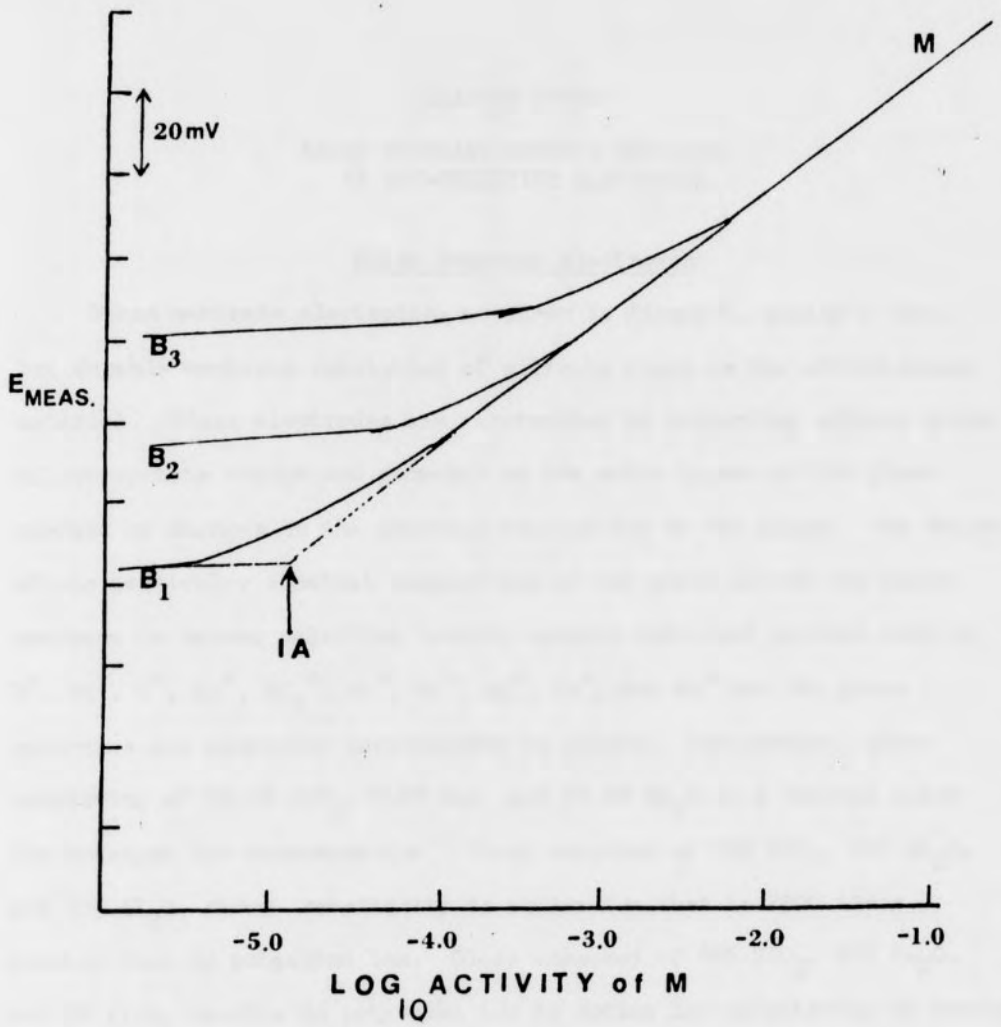


Figure 3. Illustration of Primary Ion M^{+2} Electrode Response Using Fixed Amounts of Interferent Ion B^{+2}

$E_{MEAS.}$, measured potential in millivolts; M, response of primary ion M^{+2} at fixed concentrations of interferent ion B^{+2} where the concentrations are $B_3 > B_2 > B_1$; IA, intercept activity of M^{+2}.

CHAPTER THREE

BASIC MEMBRANE SENSORS EMPLOYED IN ION-SELECTIVE ELECTRODES

Glass Membrane Electrodes

Glass membrane electrodes, as shown in Figure 4, employ a thin but durable membrane consisting of silicate glass as the active sensor material. Glass electrodes are constructed by implanting anionic sites of appropriate charge and geometry on the outer layers of the glass surface by changes in the chemical composition of the glass. The choice of the particular chemical composition of the glass allows the glass membrane to become selective towards certain univalent cations such as H^+, Na^+, K^+, Li^+, NH_4^+, Rb^+, Cs^+, Ag^+, Tl^+, and Cu^+ but the glass membranes are generally unresponsive to anions. For example, glass consisting of 72.2% SiO_2, 6.4% CaO, and 21.4% Na_2O is a typical glass for hydrogen ion determination. Glass composed of 71% SiO_2, 11% Na_2O, and 18% Al_2O_3 shows sensitivity to sodium ion that is 2500 times greater than to potassium ion. Glass composed of 68% SiO_2, 27% Na_2O, and 5% Al_2O_3 results in potassium ion to sodium ion selectivity of twenty to one. The explanation for the glass electrode response and selectivity is complicated but it involves surface ion exchange and ion diffusion principles. It should be noted that the ions do not migrate completely across the membrane in glass electrodes. Rather, the ions in the internal reference solution of the glass membrane electrode and the sample solution approache the center of the membrane, but do not migrate through a central dry glass layer.

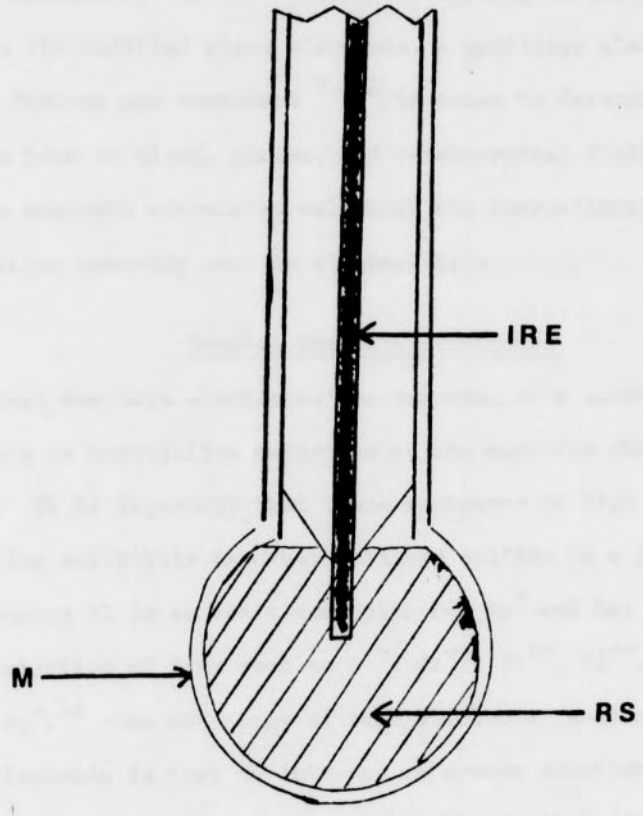


Figure 4. Diagram of a Glass Membrane Electrode

IRE, internal reference electrode (Ag/AgCl); RS, internal reference solution; M, glass membrane.

One example of the use of glass electrodes in testing biological fluids is the modified glass electrode, a capillary electrode, made by H. D. Portnoy and coworkers [9, 10] in order to determine sodium and potassium ions in blood, plasma, and cerebrospinal fluid. Their electrode analysis correlated well with the conventional flame photometry determination commonly used in clinical labs.

Crystal Membrane Electrodes

Crystal membrane electrodes are composed of a sensor made of solid-state or crystalline materials as the membrane phase as shown in Figure 5. It is important that these membranes be high ionic conductors and have low solubility in water. Silver sulfide is a good crystal matrix because it is an ionic conductor for Ag^+ and has been developed for the detection of ions such as S^{--}, Cu^{++}, Pb^{++}, Cd^{++}, I^-, Br^-, Cl^-, CN^-, and Ag^+.[11] One advantage of such electrodes such as the silver sulfide electrode is that no internal reference solution is needed if a metallic lead is connected directly to the crystal membrane. An excellent example of a crystal membrane electrode is the lanthanum fluoride electrode whose sensor is developed from a single crystal of this salt doped with rare earth material to increase its electrical conductivity.[12] Lanthanum fluoride conducts fluoride ions and its high selectivity towards fluoride is due to the size of the crystal lattice into which other ions cannot enter.

The lanthanum fluoride electrode has had considerable use in clinical analysis for determination of fluoride in blood serum in conjunction with a standard fluoride chemical test using morin-thorium

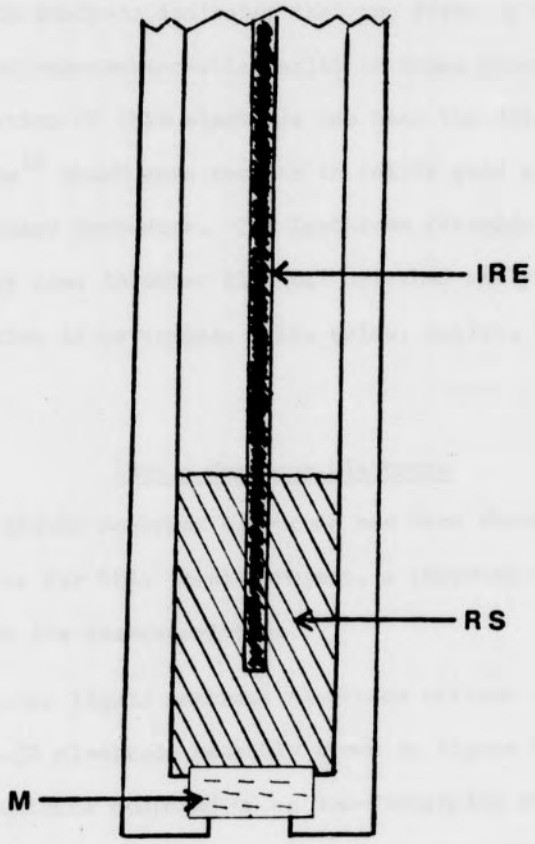


Figure 5. Diagram of a Crystal Membrane Electrode

IRE, internal reference electrode (Ag/AgCl); RS, internal reference solution; M, crystal membrane.

reagent.[13] This analysis indicated that two forms of fluoride, exchangeable and non-exchangeable, exist in human blood serum. Another application of this electrode has been the determination of fluoride in bone[14] which gave results in fairly good agreement with a clinical standard procedure. The lanthanum fluoride electrode has been extensively used in other clinical oriented analyses such as fluoride detection in beverages, milk, urine, saliva, toothpaste, etc..

Liquid Membrane Electrode

Since the liquid membrane electrode has been chosen as the thiamine detector for this thesis project, a thorough description will be given of its characteristics.

A very popular liquid membrane electrode offered commercially is the Orion 92-32 electrode assembly shown in Figure 6. This electrode consists of a membrane composed of an ion-exchanging or carrying material dissolved in a water immiscible liquid phase that is held in an inert porous support membrane. By choosing an appropriate ion-exchanging site and liquid phase, the liquid membrane can selectively respond to practically any desired ion. Because mobility of a desired ion is the essential principle of forming a membrane potential, the liquid membrane offers a fine medium for this ion mobility by providing a phase for ion diffusion.

As demonstrated in Figure 6, the membrane containing the active liquid phase is placed between two aqueous phases; the internal reference solution in which the Ag/AgCl reference electrode is immersed

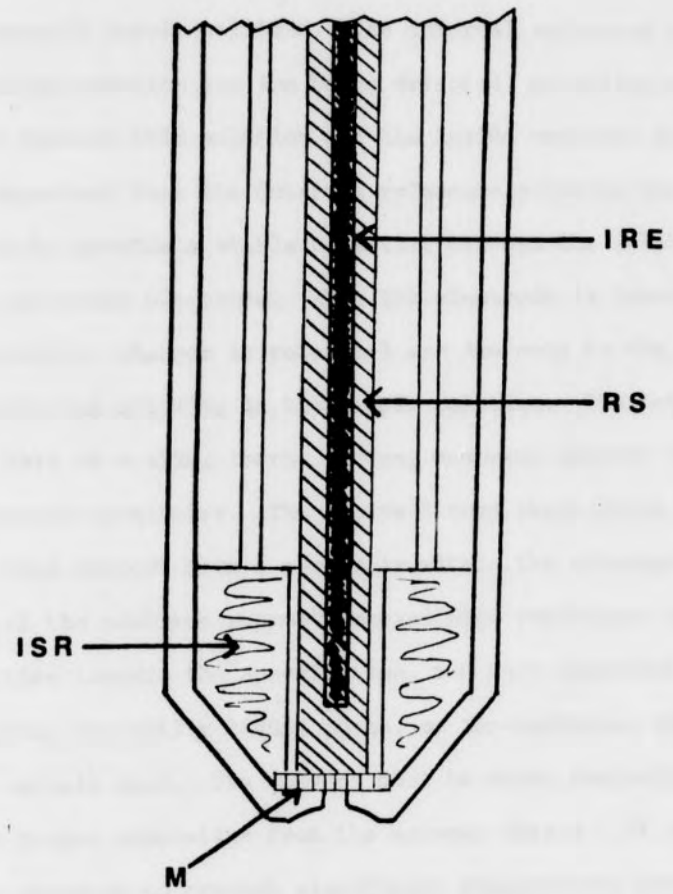


Figure 6. Diagram of a Liquid Membrane Electrode

IRE, internal reference electrode (Ag/AgCl); M, liquid membrane; ISR, ion-exchanger solution reservoir; RS, internal reference solution.

and the aqueous sample solution. The internal reference solution of the electrode contains the ion being detected, providing a stable potential between this solution and the inside membrane surface. It is also important that the internal reference solution has chloride ions so as to provide a stable potential between the solution and the internal reference electrode. When the electrode is immersed in the sample solution, changes in potential are due only to the changes in the specific ion activity in the sample solution. The active liquid phase is held in a thin, inert, porous, membrane support that usually has hydrophobic qualities. The active liquid phase wicks into the pores of this support from a reservoir within the electrode body. The thinness of the membrane support removes high resistance and shortens response time towards the specific ion, two very important features.

Choosing the active liquid phase, or ion-exchanger solution, requires certain care. The solvent must be water immiscible in order to insure proper separation from the aqueous phases. It must have a low vapor pressure to prevent significant evaporation; have a high viscosity to hinder rapid loss by outward flow across the membrane, a design that prevents contamination of the ion-exchanger solution by sample material; and have good stability for a reasonable period of time. Usually, a low dielectric constant, organic solvent provides a good active liquid phase medium. In order for the desired ion to move across the membrane, a charged "site" of opposite charge or a neutral "site" that can trap the ion must exist in the membrane solvent phase. The ion and the site become neutral, undissociated

salt molecules in the solvent. This ion-site must have a relatively large non-polar area to prevent solubility in the adjacent aqueous layers and to insure solubility in the organic, low dielectric constant, solvent phase. When the active liquid phase is in contact with the adjacent aqueous phases, an exchange equilibrium exists across the membrane interface between the ions of the aqueous phase and the ions of the ion-site molecule in the organic phase. This ion exchanging is depicted in Figure 7. The ions move through the membrane towards the solution of lower activity by driving the equilibrium at the membrane interfaces to the right. Available sites keep "latching on" to other ions of interest and continue to drive the reaction to the right until the potential across the membrane which opposes this reaction reaches a point where no further migration across the membrane is possible. The selectivity of the electrode depends upon choosing an appropriate site group that exchanges more readily with the desired ion in the presence of possible interfering ions in the sample. Also, choice of the "site" depends upon how quickly ion-exchange equilibrium can occur with the ion of interest.

In the course of using a liquid membrane electrode, it is usually best to make the desired ion concentration of the internal reference solution approximately the same as that to be analyzed in the sample solution. This prevents a large concentration gradient between sample and internal ion solutions. Also, because of the outward flow of the active liquid phase, some slight drifting of potential is usually observed. If drifting is still excessive after proper conditioning of the electrode, there could be a defect in the electrode assembly or the

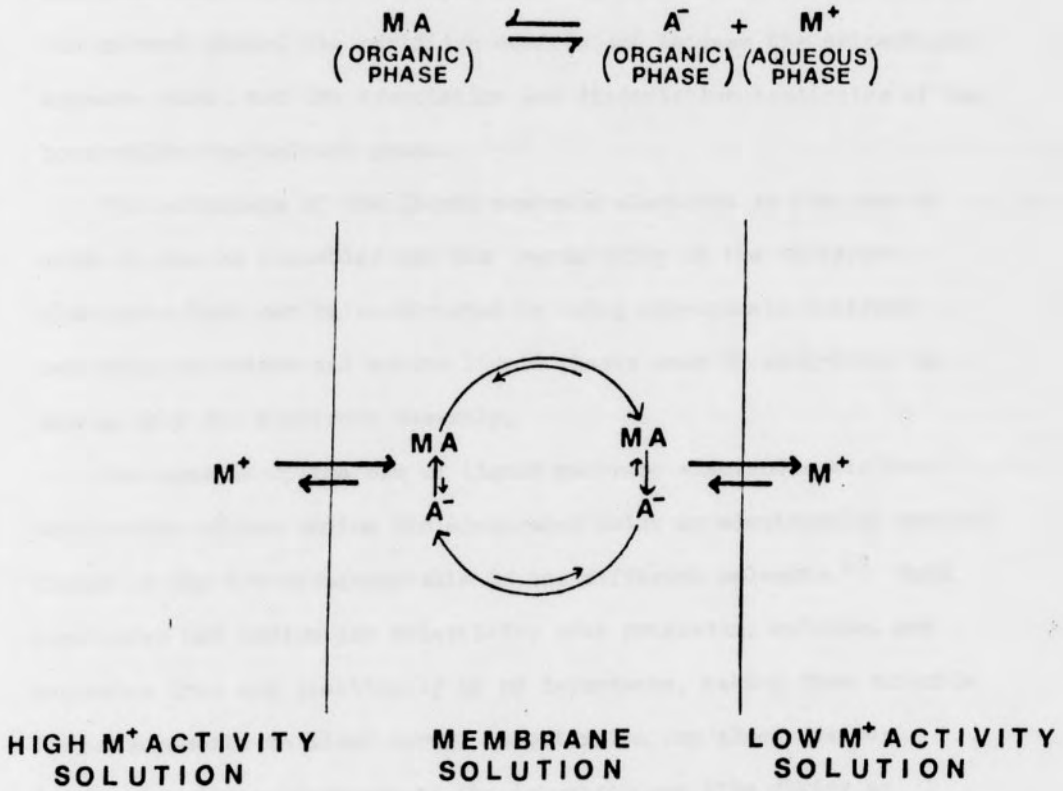


Figure 7. Diagram of Ion-exchange Equilibrium Across the Liquid Membrane Interface Between the Aqueous Phase and the Organic Phase

M^+, specific mobile ion (in this case a univalent cation); A^-, ion-exchanger site.

organic solvent used in the active liquid phase may not have the proper characteristics. It should be noted that the choice of solvent for the active liquid phase also determines selectivity of the liquid membrane electrode due to properties such as mobility of the ions in the solvent phase, the partition coefficient between the solvent and aqueous phase, and the association and dissociation tendencies of the ions within the solvent phase.

The advantage of the liquid membrane electrode is the ease of which it can be assembled and the versatility of the different electrodes that can be constructed by using appropriate internal reference solutions and active liquid phases even if restricted to having only one electrode assembly.

One example of the use of liquid membrane electrodes was the development of two sodium ion electrodes using an electrically neutral ligand as the ion-exchanger site in two different solvents.[15] Both electrodes had sodium ion selectivity over potassium, calcium, and magnesium ions and practically no pH dependence, making them suitable for measurements in blood serum. A potassium ion electrode was developed using valinomycin as the ion-exchanger site giving an electrode with a high selectivity for potassium over sodium ion and diavalent cations.[16] It was used for the direct determination of potassium ion in human serum and no significant complexing of potassium ion appeared to occur in the serum. Rechnitz and Herman[17, 18] developed a carbonate liquid membrane electrode with high selectivity towards carbonate ion activity for the direct determination of the carbon dioxide content in human serum samples.

A slight alteration of the liquid membrane electrode which employs an active liquid phase has been the development of a dry membrane composed of the ion-exchanger material imbedded in a polymeric film. This is sealed to an electrode body containing an internal reference solution similar to the liquid membrane electrode. Sometimes the ion-exchanger solution functions as a plasticizer for the polymeric film or an additional plasticizer such as di(2-ethyl hexyl)-2-ethylhexylphosphonate (DOOP) or di-n-decyl phthalate (DDP) is used. As a result, a complete ion-exchanger membrane is formed from a polymeric film containing the ion-exchanger and plasticizer in molecular dispersion with the advantages over the conventional liquid membrane electrode of having no gradual loss of the ion-exchanger solution, using only a small amount of the ion-exchanger solution for the membrane construction, and the easy replacement of an old membrane with a new one. A short review by Koryta[19] of this type of ion-exchanger membrane has recently appeared.

CHAPTER FOUR

APPLICATIONS OF ION-SELECTIVE ELECTRODES

Modified Membrane Electrodes

Many types of electrodes for clinical and biological analysis have been developed using one of the three basic ion-selective electrode sensors in combination with other analytical and clinical construction techniques. Some examples of these modified electrodes are gas-sensing electrodes, bioprobes which use biological substrates in conjunction with membrane electrodes, and microelectrodes.

The gas-sensing electrodes are comprised of a conventional membrane electrode, contacted by a solution containing ions the membrane electrode can selectively detect, which is then completely surrounded by a gas permeable, hydrophobic membrane. When the entire gas electrode is immersed in a sample solution, the gas permeable membrane allows only the free passage of the dissolved gas from the sample into the inner solution layer surrounding the membrane electrode, shifting the equilibrium of this inner solution which is detected by the membrane electrode. One example described in the literature is the determination of pCO_2 by direct measurement with a carbon dioxide gas-sensing electrode.[20]

A fairly new area that is under investigation is the bioprobe that uses a conventional membrane electrode surrounded by specific biological materials, such as enzymes or proteins, to produce sensors that selectively detect biological species in body fluids and living tissue. One bioprobe is the urea enzyme electrode in which the urease enzyme is immoblized

around an ammonia membrane glass electrode.[1] The urease hydrolyzes the urea in a biological sample to form carbonate and ammonium ions. The ammonium ions are detected by the membrane electrode and thus the cell potential is related to the urea activity in the sample. There are many other examples of these bioprobes using various combinations of biological materials and membrane electrodes. A short review of these bioprobes has been organized by G. A. Rechnitz[1] discussing the construction and use of bioprobes.

A relatively new development has occurred for ion-selective electrodes by the construction of microelectrodes.[1] These microelectrodes should respond no differently from their larger models except very special care must be taken in the engineering design. Two types of microelectrodes developed so far are the open-tip micropipet electrode and the closed-tip microelectrode. The open-tip micropipet electrode contains an active liquid ion-exchanger solution in which the membrane surface has been reduced to a single pore approximately 0.5 to 1 μm in diameter. The closed-tip microelectrode uses a glass sensor as the membrane surface and this electrode has a size range of 100 to 500 μm. This small size allows the closed-tip microelectrode to be installed in a hypodermic syringe needle along with an external reference electrode.

Biological and Clinical Uses of Membrane Electrodes

Today instrumentation has become an integral part of biological, clinical, and biomedical analysis. Many different analytical techniques are used to determine constituents in biological and medical samples.

A large portion of these analyses are carried out by automatic systems. Two advantages of using automated analysis equipment are reliability of the analysis results and the ability to analyze a large number of samples quickly. For most instrumental analyses, pretreatment of samples is necessary. The time used in this process can vary, depending upon the method chosen for analysis. Using ion-selective electrodes has many benefits. Being highly selective, they may need very little pretreatment of sample or none at all. They offer a relatively inexpensive analytical method that can give reproducible results comparable to conventional clinical methods that may be slower or require more care and maintenance. Also, membrane electrodes supply quick sample results, making them ideal to be employed in automated systems where large number of samples have to be analyzed.

One automated electrode sampler system is shown in Figure 8.[21] The sampler contains a number of samples to be analyzed. A proportioning pump pulls the sample and any other needed components simultaneously towards the electrodes. The sample solution flows through a mixing coil, a drop chamber disconnector which debubbles the sample and reduces electrical noise, and finally enters the membrane electrode and reference electrode through flow caps which have a reservoir where the sample is in contact with the electrode sensors. The sample enters and leaves by means of inlet and outlet tubing and is discarded after analysis. The system can be automatically washed to be used again for the next sample. Companies like Orion Research and Technicon have developed automatic electrode analyzers that can determine more than one type of constituent from one sample and can analyze many samples within a short time.

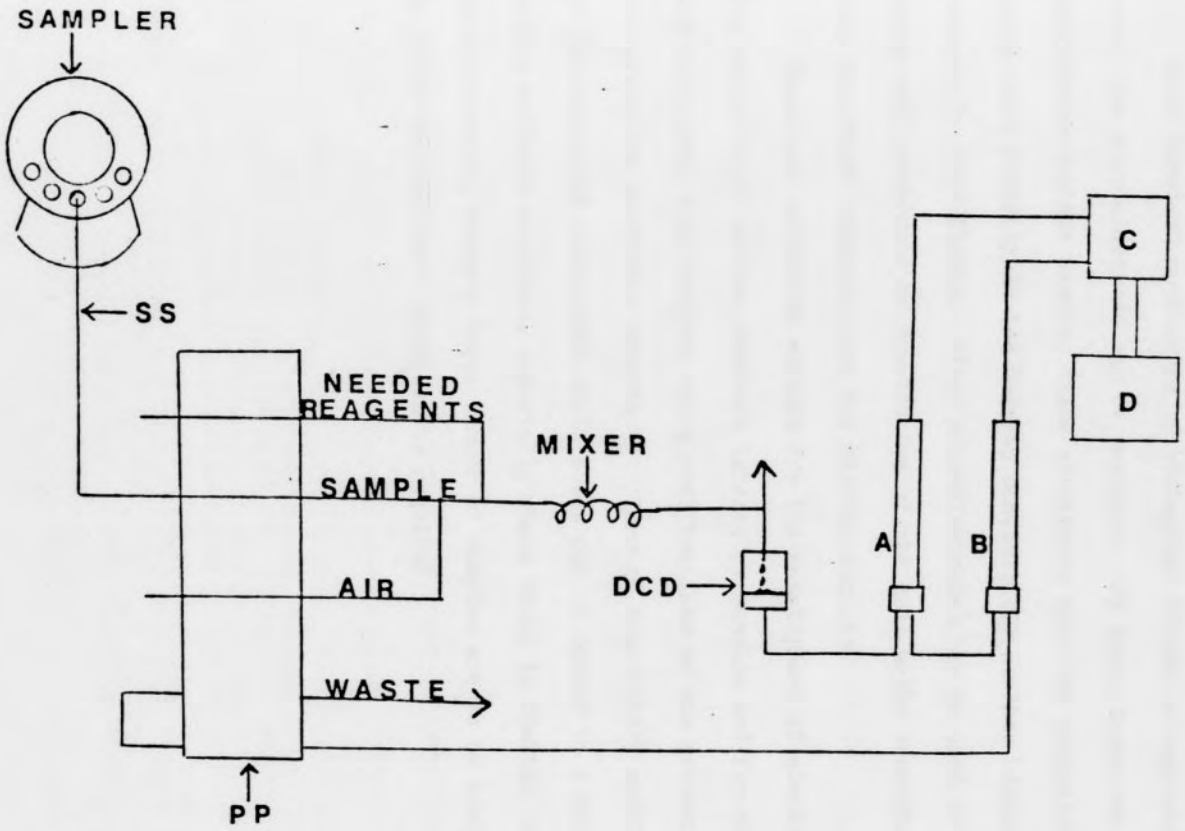


Figure 8. Schematic Diagram of an Automatic Analysis System

SS, sample solution; DCD, drop chamber disconnector; PP, proportioning pump; A, ion-selective electrode; B, external reference electrode; C, voltmeter; D, recorder.

When constant monitoring of biological fluids is required in vivo, the microelectrode can be employed. By their insertion in a hypodermic syringe needle, these electrodes have the capability of being used directly in the body to continuously monitor biochemical changes in body fluids. Also, microelectrodes can be used to directly study cell processes by penetration of the cell walls providing a very important research tool for microbiologists.

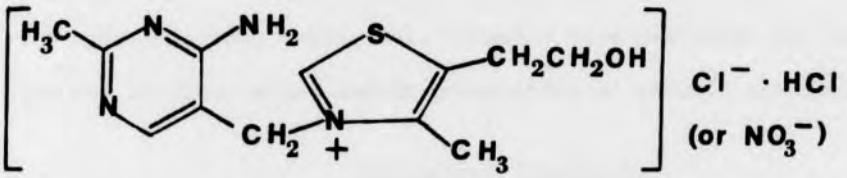
There are unlimited sources for the development of electrodes for analysis of routine chemical laboratory samples and for clinical and biological applications using modifications of the conventional ion-selective electrode techniques. Through imaginative application of ion-selective electrodes whole areas can be opened to a means of quick, reliable analysis, especially where study is limited to small sample amounts, where a large number of samples are to be analyzed, or where "on-the-spot" analysis is required.

CHAPTER FIVE

THIAMINE (VITAMIN B_1) [22, 23, 24]

Physical Properties

Thiamine can exist in the chloride hydrochloride form or the mononitrate form with the structure



Thiamine hydrocholoride has a molecular weight of 337.28 g and is formed as white crystals of monoclinic plates in rosette-like clusters with a yeasty odor and bitter taste. Practically all of the thiamine hydrochloride sold is synthetic. Thiamine hydrochloride is highly soluable in water in which one gram dissolves in about one milliliter. It absorbs up to one mole of water in air of average humidity. The water can be removed by drying at 100° C or in a vacuum over sulfuric acid. In the dry form, thiamine hydrochloride is very stable and heating at 100° C for twenty-four hours does not cause decomposition nor alter its potency. However, in aqueous solution, thiamine hydrochloride is sensitive to reduction and oxidation. In neutral or alkaline solutions it is destroyed rapidly, but in acid solutions it can be sterilized by heating. Thiamine hydrochloride occurs in natural foods and in animal

tissue in the free form or in combined form. The acid dissociation constant of thiamine is 1.58×10^{-5}. It exists in various amounts in different sources. Yeast, cereal, grains, pork, and nuts are high in thiamine hydrochloride.

Thiamine mononitrate has a molecular weight of 327.36 and is practically nonhygroscopic. 2.7 grams dissolve in about 100 ml of water at 25° C. Aqueous solutions at a pH of 4.0 show greater stability than neutral solutions. Thiamine mononitrate is much more stable than the hydrochloride, making this thiamine form preferred and recommended for use in flour mixes and in preparation of vitamin capsules.

Biological Importance

In the human body free thiamine exists in the blood plasma and cerebrospinal fluid in a concentration of about one microgram in one hundred milliliters. In the blood cells, the thiamine exists as the pyrophosphates in protein combination in a concentration of about six to twelve micrograms in one hundred milliliters. The body stores the thiamine it needs and excretes the excess. The stored thiamine is depleted in a short time and must be replaced by daily intake. Any excess thiamine is usually excreted in the urine, which contains only the free form. Normal urinal excretion of thiamine from an individual with adequate intake is at least fifty micrograms daily. Thiamine deficiency is characterized by loss of appetite and weight causing, in its advanced stages, the disease known as beriberi in which there is an increase of pyruvate and lactate levels in the blood and possible degenerate changes of several peripheral nerves.

Numerous methods have been developed to test for thiamine incorporating animal assays and microbiological, chemical and physical methods. Of the chemical methods, the preferred way at present to test for thiamine in body fluids and tissue is the oxidation of thiamine to thiochrome, which is a highly fluorescent species that can be easily measured using fluorometry.[25]

A complete review of this thiochrome method and other thiamine analysis methods has been organized by K. A. Valsalan.[26]

CHAPTER SIX

DEVELOPMENT OF A THIAMINE LIQUID MEMBRANE ELECTRODE

Background

The development of a thiamine liquid membrane electrode has been claimed by Ishibashi, Kina, and Maekawa.[27,28] Their thiamine electrode consists of an exchange site, tetraphenyl borate, in a 1,2-dichloroethane solvent. The response of the thiamine electrode is Nernstian for a bivalent species. Selectivity is high for thiamine over sodium, potassium, and ammonium ions but is inadequate for the detection of mixtures containing pyridoxine monohydrochloride (vitamin B_6). Also, the description of the sample solution preparation is totally inadequate. The authors noted that thiamine is stable only at a low pH range but do not specify how the sample solution was pH controlled or if it was pH controlled at all.

A photometric method for the analysis of thiamine based on an extraction technique has been developed by Das Gupta and Cadwallader.[29] The method employs the formation of the thiamine-bromothymol blue salt and its extraction from an aqueous into an organic phase, the best solvent being chloroform. The reaction between the bromothymol blue dye and the thiamine is considered to be a simple acid-base reaction where the dye is a strong acid and the thiamine is a base. The site of reaction in thiamine may be either the amino group or the quaternary nitrogen with the sulfonic acid group of the dye.[29,30] The concentration

of the thiamine in the aqueous solution is then determined photometrically by measuring the absorbance of the organic solution. The thiamine-bromothymol blue salt is more soluble in the organic solvent than the aqueous solution. This extraction process has been determined to be pH dependent with a pH of 6.6 giving the optimum condition for extraction. Two mathematical approaches with supporting experimental data have been performed on the effect of pH and bromothymol blue dye concentration on the extraction of the thiamine-bromothymol blue salt by an organic solvent.[30, 31] An important feature of this method is the lack of interference from other vitamins, hormones, and other biological substances.

In the course of the development of a thiamine liquid membrane electrode, the thiamine electrode produced by Ishibashi, Kina, and Maekawa[27, 28] and the extraction method of Das Gupta and Cadwallader[29] need to be considered. The liquid membrane electrode of Ishibashi et al. has low selectivity for thiamine over pyridoxine hydrochloride due possibly to the solvent used for the ion-exchanger solution. The lack of information concerning the pH dependence of the electrode's response towards thiamine concentration is a very important factor if the electrode is to be used effectively in practical analysis procedures.

For the thiamine extraction method, pH dependence was noted for the extraction process of thiamine-bromothymol blue salt with an organic solvent and lack of interference from other common clinical substances was observed. This extraction method offers an approach to a thiamine liquid membrane electrode by its use of an organic solvent (a plausible active liquid phase), by the availability of a possible ion exchange site

in the organic solvent (the thiamine-bromothymol blue salt), and, finally, by its high selectivity over the interfering substances commonly found in commercially prepared vitamin capsules.

Experimental

Apparatus.

The apparatus used for study of the thiamine liquid membrane electrode consists of a high impedance voltmeter, a commercially available liquid membrane electrode assembly, a double junction external reference electrode, a pH meter, a pH microelectrode, a constant temperature circulator, a jacketed beaker with a magnetic stirrer motor and stirring bar, a micropipet, and a ten milliliter full capacity buret.

The sample solution is contained in a jacketed beaker allowing a heat controlled transfer liquid to pass over the walls of the ion-selective electrode cell and condition the sample solution to a desired temperature. The heat transfer liquid used is water and it is controlled and circulated using a Haake Constant Temperature Circulator (Model FK). The sample is stirred by means of a magnetic stirrer motor and stirring bar.

The liquid membrane electrode is the Ionalyzer divalent cation electrode body, Model 92-32, developed by Orion Research Incorporated which uses thin porous membrane supports to hold the active liquid membrane phase. The external reference electrode is the 4857-H10 Double Junction Reference Electrode by Arthur H. Thomas Company. This contains a calomel element in an inner section filled with saturated aqueous potassium chloride which is connected through a porous ceramic

plug to the outer section containing a 1.0 M aqueous sodium chloride solution. The junction with the sample is made through a porous ceramic plug in the tip of the electrode. The liquid membrane electrode and external reference electrode are both positioned at an angle of 20° with respect to the vertical by an Orion electrode holder in order to diminish the chance of air bubbles getting trapped under the electrodes. Both electrodes are easily connected to the appropriate terminals in the voltmeter.

The voltmeter is an Orion Research Model 801 all-electronic, high-input-impedance digital pH/mv meter that supplies a pH range from 0.000 to 13.999 in 0.001 pH unit increments with a repeatibility of ± 0.001 pH unit. Also, when adjusted to the millivolt mode, the voltmeter supplies a range of +999.9 to -999.9 in 0.1 millivolt increments with a ±0.1 millivolt repeatibility. The data output is available as binary-coded decimals and the recorder output is adjustable from 0 to 100 millivolts for ±1000 millivolts or ±7 pH units.

The pH electrode is a Fisher Scientific Microprobe Combination Electrode, one fourth-inch diameter version (Cat. No. 13-639-92) with the reference element solution being a 4 M potassium chloride-saturated silver chloride aqueous solution. The pH meter used is a pH/pIon electrometer with digitalizer/indicator (Models EU-200-30 and EU-200-62, respectively) produced by Heath Company. Some of its features are ability to select cation, anion, or millivolt modes, automatic pH slope control, manual temperature control, or an optional automatic temperature compensation control.

Minute volumes of solution are measured and dispensed using a Manostat Digi-Pet ultra-micro pipet (Manostat Corp., Cat. No. 71-632-00) equipped with a 1.0 ml full-capacity reservoir. This micropipet is able to dispense volumes of solutions down to ±0.0001 ml when using the 1.0 ml reservoir. Dispensing of solutions by this micropipet should be done by immersing the capillary tip of the reservoir into the sample. The larger volumes of solution are measured using a 10.0 ml capacity buret with a 0.02 milliliter gradient scale.

The entire sample solution is covered with a layer of parafilm (American Can Company, Marathon Products, Neenah, Wis.), a maleable plastic wrapping film, after the liquid membrane electrode, the external reference electrode, and the pH electrode are immersed in the sample solution. A small hole is bored through this parafilm layer to allow entry of the micropipet and buret tips into the sample solution.

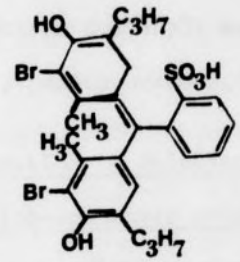
Reagents.

The water used for all aqueous solution preparations was distilled in a Barnstead still (Model SM-10, Barnstead Still and Sterilizer Company, Inc., Forest Hills, Boston, Mass.). This water was passed through a mixed bed of ion-exchange column (Barnstead Hose Type Cartridge, Fisher Scientific Company) for removal of remaining inorganic impurities and later purified by a second distillation in a vented all-glass distillation apparatus for removal of organic impurities. The water was collected and stored in a closed Nalgene Container (Nalge Sybron Corporation) to prevent contamination from carbon dioxide.

The water soluble bromothymol blue dye (Matheson Coleman and Bell), the polyvinyl chloride (Aldrich Chemical Company), and the

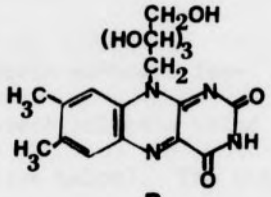
di-n-decyl phthalate (Eastman Corporation) were used without further purification. Sigma Chemical Corporation supplied the d-pantothenic acid (d-calcium pantothenate or vitamin B_5) stored desiccated at 0 to 0.5°C, thiamine hydrochloride (aneurine or vitamin B_1) stored in the dark, nicotinamide (niacinamide or nicotinic acid amide), riboflavin (vitamin B_2) protected from light, and pyridoxine monohydrochloride (vitamin B_6) stored in the dark and also desiccated. All Sigma Chemical Corporation products, unless specifically indicated, were not necessarily the anhydrous form. The ascorbic acid (vitamin C) was purchased from a local pharmacy (Rite-Aid Corp.) as commercial capsules. No list of other possible chemicals, such as binders or additives came with the capsules. The structures for the dye and the chemicals obtained from Sigma Corporation are given in figure 9. All other solid chemicals employed were either certified analytical reagents or certified A. C. S. chemicals of proper analytical grade.

The standard sodium hydroxide solutions and the standard hydrogen chloride solutions were prepared using certified concentrated standard 10.000 ± .005 normal solution by Fisher Scientific Company. The solutions for the internal reference element of the pH electrode and double junction reference electrode were a 4 M potassium chloride solution saturated with silver chloride (Corning Instruments, Inc.) and a saturated potassium chloride solution (Beckman Instruments, Inc.), respectively. Nitrobenzene (Fisher Certified Reagent, Fisher Scientific Company) and the tetrahydrofuran (Matheson Coleman and Bell, classified as suitable for histological use) were used directly without further purification. Trifluoroacetyl-p-butylbenzene was prepared by Harvey B.

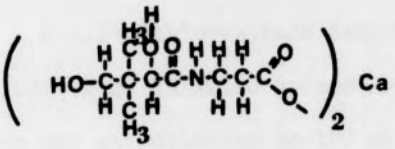


(In acid)

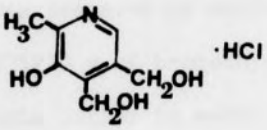
A



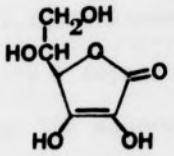
B



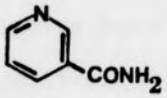
C



D



E



F

Figure 9. Diagram of Molecular Structures for Bromothymol Blue Dye Salt (A), Riboflavin (B), d-pantothenic acid (C), Pyridoxine Monohydrochloride (D), Ascorbic Acid (E), and Nicotinamide (F).

Herman.[17] Chloroform (Fisher Certified, Spectroanalyzed, Fisher Scientific Company), and 1,2-dichloroethane (Matheson, Coleman, and Bell), purity unknown, were fractionally distilled before use.

Preparation of Solutions.

Ion-exchanger solutions. A number of liquid membrane ion-exchanger solutions were prepared from different solvents using the thiamine-bromothymol blue extraction method (see below). The thiamine was always in excess as compared to the bromothymol blue dye and all ion-exchanger solutions were stored in a freezer when not in use.

A 1,2-dichloroethane ion-exchanger solution was prepared by adding 0.16864 g of thiamine hydrochloride hydrate to 0.031220 g of bromothymol blue dye and diluting to 100 ml with pH 6.6 phosphate buffer to make an ion-exchanger solution of 1×10^{-3} M thiamine-dye salt. 100 ml of purified 1,2-dichloroethane were added and stirred vigorously for one hour. Using a separatory funnel, the organic phase (bottom layer) was removed and the aqueous layer was extracted with an additional volume of 100 ml of 1,2-dichloroethane with vigorous stirring for forty-five minutes and then separated. The organic solvent extracts were collected, passed through a dry filter paper to remove any emulsified aqueous phase, and evaporated in a fractional distillation apparatus to less than 50 ml. This solution was then allowed to cool and was passed through another dry filter paper into a 50 ml volumetric flask where it was diluted to the mark with purified organic solvent. A precipitate indicated a saturated solution of the acid-dye salt. This ion-exchanger solution was centrifuged to remove the orange-red precipitate which was dried for infrared analysis. The ion-exchanger solution was yellow. An

infrared spectrum of the orange precipitate was determined and compared to the infrared spectra of the pure thiamine hydrochloride hydrate and the bromothymol blue dye used for the ion-exchanger preparation.

The potassium bromide pellet technique was used for all three samples. By comparing the three spectra, it was noted that the major infrared peaks characterizing the thiamine hydrochloride hydrate and the bromothymol blue dye were also found in the orange precipitate spectrum indicating that it was the thiamine-bromothymol blue salt. The major peaks for the thiamine hydrochloride hydrate sample were at 1655, 1610, 1530 and 1045 cm^{-1}. The major peaks for the bromothymol blue dye were at 3490, 2960, 1610, 1090, 1020 and 620 cm^{-1}.

The nitrobenzene ion-exchanger solution was prepared by adding 0.17764 g of thiamine hydrochloride hydrate to 0.03122 g of bromothymol blue dye and diluting with 50 ml of pH 6.6 phosphate buffer to make an ion-exchanger solution of 1×10^{-3} M thiamine-dye salt. 50 ml of nitrobenzene were added to the aqueous solution and stirred vigorously for one and one-half hours. Using a separatory funnel, the organic phase (bottom layer) was removed and passed through a dry filter paper to remove any emulsified aqueous phase. The ion-exchanger solution was yellow.

The trifluoroacetyl-p-butylbenzene ion-exchanger solution was prepared from 0.03553 g of thiamine hydrochloride hydrate to 0.00646 g of bromothymol blue dye and diluting these compounds in 10 ml of pH 6.6 phosphate buffer and 5 ml of trifluoroacetyl-p-butylbenzene solution to make an ion-exchanger solution of 2×10^{-3} M thiamine-dye salt. This mixture was stirred vigorously for five hours and allowed to stand

overnight. Separation was not very complete. The solvent phase was emulsified with the aqueous phase and showed precipitate formation. The solvent phase was centrifuged and the organic phase was removed and passed through dry filter paper. The pale yellow color of the ion-exchanger solution indicated that extraction was incomplete.

The chloroform ion-exchanger solution was prepared by adding 0.16864 g of thiamine hydrochloride hydrate to 0.03122 g of bromothymol blue dye and diluting to 100 ml with pH 6.6 phosphate buffer to make an ion-exchanger solution of 1×10^{-3} M thiamine-dye salt. 100 ml of purified chloroform were added to the aqueous solution and the mixture was stirred vigorously for forty-five minutes. Using a separatory funnel, the aqueous phase (top layer) was removed, extracted with another 100 ml of chloroform, and the mixture was stirred vigorously for another forty-five minutes. The aqueous phase was again removed and extracted with an additional 100 ml of chloroform with one hour of stirring. All three chloroform extracts were collected, passed through a dry filter paper, and evaporated using a fractional distillation apparatus until less than 50 ml of solution remained in the reaction pot. This was allowed to cool and was placed in a 50 ml volumetric flask which was diluted to the mark with purified chloroform. This ion-exchanger solution was yellow.

The octanol-1 ion-exchanger solution was prepared from 0.17764 g of thiamine hydrochloride hydrate, 0.03122 g of bromothymol blue dye, and 50 ml of pH 6.6 phosphate buffer to make an ion-exchanger solution of 1×10^{-3} M thiamine-dye salt. 50 ml of octanol-1 was added to this aqueous solution, the mixture was stirred vigorously for one hour, and

the solution was placed in a separatory funnel where extraction looked nearly complete since the aqueous phase (bottom layer) was very pale yellow while the organic phase had a clear, dark orange appearance. The organic phase was removed and passed through a dry filter paper.

The following mixed ion-exchanger solutions were prepared by combining the chloroform and octanol-1 ion-exchanger solutions: 1:1, 2:1, and 4:1 chloroform/octanol-1 by volume. For all three preparations, cloudiness appeared for a brief time upon initial mixing but disappeared after further mixing. When the ion-exchanger solutions were placed in a freezer, aqueous balls of blue color appeared in the organic ion-exchanger solutions. The blue color was probably due to the dye in the solutions. In the chloroform-octanol-1 and in the octanol-1 ion-exchanger solutions, the blue aqueous balls would periodically form while the solutions were stored in the freezer and had to be removed. All three chloroform-octanol-1 ion-exchange solutions should have been 1×10^{-3} M thiamine-bromothymol blue salt if both the pure chloroform and octanol-1 ion-exchanger solutions were 1×10^{-3} M.

The development of polymeric films as the membrane sensor for the liquid membrane electrode body was attempted. The goal of this construction was to produce a polymeric membrane that could be easily sealed in the Orion Research 92-32 liquid membrane electrode body just as the conventional porous membrane supports were sealed. This polymeric film would need the characteristics of thinness, durability, and plasticity for forming good seals. A general procedure for forming

polyvinyl chloride (PVC) membranes was developed with some small changes from reference methods.[32, 33, 34] 35 mm (i.d.) glass ring (10 ml volume capacity) and a 30 mm (i.d.) glass ring (4 ml volume capacity) were cut from glass test tubes. Two glass rings allowed the formation of PVC membranes of different thickness. The edge of one side of the ring was smoothed with sandpaper and then sealed on a glass plate using a minimum amount of Non-Aq stopcock grease (Fisher Scientific Company), an organic insoluble grease. The entire edge of the glass ring in contact with the glass plate was coated with hot paraffin wax and allowed to cool in order to keep the glass ring in position on the glass plate. The stopcock grease was used to prevent the paraffin from dissolving in the organic solvent used for the PVC membrane construction. The PVC membrane solution prior to hardening contained various proportions of 1,2-dichloroethane ion-exchanger solution, PVC, and any needed plasticizer. All were dissolved in tetrahydrofuran (THF). THF was added to bring the entire membrane preparation into the liquid form. This final solution was then poured into the glass rings. Each glass ring was covered with a layer of filter papers (five to ten sheets) and weighted down with a heavy object so as to enable very slow evaporation of the THF. This solution was then allowed to sit undisturbed for at least forty-eight hours. The resulting solid PVC membrane was peeled from the glass and the underside of the membrane was allowed to dry thoroughly. The PVC membrane foils were made by cutting the large membrane with a cork borer to fit the diameter of a conventional Orion porous membrane electrode body.

PVC membranes using the 1,2-dichloroethane ion-exchanger solution as the plasticizer were prepared using the following amounts of materials.[32,33,34] The first attempt used 0.66 g of the 1,2-dichloroethane ion-exchanger solution and 0.28 g of PVC dissolved in 10.0 ml of THF. 6.4 ml of this mixture was poured into the 35 mm glass ring and the remaining 3.6 ml into the 30 mm glass ring. The second attempt used 0.33 g of 1,2-dichloroethane ion-exchanger solution and 0.13 g of PVC dissolved in 8.3 ml of THF. 5.3 ml of this mixture was poured into the 35 mm glass ring and the remaining 3 ml was poured into the 30 mm glass ring. After testing the resulting PVC membrane, it appeared that using 1,2-dichloroethane ion-exchanger solution as the plasticizer was not effective. The membrane produced had toughness and durability but lacked the plasticity needed to form a good seal in Orion 92-32 liquid membrane electrode body.

PVC membranes using plasticizers, such as di(2-ethylhexyl)-2-ethylhexylphosphonate or didecyl phthalate, have been developed.[35] A PVC membrane containing di-n-decyl phthalate (DDP) was prepared using the following materials. The first attempt used 0.33 g of 1,2-dichloroethane ion-exchanger solution, 0.04 g of PVC and 0.33 g of DDP, all dissolved in 8.3 ml of THF. 5.3 ml of this mixture was poured into the 35 mm glass ring and the remaining 3 ml was poured into the 30 mm glass ring. These PVC membranes had a soft highly rubbery surface that was easily breakable due to no supportive properties. The second attempt used 0.33 g of 1,2-dichloroethane ion-exchanger solution, 0.20 g of PVC, and 0.16 g of DDP all dissolved in 8.3 ml of THF. 5.3 ml of this mixture was poured into the 35 mm glass ring and the remaining 3 ml was poured into the 30 mm

glass ring. These PVC membranes had slight flexibility with good supportive characteristics and the necessary plasticity to make a good seal in the Orion liquid membrane electrode body.

Buffer Solutions. Standard aqueous pH buffers were prepared according to R. G. Bates[36] to calibrate the pH electrode and pH meter. A pH 4.008 buffer was prepared by making a 0.05 M potassium hydrogen phthalate solution. The potassium hydrogen phthalate was dried at a temperature of 100°C for three hours prior to use. This buffer solution was kept from accidental contamination with strong acid or alkali. A pH 6.865 buffer solution was prepared by making a 0.025 M potassium dihydrogen phosphate and a 0.025 M disodium hydrogen phosphate solution. Both compounds were dried for at least three hours at 110°C and cooled in a desiccator. A pH 9.180 buffer solution was prepared by making a 0.01 M borax (sodium borate) solution. This buffer solution was stoppered tightly as the absorption of only 0.2% carbon dioxide by the solution causes the pH to be altered by 0.001 unit.

The aqueous buffer solutions employed for experimental analysis of the different liquid membrane electrode systems were prepared in a manner similar to the directions in the "United States Pharmacopeia" (USP).[37] The buffer solutions studied had pH values of 4.0, 6.0, 6.6, and 8.0. According to the USP method, 50 ml of 0.2 M potassium dihydrogen phosphate solutions or 50 ml of a 0.2 M potassium hydrogen phthalate solution with appropriate amounts of 0.2 M sodium hydroxide solution were used in the buffer preparations to make 0.05 M potassium dihydrogen phosphate solutions or 0.05 M potassium hydrogen phthalate solutions with various concentrations of sodium hydroxide. These salt

concentrations were proportionally increased in order to obtain stronger buffer solutions containing 0.1 M potassium dihydrogen phosphate or 0.1 M potassium hydrogen phthalate with the proportionally increased concentrations of sodium hydroxide. A pH 4.0 buffer solution was prepared by making a 0.1 M potassium hydrogen phthalate and a 0.0008 M sodium hydroxide solution. A pH 6.0 buffer solution was prepared by making a 0.1 M potassium dihydrogen phosphate and a 0.01128 M sodium hydroxide solution. A pH 6.6 buffer solution was prepared by making a 0.1 M potassium dihydrogen phosphate and a 0.03548 M sodium hydroxide solution. A pH 8.0 buffer solution was prepared by making a 0.1 M potassium dihydrogen phosphate and a 0.0937 M sodium hydroxide solution using water that had been boiled and cooled prior to use in order to eliminate any dissolved carbon dioxide that could interfer with this buffer preparation. The potassium dihydrogen phosphate and potassium hydrogen phthalate were dried in an oven at 100°C for at least three hours and cooled in a desiccator before use in all these buffer preparations.

Thiamine Hydrochloride Solutions. In the preparation of solutions containing thiamine hydrochloride, a problem arose concerning the high hygroscopic quality of this compound. Two methods were utilized to determine the amount of water absorbed by the thiamine hydrochloride in the reagent bottle. In one method a known amount of thiamine hydrochloride from the reagent bottle was heated at 100°C for twenty-four hours. It was cooled in a desiccator and reweighed to determine the loss of water upon heating of the sample. It was calculated that

for 1 mole of thiamine hydrochloride, there was 0.8 mole of water. However, this value may not be correct since upon weighing of the thiamine hydrochloride after heating the weight of the sample gradually increased, indicating absorption of water from the atmosphere by the sample. The other method to analyze the hydrated thiamine hydrochloride was to use a thermobalance (Perkin Elmer, TGS-1) with a differential scanning calorimeter (Perkin Elmer DSC-1B). The sample was heated to a maximum temperature of 120°C. The amount of water detected by this method was approximately 0.6 mole of water for each mole of thiamine hydrochloride. However, in this instrumentational method, helium is passed through the system, and the thiamine hydrochloride sample showed a weight loss at room temperature even before heating occurred. This indicated that the thiamine hydrochloride forms a weak complex with water since the water was easily evaporated by the constant helium flow over the sample. After careful evaluation of both methods employed, it was concluded that reagent thiamine hydrochloride is definitely a monohydrate. Therefore, the thiamine hydrochloride was always kept in a desiccator and stored in the dark until needed.

Because of the hydrogen chloride complexed to the thiamine, all aqueous solutions prepared from thiamine hydrochloride had to be monitored with a pH electrode and meter due to the formation of an acidic solution upon addition of the salt to water. Appropriate steps had to be taken to adjust the pH value of the thiamine hydrochloride solutions to the desired value.

The Liquid Membrane Electrode's Internal Reference Solution.

The internal reference solution of the liquid membrane electrode consisted of a 0.001 M thiamine and a 0.1 M sodium chloride solution. The pH of this solution was adjusted to the same pH value as that of the sample solution to be analyzed. The following method was used to prepare the internal reference solution. The appropriate amount of thiamine hydrochloride, taken directly from the reagent bottle, was weighed to make 100 ml of a 0.001 M solution. Likewise, the appropriate amount of sodium chloride, dried at 100°C for at least three hours and cooled in a desiccator, was weighed to give 100 ml of a 0.1 M solution. These were quantitatively mixed and diluted to about 50 ml with water. The pH electrode was carefully immersed in this solution and the solution was stirred. A 0.01 M sodium hydroxide solution was added until the desired pH of the solution was attained. The amount of sodium hydroxide solution added was recorded, and the thiamine-sodium chloride solution was diluted to 100 ml in a volumetric flask.

Sample Solutions. Preparation of initial sample solutions for analysis by the liquid membrane electrode system was accomplished by having an initial volume of sample containing 100 ml of a suitable buffer solution by itself or with previously added interfering substances.

For blank sample solutions containing no interfering substances, 100 ml of a buffer solution was added to the jacketed heat controlled beaker and logarithmic amounts of a thiamine stock solution of about 0.1 M concentration or other types of stock solutions were added in milliliter increments using the micropipet or the 10 ml capacity buret.

These milliliter additions of stock solution were 0.01, 0.01, 0.03, 0.05, 0.1, 0.3, 0.5, 1.0, 3.0, 5.0, 10.0, etc. in which the total logarithmic volumes of stock solution in milliliters present in the sample solution were 0.01, 0.02, 0.05, 0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, etc. This method of adding a stock solution to an initial volume of sample gave a practical range of concentrations of the ion of interest that the liquid membrane electrode could analyze.

A 0.1 M sodium chloride stock solution was prepared by dissolving 2.92214 g of oven dried sodium chloride in 500 ml of water and was added to a pH 6.0 buffer solution in the volume increments as given above to make a range of sample solutions with different sodium chloride concentrations.

Sample solutions containing possible interfering substances were made using the type of substances commonly found in commercially available vitamin preparations. For regular vitamin capsules, each capsule usually contained 15 mg of thiamine mononitrate, 10 mg of riboflavin, 5 mg of pyridoxine monohydrochloride, 50 mg of nicotinamide, 10 mg of d-calcium pantothenate, and 300 mg of ascorbic acid. These amounts were found in two commercially popular vitamin preparations named Allbee with C (A-H Robins Company) and B-Complex with Vitamin C (Rite-Aid Company). In all cases, four times the amount specified in each commercial vitamin capsule preparation were added to 100 ml of sample solution.

An interference sample solution of riboflavin, pyridoxine monohydrochloride, and d-calcium pantothenate was prepared by combining these substances and diluting to one 100 ml with pH 6.0 buffer solution.

Because it was believed that nicotinamide may interfer with the thiamine response of the liquid membrane electrode as noted by Das Gupta and Cadwallader,[29] the nicotinamide and ascorbic acid interference sample solution was made separately in a pH 6.0 buffer solution. Due to the ascorbic acid, the buffer capacity of the solution was exceeded and neutralization of the interference substances was required before final dilution with the buffer solution. The interference substances were added and diluted to about 25 ml with pH 6.0 buffer solution and neutralized to pH 6.0 with additions of 1.0 M sodium hydroxide solution recording the amount of sodium hydroxide used. The mixture was then diluted to 100 ml with the pH 6.0 buffer solution.

A separate interference sample solution was prepared for both the nicotinamide and the ascorbic acid. The nicotinamide sample solution required no neutralization and was mixed directly with 100 ml of pH 6.0 buffer solution. The ascorbic acid sample solution was neutralized with 1.0 M sodium hydroxide in the manner as stated above. The amount of sodium hydroxide added was recorded. This interference solution was then diluted to 100 ml with the pH 6.0 buffer solution.

A 0.0420 M sodium chloride interference sample solution was prepared by adding 0.24780 g of oven dried sodium chloride to 100 ml of pH 6.0 buffer solution.

All the above interference sample solutions were used just for the initial sample solution to which a thiamine stock solution of about 0.1 M concentration was added in the volume increments stated previously to make a range of sample solutions with different thiamine concentrations that could be analyzed by a liquid membrane electrode.

The Thiamine Stock Solution. The preparation of a 0.1 M thiamine stock solution was performed by two methods.

Method 1 assumed the thiamine hydrochloride to be the hydrated form when taken directly from the reagent bottle and the appropriate amount of thiamine hydrochloride hydrate was weighed to make a 0.1 M thiamine solution in a total volume of either 100 ml or 200 ml depending upon the amount of stock solution needed for an analysis. This method did not give precise concentrations of thiamine stock solution but in the preliminary task of finding a liquid membrane electrode that would show good thiamine response this was not critical.

Method 2 used a more precise manner in obtaining a thiamine stock solution. A beaker equipped with a cap was employed. The beaker was heated in an oven to remove all moisture present on its walls, and then cooled in a desiccator for one hour. The beaker was touched only with a pair of tongs or for a short time with tissue paper. After the cap was placed on the empty beaker, both were weighed accurately. The approximate amount of thiamine hydrochloride hydrate needed to make a 0.1 M solution in a total volume of 100 ml or 200 ml was taken from the reagent bottle, added carefully to the beaker, and then oven heated at 100°C for three hours. The beaker and sample were cooled in a desiccator for about one hour, after which the same cap was positioned tightly on the beaker, and the entire assembly accurately weighed. The actual weight of the unhydrated thiamine hydrochloride was then calculated.

After a thiamine hydrochloride sample had been weighed by either methods 1 or 2, the thiamine hydrochloride was then neutralized to the

pH value of the initial sample solution to be tested. This sample solution always contained a buffer and, therefore, the thiamine stock solution had to be neutralized to the appropriate pH in order not to extend the capacity of the buffer once it was added. The neutralization of the thiamine stock solution was similar to that method used for the thiamine-sodium chloride internal reference solution. The thiamine hydrochloride was quantitatively diluted to about 50 ml with water. The pH electrode was immersed in this solution. The solution was stirred. 1.0 M sodium hydroxide was added until the desired pH of the solution was attained. The amount of sodium hydroxide was recorded. This thiamine solution was quantitatively transferred into an appropriate volumetric flask and diluted to the mark with water.

Methods of Analysis.

The liquid membrane electrode systems used in this project consisted of initial data of volume of a stock solution added to an initial volume of sample solution and the corresponding measured potentials in millivolts. To use these data effectively, a working curve must be developed in order to determine the kind of response the liquid membrane electrode has towards the concentration of the ion of interest, which can be thiamine or any other substance. Also, a working curve indicates how reproducibly the electrode responds to the different concentrations of the ion of interest. If the liquid membrane electrode is responding in the desired manner, calibration working curves have to be implemented for a wide range of concentrations of the ion of interest. Working curves have to

be constructed to determine the effect of interference substances in the sample solution on the response of the liquid membrane electrode towards the ion of interest.

To form these working curves, the measured potential in millivolts versus the corresponding $\log_{10}$ activities of the ion of interest have to be evaluated. The activity of an ionic substance is related to all the ionic species present in the sample solution. For example, in the addition of a thiamine stock solution to a sample solution, the activity of the thiamine is a function not only of the added thiamine and sodium chloride from the stock solution, but also of the salts of the buffer solution and any other ionic interference substances that are present.

Two Fortran IV programs have been developed to easily convert the amounts of stock solution of the ion of interest added to a sample solution into the log activities of the ion of interest. Each log activity value and its corresponding measured potential are used in a linear regression method to determine the slope, the intercept, the standard deviation of these values, and the correlation coefficient for the plot of the measured potential versus log activity of the ion of interest. These programs are called SALTBI and SALTIV.

Program SALTBI (Appendix A) is used for sample solutions containing only a buffer, one ion of interest, and only one additional ionic salt.

Program SALTIV (Appendix B) is used for sample solutions containing a buffer and interference substances numbering no more than five. Similar to program SALTBI, one ion of interest and one additional ionic salt can be accomodated in this program.

Both programs utilize Davis equation[2] for determining the activity coefficient, and the ionic strength contributed by each ionized salt in the sample solution is obtained by the factor method described on page 8. Two subroutines, FREE and LINFIT, are used. Program FREE[38] (Appendix C) allows free input format of data values for the SEL 72 computer system from any control unit and assigns the input value to a variable in the main program. Program LINFIT (Appendix D) is the subroutine employing the linear regression method using the calculated log activity of the ion of interest and its corresponding measured potential.

From these Fortran IV programs, the working curve can be drawn by plotting the measured potential versus the corresponding log activity of the ion of interest. It is these curves that show whether a liquid membrane electrode is responding in a suitable manner with or without interference substances present and whether the electrode response depends upon certain conditions such as pH.

The General Procedure for an Experiment Employing a Liquid Membrane Electrode Cell.

The general format of performing an experiment with a liquid membrane electrode was as follows:

1. Assemble the Orion liquid membrane electrode according to the Orion Research 92-32 manual and choose the appropriate porous membrane support to contain the ion-exchanger solution.

2. Position the electrode upright without the top cap and add the desired ion-exchanger solution through the larger outside hole on top of the electrode leading to the liquid membrane solution reservoir

in the outside chamber of the electrode. Be sure of proper wicking of the membrane support by the ion-exchanger solution by holding electrode overhead and observing the membrane. As the membrane wicks the ion-exchanger solution from the reservoir it should become partially translucent or wet-looking. Place the washer over the ion-exchanger solution filling hole and, using a syringe with the special nozzle for this electrode, add the internal reference solution in the center filling hole on top of the electrode. Since this solution will be emitted out of the electrode from the vent hole, it is necessary to catch this solution with a tissue. This internal reference solution should be sufficiently flushed through the inner electrode chamber to remove any trapped air bubbles. Replace the top cap containing the lead for the electrical connection between the internal reference electrode in the inner chamber of the liquid membrane electrode and the voltmeter, and connect the lead to the appropriate voltmeter terminal.

3. Once assembled, the liquid membrane electrode can be used for long periods of time with the same ion-exchanger solutions. However, before each experiment using the electrode, a fresh internal reference solution is prepared because of the possible decomposition of the thiamine in the old internal reference solution. This fresh reference solution replaces the old solution in the inner chamber of the electrode by flushing the chamber five to six times.

4. The liquid membrane electrode is conditioned before use.

5. Prepare the external double junction reference electrode for use by adding the appropriate solutions of saturated potassium chloride

and 1.0 M sodium chloride and connect the electrode to the proper terminal on the voltmeter. Since the sodium chloride solution contacts the sample solution through a porous ceramic plug, it is replaced with new sodium chloride solution by suitable flushing of the chamber just before the reference electrode is used. Store the double junction reference electrode in water. Also prepare the pH electrode for use by conditioning it in a buffer solution.

6. Start the constant temperature circulator and maintain a temperature of 25°C.

7. Place the magnetic stirring bar and 100 ml of sample solution into the jacketed beaker. Rinse the liquid membrane electrode with water and blot dry with paper tissue. Place the electrode in the sample solution and stir this solution at constant speed throughout the experiment. Check for air bubbles trapped under the bottom electrode cap where the membrane sensor is located. If air bubbles form, tap the electrode to remove them.

8. Rinse with water and blot dry the external double junction reference electrode. Place this electrode into the sample solution and turn on the voltmeter to the millivolt mode. If the measured potential is stable, showing little drift, then continue. If the measured potential is not stable but fluctuates widely, then check for air bubbles under the electrode cap or in the internal reference solution chamber of the liquid membrane electrode.

9. Rinse with water and carefully blot dry the pH electrode. Place this electrode into the sample solution and turn on the pH meter.

10. Cover the jacketed beaker and its sample solution with a layer of parafilm.

11. Wait for the voltmeter and the pH meter to give stable readings.

12. Bore a hole in the parafilm and begin to add the desired volumes of stock solution.

13. Record the amount of stock solution dispensed into the sample solution upon each addition, the total amount of stock solution added to the sample solution, the measured potential (in millivolts) and the pH of the sample solution after each stock solution addition, and the description of the liquid membrane electrode response.

14. After the range of concentrations of the ion of interest has been analyzed by the liquid membrane electrode, shut off the instrument, clean all glassware and equipment, and properly store all the electrodes.

The Progressive Procedure for Determining the Most Suitable Liquid Membrane Electrode for Thiamine Analysis.

The first ion-exchanger solution extensively studied in this investigation was the 1,2-dichloroethane ion-exchanger solution. The liquid membrane electrode employed an Orion Research divalent cation membrane (Model 92-32-04) as the support for the ion-exchanger solution.

To determine the pH dependence of the liquid membrane electrode's response towards thiamine concentration, the initial sample solutions contained no interference substances and three different pH buffer solutions were employed for analysis. The liquid membrane electrode was tested for its response in a range of thiamine concentrations in sample solutions of pH 4.0, 6.0, and 8.0 to determine which pH gave the optimum response. The thiamine-sodium chloride internal reference solution and the thiamine

stock solution (prepared according to Method 1, page 52) were adjusted to the pH value of the buffer solution in the initial sample solution. The liquid membrane electrode was conditioned in the appropriate buffer solution containing some thiamine stock solution. Two consecutive trials using thiamine stock solution additions were performed for each pH analysis to study electrode reproducibility. The working curves of potential (millivolts) versus the $\log_{10}$ activity of thiamine were constructed using program SALTBI and the slope, intercept, standard deviations, and correlation coefficient were calculated. In using this program, it was assumed that thiamine is a divalent ion, which may or may not be true, at the pH values studied.

All the working curves for each pH studied had gradually increasing slopes, the degree of slope depending on the pH used. The pH 6.0 sample solutions had a higher slope value than the sample solutions of pH 4.0 and pH 8.0. A table of the results are given below.

Table 2

pH Dependence of the
1,2-dichloroethane Liquid Membrane Electrode

pH Studied	Reproducibility	Slope for 5×10^{-3} to 3×10^{-2} M Thiamine (mv/decade)	Measured Potential
4.0	2 mv	13	stable
6.0	3 mv	17	stable
8.0	2 mv	14	stable

In Table 2, slope values were determined for the thiamine concentration range of 5×10^{-3} to 3×10^{-2} M because, in this range, the largest potential change per logarithmic addition of stock solution was observed. However,

small potential change response was observed between the lower thiamine concentrations which gradually increased as the thiamine concentration increased. Highest sensitivity of the liquid membrane electrode towards thiamine was obtained at a pH of 6.0. This pH appeared to be the best condition to study subsequent liquid membrane electrodes.

Because thiamine sensitivity was not sufficient for the 1,2-dichloroethane liquid membrane electrode to make it practical, a number of other liquid membrane electrodes were studied using the following ion-exchanger solutions: pure chloroform, pure octanol-1, 1:1, 2:1, and 4:1 chloroform-octanol-1, nitrobenzene, trifluoroacetyl-p-butylbenzene, and two PVC polymeric membrane films using di-n-decyl phthalate as plasticizer. All the liquid membrane electrodes employed the Orion Research divalent cation porous membrane support (Model 92-32-04) except, naturally, for the PVC membrane films that are self-contained ion-sensitive matrices requiring no additional membrane supports. The initial sample solution was the pH 6.0 buffer solution. The thiamine stock solutions were prepared according to method 1 on page 52. The thiamine-sodium chloride internal reference solutions and thiamine stock solutions were neutralized to pH 6.0. The liquid membrane electrodes were conditioned in fresh pH 6.0 buffer solutions containing some thiamine stock solution for at least two hours before use. During storage, the electrodes were either placed in thiamine buffer solutions as described above or allowed to remain in the atmosphere. Two consecutive trials using thiamine stock solution additions to the sample solution were performed for each electrode to

study thiamine response and reproducibility. The working curves of the potential (millivolts) versus the $\log_{10}$ activity of thiamine were constructed using program SALTBI. The thiamine was assumed to be a divalent cation at this pH.

A summary of the results obtained using these different liquid membrane electrodes is given in Table 3, on page 62.

The pure chloroform, pure octanol-1, and chloroform-octanol-1 electrodes showed an increasing potential change between each thiamine stock solution addition. No electrode gave a linear Nernstian response for any thiamine concentration range. As a result, the working curves of potential (millivolts) versus $\log_{10}$ activity of thiamine were indeed curves and not straight lines. The pure chloroform electrode gave good response with different thiamine concentratims but reproducibility was extremely poor. The pure octanol-1 electrode responded slightly better towards thiamine if stored in air but the response was still only fair. The advantage of this electrode was its great stability with no potential drift. The chloroform-octanol-1 combination electrodes were devised to capture the best characteristics of the two solvents and to find the combination that would possess the best response towards thiamine concentration, potential stability, and acceptable reproducibility. The 1:1 chloroform-octanol-1 liquid membrane electrode proved to best suit these requirements.

The nitrobenzene liquid membrane electrode had poor thiamine sensitivity. Incompatibility with the Orion divalent cation membrane support was the probable reason for this. The divalent cation membrane

Table 3

Results for Various Liquid Membrane Electrodes Studied at pH 6.0

Liquid Membrane Electrode	Reproducibility	Thiamine Conc. Range and Slope (mv/decade)		Remarks
pure chloroform	poor	$5 \times 10^{-3} - 2 \times 10^{-2}$	≈34	substantial potential drift
pure octanol-1	excellent	$5 \times 10^{-3} - 3 \times 10^{-2}$	≈20-27	no potential drift
1:1 chloroform-octanol-1	excellent	$5 \times 10^{-3} - 3 \times 10^{-2}$	≈40	no potential drift
4:1 chloroform-octanol-1	fair	$5 \times 10^{-3} - 3 \times 10^{-2}$	≈43	slight potential drift at low thiamine conc.
2:1 chloroform-octanol-1	fair	$5 \times 10^{-3} - 3 \times 10^{-2}$	≈42	slight potential drift at low thiamine conc.
nitrobenzene	not determined	not determined		possible membrane incompatibility
trifluoroacetyl-p-butylbenzene	not determined	not determined		no response to thiamine
polymeric films with DDP:				
thick PVC membrane	not determined	not determined		symptoms of high resistance across membrane
thin PVC membrane	not determined	not determined		symptoms of high resistance across membrane

(excellent= ±1mV, fair= ±3mV, poor≥ ±3mV)

support used appeared to deteriorate from contact with nitrobenzene. Another membrane support was prepared from Duralon, a nylon based support with a pore size of 1.0 μm, made by Millipore Corporation. A nylon membrane, according to Orion Research reports, is suitable with nitrobenzene but problems developed with this liquid membrane due to leakage of ion-exchanger solution from the electrode body, probably as a result of the poor sealing characteristics of the nylon membrane support. A teflon membrane support with the product name Mitex, made by Millipore Corporation, was also tried with the nitrobenzene ion-exchanger solution, but wicking of this solution into the pores of the teflon support was unsuccessful. Finally, a Uni-pore membrane support (Bio-Rad Laboratories, 0.03 μm pore size) was prepared, but was completely decomposed by the nitrobenzene solvent.

The trifluoroacetyl-p-butylbenzene ion-exchanger solution was simply not suitable as an ion-exchanger solution and response was poor towards thiamine detection.

The polymeric films were taken from the preparations using di-n-decyl phthalate as the plasticizer as stated on page 45. The PVC membranes used were obtained from the products of the second attempt. These PVC membranes sealed well into the Orion Research liquid membrane electrode. The PVC membrane from the 35 mm glass ring appeared to have a higher resistance across the membrane than the PVC membrane from the 30 mm glass ring. This was expected since the former membrane was thicker than the latter.

Considering all the liquid membrane electrodes studied, the best electrode developed appeared to be the 1:1 chloroform-octanol-1 liquid membrane electrode. It was this electrode that was extensively studied for pH dependence, response towards thiamine, reproducibility, and interference from the substances commonly found in commercial preparation of vitamin capsules.

The 1:1 Chloroform-Octanol-1 Liquid Membrane Electrode.

For all experiments dealing with the 1:1 chloroform-octanol-1 liquid membrane electrode, the electrode was exposed to the atmosphere until just prior to use when the electrode was conditioned in a pH 6.0 buffer solution with a small amount of thiamine stock solution that has been neutralized to pH 6.0. All thiamine stock solutions were prepared according to method 2 on page 52 in order to have precise thiamine concentrations for the study of this chosen liquid membrane electrode.

The pH dependence of the 1:1 chloroform-octanol-1 liquid membrane electrode towards thiamine response was determined by varying the pH of a 0.01 M thiamine sample solution and observing the measured potential. This initial sample solution gives a pH value of approximately three because of the acidic nature of the thiamine hydrochloride, therefore, supplying an initial sample solution that can be directly analyzed without further preparation. The pH of the sample solution was varied by adding various amounts of 1.0 M sodium hydroxide. The measured potential, the pH, and the amount of sodium hydroxide solution added to the sample solution were recorded.

Because of the high concentration of the sodium hydroxide, only small amounts had to be used to vary the pH of the sample solutions. This was beneficial in that, by the end of the pH analysis, a total of approximately one milliliter of sodium hydroxide solution was needed to adjust the entire pH range of the sample solution studied. This meant that the thiamine concentration was not changed drastically by volume change indicating that the measured potential change was directly due to pH variation. Due to the small amounts of sodium hydroxide solution used, the Manostat micropipet could be used to dispense the sodium hydroxide solution into the sample. A working curve of potential (millivolts) versus pH was constructed and is shown in Figure 10. Reproducibility is not good for the two consecutive trials made, but for both trials a minimum in the curve is noted that correlates with the pH value of approximately 6.5, the value Das Gupta and Cadwallader[29] obtained as the pH range for best extraction of thiamine into the organic solvent as thiamine-bromothymol blue salt. This minimum of measured potential would be expected at the best solution pH due to the consideration of the partition coefficient of thiamine between the aqueous sample phase and the active liquid organic phase of the liquid membrane electrode[39]. The best pH range for detection of thiamine by the 1:1 chloroform-octanol-1 liquid membrane electrode was between 5.5 to 7.5 where there is only about a one millivolt difference. This is in partial agreement with the optimum pH value of 5.2 to 6.6 calculated by Das Gupta, Cadwallader, Herman, and Honigberg[30] for the extraction method. The 1:1 chloroform-octanol-1 liquid membrane electrode was therefore studied at a pH of 6.0.

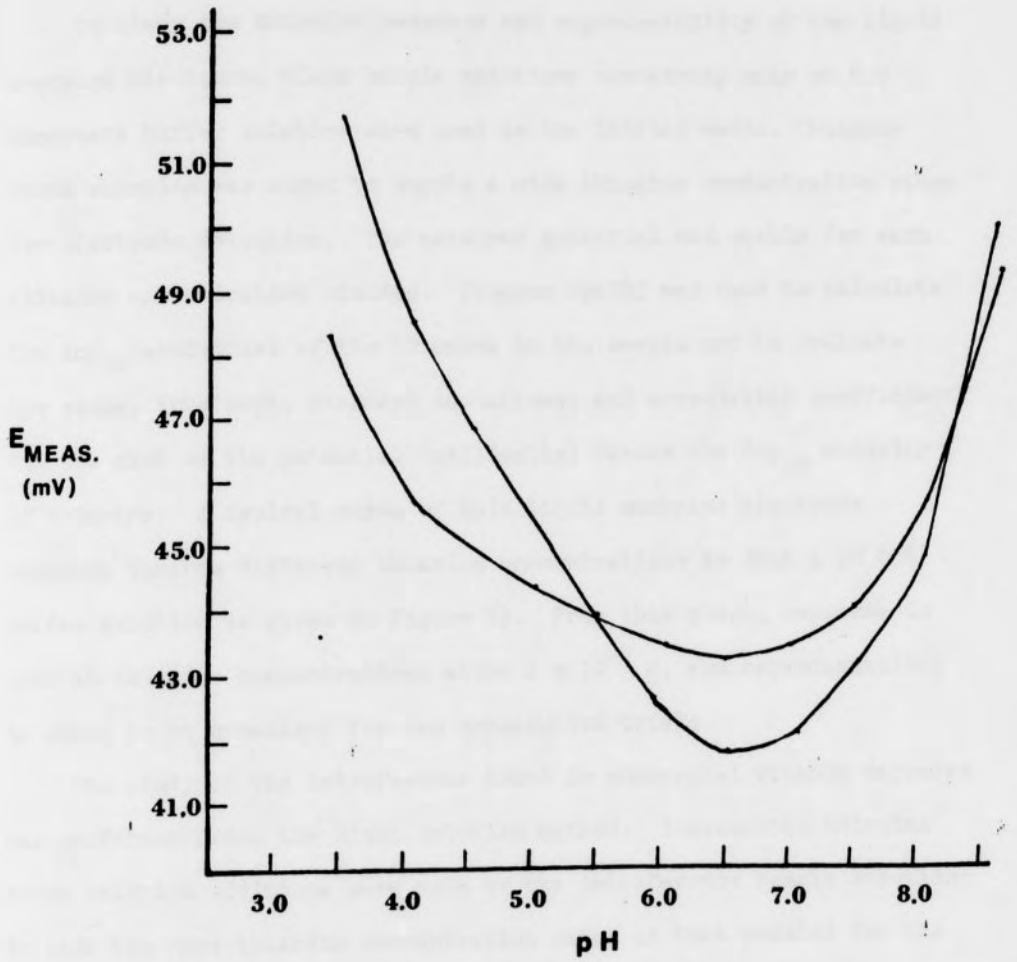


Figure 10. pH dependence of the 1:1 chloroform-octanol-1 liquid membrane electrode (two separate trials).

To study the thiamine response and reproducibility of the liquid membrane electrode, blank sample solutions containing only pH 6.0 phosphate buffer solution were used as the initial media. Thiamine stock solution was added to supply a wide thiamine concentration range for electrode detection. The measured potential was stable for each thiamine concentration studied. Program SALTBI was used to calculate the $\log_{10}$ activities of the thiamine in the sample and to evaluate the slope, intercept, standard deviations, and correlation coefficient for the plot of the potential (millivolts) versus the $\log_{10}$ activity of thiamine. A typical curve of this liquid membrane electrode response towards different thiamine concentrations in just a pH 6.0 buffer solution is given in Figure 11. From this graph, response is good at thiamine concentrations above 1×10^{-3} M, and reproducibility is shown to be excellent for two consecutive trials.

The study of the interference found in commercial vitamin capsules was performed using the mixed solution method. Incremental thiamine stock solution additions were made to the interference sample solutions to give the same thiamine concentration range as that studied for the blank buffer sample solutions. The initial interference sample solutions were prepared just prior to use in order to avoid possible decomposition of the interference substances. Thiamine solutions in a pH 6.0 phosphate buffer were analyzed before the solutions with the interference substances in order to construct working curves to calibrate the liquid membrane electrode. These calibration curves were compared to the curves developed from the interference sample solutions in order to evaluate the extent of interference from the different substances studied. Program SALTIV was used to calculate

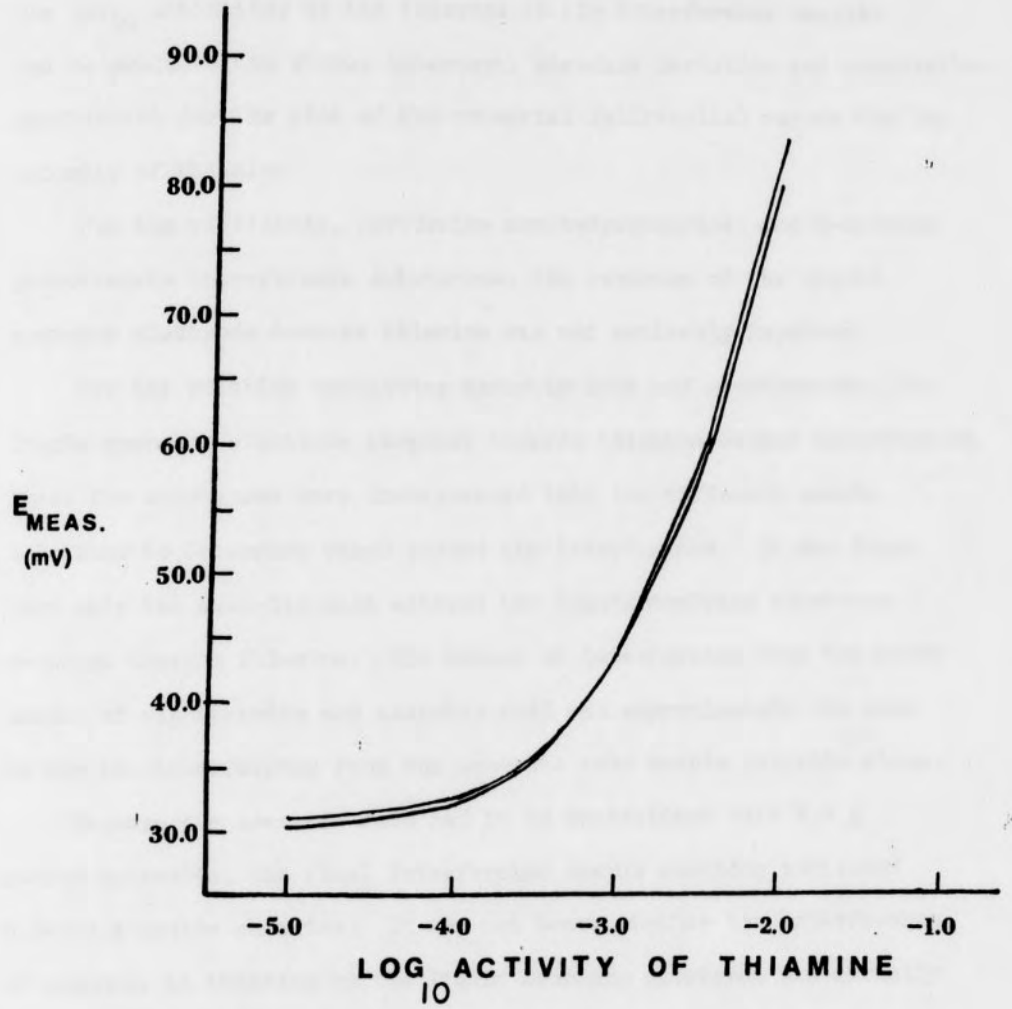


Figure 11. A working curve illustrating the response and reproducibility of the 1:1 chloroform-octanol-1 liquid membrane electrode towards different thiamine concentrations (two separate trials).

the $\log_{10}$ activities of the thiamine in the interference samples and to evaluate the slope, intercept, standard deviation and correlation coefficient for the plot of the potential (millivolts) versus the log activity of thiamine.

For the riboflavin, pyridoxine monohydrochloride, and d-calcium pantothenate interference substances, the response of the liquid membrane electrode towards thiamine was not noticably impaired.

For the solution containing ascorbic acid and nicotinamide, the liquid membrane electrode response towards thiamine showed interference. These two substances were incorporated into two different sample solutions to determine which caused the interference. It was found that only the ascorbic acid altered the liquid membrane electrode response towards thiamine. The amount of interference from the mixed sample of nicotinamide and ascorbic acid was approximately the same as for the interference from the ascorbic acid sample solution alone.

Because the ascorbic acid had to be neutralized with 1.0 M sodium hydroxide, the final interference sample solution contained 0.04240 M sodium chloride. It was not known whether the interference of response to thiamine by the liquid membrane electrode was actually due to the ascorbic acid or to the sodium chloride produced from the neutralization of the ascorbic acid. Another trial was made to determine if interference was due to the sodium chloride by preparing a sample solution of 0.04240 M sodium chloride solution in the pH 6.0 buffer solution. Using program SALTIV, the working curve for this trial is shown in Figure 12 along with a calibration working

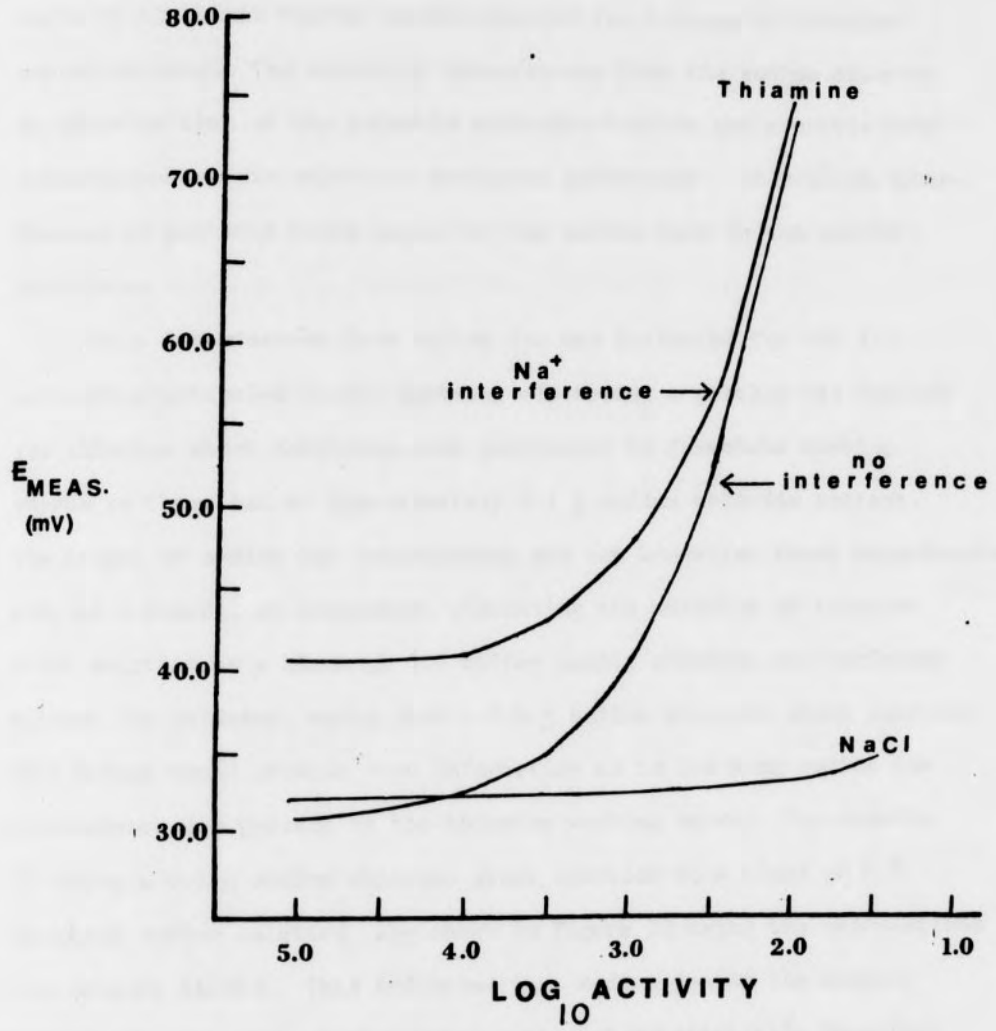


Figure 12. A working curve illustrating the response of the 1:1 chloroform-octanol-1 liquid membrane electrode towards thiamine with and without sodium ion interference and towards sodium ion without thiamine.

curve of the blank buffer sample solution for a range of thiamine concentrations. The amount of interference from the sodium chloride is close to that of the ascorbic acid-nicotinamide and ascorbic acid interference sample solutions evaluated previously. In reality, interference is probably being caused by the sodium ions in the sample solutions.

Since interference from sodium ion was indicated for the 1:1 chloroform-octanol-1 liquid membrane electrode, a problem was implied for thiamine stock solutions used previously to formulate working curves as these had an approximately 0.1 M sodium chloride content. The extent of sodium ion interference was not known for these experiments and, as a result, an experiment simulating the addition of thiamine stock solution to a blank pH 6.0 buffer sample solution was performed without the thiamine, using just a 0.1 M sodium chloride stock solution. This method would provide some information as to how much sodium ion interference was present in the thiamine working curve. The results of adding a 0.1 M sodium chloride stock solution to a blank pH 6.0 phosphate buffer solution are shown in Figure 12 using the calculations from program SALTBI. This indicated that sodium ion in the amounts found in the thiamine stock solutions did not substantially interfere with this liquid membrane electrode response towards thiamine, and proved that the electrode is actually responding towards thiamine for the concentration range studied. It was established that the liquid membrane electrode started to be affected by sodium ions at a high concentration level beginning approximately at 0.4 M. This explained why the measured potential shifted when a 0.0424 M sodium chloride

in the pH 6.0 buffer sample solution was used. The initial sodium ion level from the added interference and from the buffer solution itself exceeded the sodium ion concentration where interfering response by the liquid membrane electrode starts. Before addition of any thiamine stock solution, the sodium ion interfering sample solution already has a sodium ion concentration of 0.05 M.

CHAPTER SEVEN

DISCUSSION AND CONCLUSIONS

The ion-exchanger solution using pure chloroform as the solvent produces a liquid membrane electrode that gives large measured potential drifting even though in combinations with octanol-1 the measured potential was more stabilized for the different thiamine concentrations analyzed. The probable reason for this potential drift is the partial solubility of chloroform in water. It is known that for liquid membrane electrodes slight potential drifting is caused by leakage of the ion-exchanger solution into the sample solution. Usually these ion-exchanger solvents are highly viscous and leaking is therefore slow. But the chloroform has a very low viscosity and this, in conjunction with its solubility in water, makes a poor solvent for the active liquid phase of a liquid membrane electrode. Once the chloroform is combined with the octanol-1 in any of the amounts indicated, potential drifting is substantially decreased. Apparently, the octanol-1, a highly viscous solvent, is able to combine with the chloroform to form a solvent with octanol-1's potential stability and chloroform's high thiamine extraction capability as described by Das Gupta and Cadwallader.[29]

When employing the 1:1 chloroform-octanol-1 liquid membrane electrode, the pH of the sample solutions must be controlled by a buffer solution, a common treatment for clinical analysis. The best pH is 5.5 - 7.5. The graph showing the pH dependence of the electrode

was not symmetrical, probably due to the probable decomposition of thiamine in alkaline solutions. Alkalinity could also explain the irreproducibility found for two consecutive trials. Immersion of the electrode in an alkaline solution could be destructive to the liquid membrane making the electrode response for the second trial different than the first. The 1:1 chloroform-octanol-1 liquid membrane electrode has the capability of being utilized for at least five hours without recalibration. Within this time, the electrode showed a relative precision of about 2.0%. Also, the response time is relatively fast, taking up to one minute for low thiamine concentrations and about thirty seconds for high thiamine concentrations. The quick response indicates that the thiamine-bromothymol blue ion-exchanger site in the active liquid phase has the necessary mobility in the solvent used in this electrode. A feature of the electrode that should be noted is its non-ideal Nernstian response towards thiamine. The thiamine seems to behave neither as a univalent or a divalent species. According to Ishibashi, Kina, and Maekawa,[27] their liquid membrane electrode responded to thiamine as a bivalent species. This does not necessarily prove thiamine to be a bivalent species at the pH range studied. Nevertheless, in practical usage it is more important for the liquid membrane electrode to respond reproducibly and selectively than to respond in a Nernstian manner even though it is advantageous to possess both in an electrode system.

For practical application in determining thiamine concentration in commercially available vitamin capsules, one capsule per 20-25 ml

of pH 6.0 buffer solution would be necessary for analysis by the 1:1 chloroform-octanol-1 liquid membrane electrode. For convenience in these experiments, the amount of interference substances analyzed in the sample solutions in the project have been increased proportionally to equal the amount found in four capsules dissolved in 100 ml of pH 6.0 buffer solution which would contain a thiamine concentraion of approximately 1×10^{-3} M. The sodium ion concentration in the sample solutions must be known if the thiamine is to be analyzed properly due to sodium ion interference above 0.04 M. To assure no substantial interference from sodium ion in the sample solutions, sodium ion should be less than 0.04 M if possible. Potassium ion interference is expected to be similar to sodium ion interference based on the same degree of sodium and potassium interference found in the liquid membrane electrode developed by Ishibashi, Kina, and Maekawa.[27] The selectivity constant for thiamine in the presence of sodium and potassium ions was calculated to be about 1×10^{-1}. The great advantage of this 1:1 chloroform-octanol-1 liquid membrane electrode compared to the one developed by Ishibashi, Kina, and Maekawa is the high selectivity for thiamine over pyridoxine monohydrochloride (vitamin B_6), making it a more practical electrode for realistic clinical analysis. No interference is expected from any substances found in commercial vitamin capsules with the possible exception of chemical binders which were not tested.

For further studies with the 1:1 chloroform-octanol-1 liquid membrane electrode, thiamine mononitrate stock solutions could be used to calibrate the electrode. Three important features of using thiamine mononitrate are

that it would not require as much preparation for weighing because of its low hygroscopicity, the thiamine mononitrate stock solution would not have the initial acidity of its thiamine hydrochloride counterpart, possibly eliminating the neutralization step with sodium hydroxide, and, finally, this species is used more extensively in pharmaceutical preparations than thiamine hydrochloride because of its better time stability so that calibration of the liquid membrane electrode would be attained using the same thiamine form as used in these pharmaceutical preparations. Further analyses of different pharmaceutical preparations containing thiamine may be examined with the corresponding interference study of other substances found in these preparations. Also, in the future, it may be possible to analyze blood samples after proper study of the liquid membrane electrode for this type of analysis.

A new area that requires investigation is the finding of a better solvent for the ion-exchanger solution that will increase electrode response at an even lower thiamine concentration while at the same time maintain response and reproducibility, making it a more practical electrode to use for further clinical analysis.

PART II

AUTOMATION OF THE ION-SELECTIVE ELECTRODE APPARATUS

CHAPTER ONE

INTRODUCTION

In the development and utilization of ion-selective electrodes for analytical studies, it is necessary that a large number of routine samples be analyzed. There are usually two methods used for determining the potential response of ion-selective electrodes for the various concentration ranges of the ion of interest. One is to prepare the solutions of the ion before analysis and the other is to systematically add a stock solution of the ion to an initial volume of solvent to obtain the range of concentrations desired. This latter method is probably the better of the two, since the electrode is continously immersed in the solution for all the concentration ranges and any error due to the removal and cleaning of the electrode as performed in the first method is eliminated. For this reason, the second method has been choosen for our current research in the analysis of ion-selective electrodes. In this procedure, the manual addition of a stock solution into a reaction cell and manual recording of the potential would be tedious and time consuming. To solve this difficulty, a hard-wire automatic pump unit for solution addition has been developed which handles addition of a stock solution of desired volumes either by a manual control or by an automatic control. In conjunction with this unit, a data recording system was developed to store the potential reading of the electrode cell for the different concentrations studied.

Some of the advantages of this particular system are the easy

control of the volumes of solution being delivered to the reaction cell and the reproducibility of these volumes for each different test trial. The system gives accurate data which are stored for later evaluation and, when in automatic control, stops when all desired solution concentrations have been analyzed. This automatic control allows the analyst to perform other duties while the pump is operating in this mode because no supervision is required.

CHAPTER TWO

CIRCUIT DESIGN AND OPERATION

Figure 13 shows the block diagram of the automatic pump system. Each unit's function and implementation are as follows. The pump delivery system was tested with two types of pump: syringe and peristaltic. During a solution addition, the pump delivery system emits a certain volume of stock solution each time a pulse is sent to it from the auto pump unit. The amount of solution displaced into the reaction cell depends on the length of this pulse. The voltmeter is used to measure the potential difference between a reference electrode and the appropriate ion-selective electrode for the different solution concentration ranges being determined. The automatic pump unit consists of two sections, the decision unit and the pulse generator. The decision unit determines the moment to add stock solution, while the pulse generator responds to the information provided by the decision unit by sending pulses of the appropriate length and timing required by the pump delivery system. The timer unit consists of a quartz oscillator and scaler. The rate of the output time pulses are programable by a series of switches which select the scale to be utilized. The amplifier and filter unit is designed to amplify the output signal of the voltmeter and to simultaneously filter out noise that could interfer with the decision section of the auto pump unit. The amplifier and filter unit contains several amplification selections and a separate control for several filter selections. The advantage of this unit is the compactness of its structure and convenience of signal

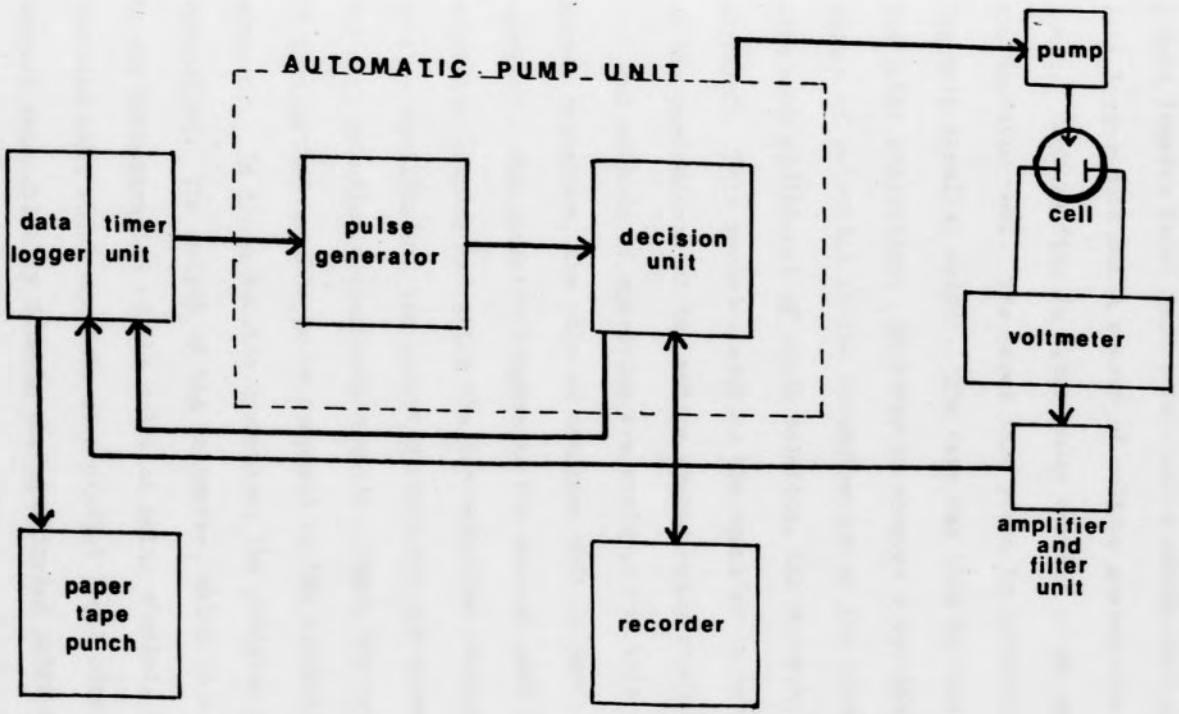


Figure 13. Block Diagram of the Automatic Pump System

regulation in one package. The data recording system incorporates a data logger, paper tape punch, and a strip-chart recorder. The data logger puts out a record of voltage analog data to a paper tape punch. Each reading is taken under control of an external pulse from the decision unit. The paper tape punch is interfaced for the data logger's parallel output. The tape can then be used as data storage for later evaluation. In order to observe a continuous scan of the change of potential as the concentration of the solution is changed with each additional of stock solution, the strip-chart recorder is employed. This recorder enables the operator to have a visual account of the performance of the entire system being studied.

Two methods of operation are employed for this system. In the manual operation, the role of decision unit is performed by a human operator. The operator behaves as the control unit by viewing the voltmeter display and after the ion-selective electrode potential attains equilibrium, the operator activates the pulse generator for the next solution concentration study. Thus, the equilibrium detection by this method is able to be compared to the automatic operation for accuracy. In the automatic operation, the decision unit is hardware-controlled. The output of the voltmeter, which is a voltage related to the concentration of the solution being studied, is sent to the decision unit which consists of a digital logic circuit. This logic circuit automatically detects the equilibrium point of the reaction cell by means of a differentiation circuit and compares the differentiator output to a reference voltage. When equilibrium is attained, the pulse generator is activated for the next solution addition.

A schematic diagram of the digital logic circuit comprising both the pulse generator and decision unit used in the auto pump unit is shown in Figure 14 and Figure 15. Table 4 gives the symbol designations for these figures.

In the pulse generator, each G1 gate acts as a divide-by-two gate for the clock input pulse to each gate. As a result, each gate G1 doubles the length of the pulse of the preceding gate G1, thus, giving the different pulse lengths. The reason for this pulse-doubling operation is related to the Nernst equation where the solution concentration factor is logarithmic. The output of each of these seven G1 gates is placed in a data selector/multiplexer integrated circuit controlled by a decade counting unit. This binary coded count selects which pulse length of a particular G1 gate output is to be sent to the pump delivery system. The combination of the decade counting unit and data selector/multiplexer allows each different length pulse to be associated with a binary count number from the decade counting unit. This decade counting unit has a count range from zero to nine, and every time an output pulse from the multiplexer ends, a monostable multivibrator connected to the clock input of the decade counting unit commands the decade counting unit to increase one count. If one particular pulse length is desired for every solution addition, the count associated with that pulse length can be selected, and a multiplexer pulse control switch can be thrown to "on" position to keep the decade counting unit at that desired count.

Contained within the decision unit is a manual reset push button

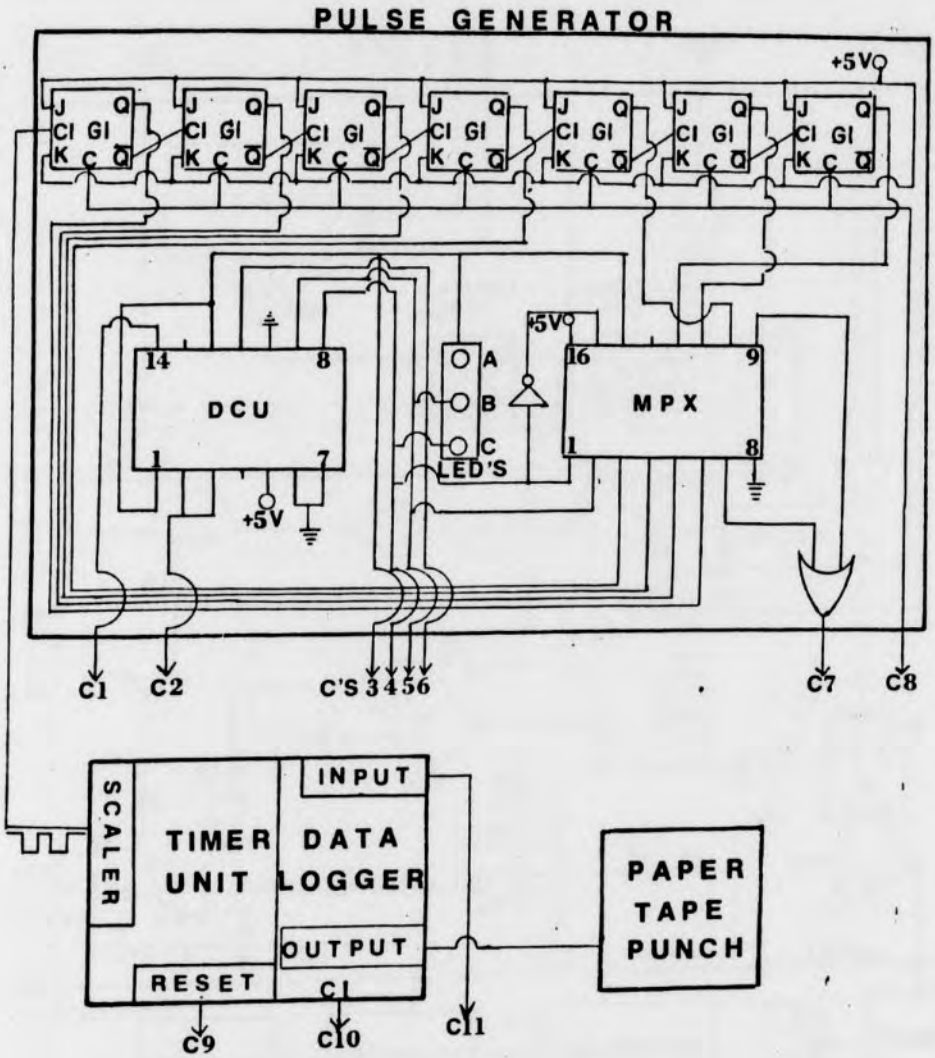


Figure 14. Schematic Diagram of the Circuit Design for the Pulse Generator of the Automatic Pump Unit

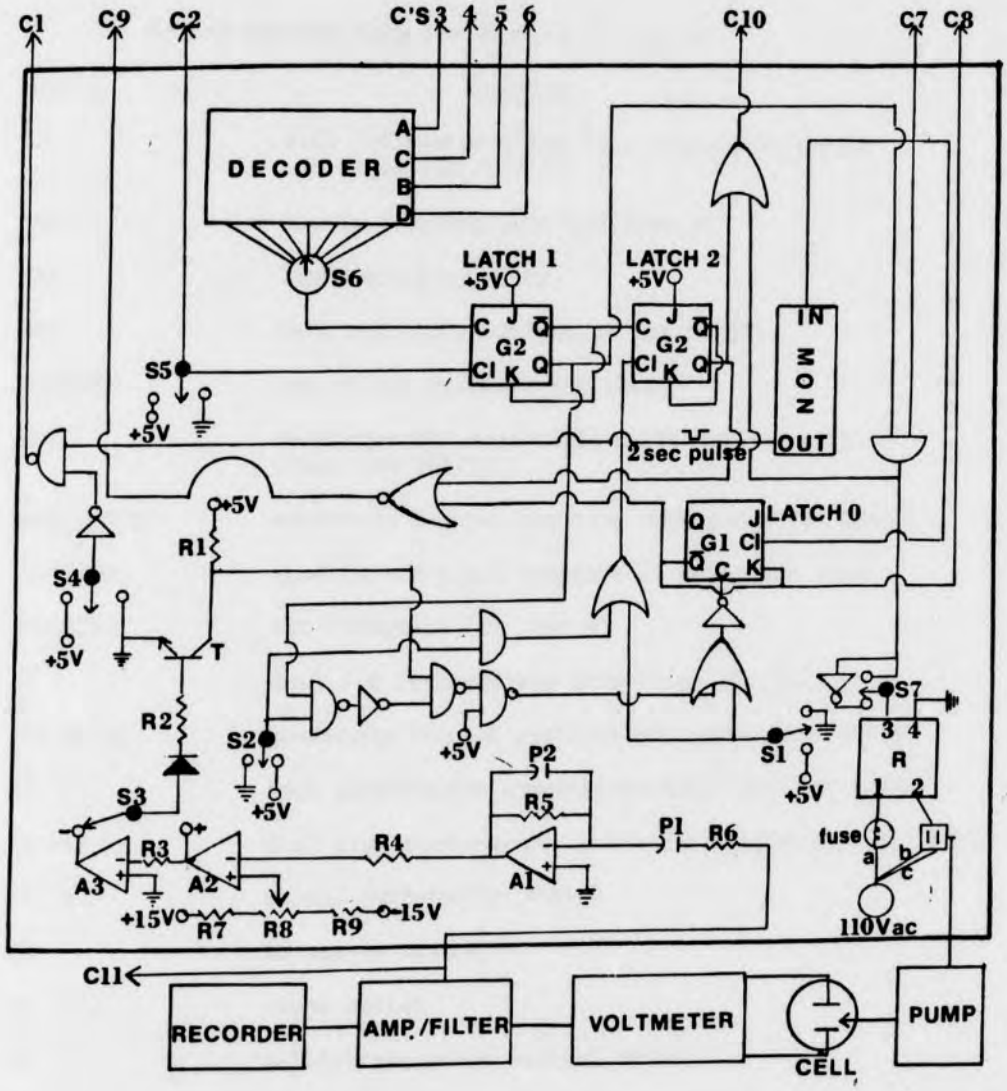


Figure 15. Schematic Diagram of the Circuit Design for the Decision Unit of the Automatic Pump Unit

Table 4

Symbol Designations for Figures 13 and 14

Symbol	Function
G1	dual J-K master-slave flip flops with preset and clear (SN 7476 N)
DCU	decade counting unit (SN 7490 N)
LED	light emitting diode
MPX	data selector/multiplexer (SN 74153)
DECODER	one of ten decoders (SN 7442)
MON	retriggerable monostable multivibrator with clear (SN 74123)
NAND GATES	quadruple 2-input positive NAND gates (M 74H00J)
OR GATES	quadruple 2-input positive OR gates (DM 7432)
INVERTER	hex inverters (SN 7404 N)
G2	dual J-K master-slave flip flops (SN 7473 N)
NOR GATES	quadruple 2-input positive NOR gates (SN 7402 N)
A1	high performance operational amplifier (SN 72307)
A2 and A3	dual high performance operational amplifier (SN 72747)
S1	manual push-button switch
S2	automatic switch
S3	slope switch
S4	multiplexer pulse control switch
S5	manual reset push-button switch
S6	termination selector switch
S7	relay switch
R1	2.2 k ohm resistor
R2	1 k ohm resistor

Table 4 (con't.)

Symbol	Function
R3 and R4	20 k ohm resistor
R5	10 k ohm resistor
R6 and R8	100 k ohm resistor
R7 and R9	500 k ohm resistor
P1	10 μF capacitor
P2	0.1 μF capacitor
T	transistor (T-336-2 T-1811)
R	Teledyne relay (P/N 611-1)
a	hot line
b	ground
c	neutral
C	clear
Cl	clock
J and K	inputs
Q and $\overline{Q}$	outputs
C1 to C11	connection leads

switch used to reset the decade counting unit back to the zero count. This switch not only resets the decade counting unit, but it also prepares the latch 1 circuit used in conjunction with the termination selector for operation. The selector allows the operator to set the count, representing a certain length pulse, that will automatically stop the operation of the auto pump unit after that count pulse is finished. The selector originates from a "one of ten" decoder circuit which transfers the binary code of the decade counting unit, from zero to nine, into ten separate pulses. When the reset switch is engaged, the Q of the latch 1 circuit is one (+5 V) as long as the termination selector is not in the shut-off state after the last count pulse. When this shut-off state does occur, the Q of latch 1 changes to 0 (ground), disabling with an AND gate any further count pulses from the multiplexer output to the pump delivery system gate. This enables the pump to respond to only the desired pulses. The recycling of the pulse count can only be started again when the reset switch is engaged once more.

The data logger takes a voltage reading only if its clock is triggered from 0 to 1. This triggering is accomplished whenever the pump is switched on because it is understood that the pump will only add the stock solution when potential equilibrium in the cell is attained. Unfortunately, this works well for all the pulse counts except when the last pulse count is finished at which time the pump, as explained above, is shut-off permanently due to the latch 1 circuit. To compensate for this circuit limitation, the latch 2 circuit was

devised especially to trigger the data logger clock at the end of the last pulse count. When the $\overline{Q}$ of the latch 1 circuit goes from 0 to 1 with the completion of the last pulse count as decided by the termination selector, the Q of latch 2 remains 0 until the latch 2 clock is triggered from 0 to 1 when the cell potential reaches equilibrium, Q goes to 1 and the last data point is recorded. It should be noted that the latch 2 Q can only be triggered this one time because no matter how many times the latch 2 clock is triggered the Q still remains in the 1 state until the reset switch is engaged and the Q returns to 0.

The starting switch to enable and disable the pulse generator is actually a dual system, with both manual and automatic control. Both are connected to latch 0 where the clear is initially in a 1 state and the clock in a 0 state. When the manual or auto control switch goes from 1 to 0, the clear of latch 0 goes from 1 to 0 and back to 1 again, but the $\overline{Q}$ of latch 0, which is initially at 0, is set to 1. The NOR gate is connected not only to the $\overline{Q}$ of latch 0 but also to the Q of latch 2 which is initially at 0 until the end of the last pulse count when potential equilibrium is reached as described above. When the $\overline{Q}$ of latch 0 goes from 0 to 1. The reset of the timer unit, initially at 1, is grounded and the clock starts. Simultaneously, the clear of the G1 gates in the pulse generator goes to 1 which starts the divide-by-two timers. The $\overline{Q}$ of latch 0 goes back to its original state when the end of the pulse from the multiplexer is finished. It is then necessary to have the automatic or manual switch turn on again in order to have the next pulse start

the pump delivery system. When the last pulse count is finished, the Q of latch 2 (as explained above) goes from 0 to 1 which starts the timer unit by bringing the reset to 0 through the NOR gate but it does not start the pulse generator G1 gates as these depend on the $\bar{Q}$ of latch 0. Because the data logger clock is established around the same timer unit, the Q of latch 2 starts the timer unit only for the purpose of allowing the last potential to be recorded by the data logger, since this timer unit has to be off reset to be in the recording mode.

The manual control is merely a push button switch, which the operator depresses when a pulse is needed to start the pump. The automatic control, however, is basically a differentiator circuit with two comparators and an interfacing transistor switch for conversion of voltage into proper logic voltage. The output of the voltmeter is amplified and filtered and is connected to the differentiator circuit composed of two resistors and two capacitors and a 307 integrated circuit operational amplifier, A1. The gain of this differential circuit is dependent on the 10 μF capacitor and the 10 mΩ resistor. The 0.1 μF capacitor and the 100 kΩ resistor are connected only for additional filter purposes necessary for an operational amplifier differentiator circuit. When the output of the A1 operational amplifier reaches the reference level of comparator A2, a 747 operation amplifier like A3, the comparator output has been adjusted to go to -15 V from +15 V for reasons that will be explained later. In the study of ion-selective electrode systems, it is known that the change of potential increases for cations or decreases for anions as solution concentration

increases. Because of this principle, two operational amplifiers, A2 and A3, are utilized for the comparator switch. A2 is used for an increasing potential signal with an increase of solution concentration and A3, serving as an inverter, is used for a decreasing potential signal with an increase of solution concentration. Either one can be enabled by employing the slope switch indicating + for the A2 and - for the A3. When a certain volume of solution is added to the reaction vessel, the amplified and filtered output voltage of the voltmeter changes to a large positive or negative voltage depending on the direction of the potential change, and the comparator changes to a +15 V since the reference voltage of the comparator has been adjusted to be approximately 0 V. The comparator is usually not set exactly on zero because the noise in the entire system has to be accounted for and, therefore, the comparator has to be adjusted to respond to a voltage just outside that level of noise. When the voltmeter responds to the equilibrium potential of the reaction cell, the change of amplified voltage to the differentiator circuit is zero, making the output of the differentiator circuit likewise zero, which is detected by the comparator with a reference voltage of approximately zero. This zero differentiator circuit output triggers the comparator from +15 V, the state of the comparator at non-equilibrium potential, into a -15 V, the state of the comparator at equilibrium potential. The comparators A2 and A3 are connected to the transistor switch, which allows the comparator signals to be converted into digital logic voltage of ground and +5 V. The diode installed before the transistor allows only the positive voltage through to the transistor switch.

For either A2 or A3, when the output is +15 V, indicating non-equilibrium potential, the +15 V is passed to the base of the transistor, giving it a low resistance which allows the current from the + 5 V power supply to flow through the transistor. Therefore, the output at the collector going into the logic circuit is at ground. This ground signal does not allow the pulse generator to be activated. However, when the comparator output is -15 V, indicating equilibrium potential, the -15 V is not allowed through the diode, making the transistor posses a high resistance. Thus, the output of the collector going into the logic circuit is +5 V, which activates the pulse generator under the following conditons. The logic circuit of the automatic control is constructed so that the +5 V transistor trigger pulse cannot activate the pulse generator unless the automatic switch is thrown on and unless the Q of latch 1 is 1, which is its state before the end of the desired last pulse count. When this last pulse finishes, the transistors trigger pulse is unable to pass into latch 0, which sets off the pulse generator, because the Q of latch 1 is switched to 0. However, the transistor trigger pulse can clock into the latch 2 circuit which triggers the data logger to record the last amplified potential reading of the voltmeter when potential equilibrium is reached. The advantage of the latch 2 circuit can be seen in that no matter how frequently the transistor trigger pulse attempts to reclock the latch after the last recording, its Q never changes state until the reset button is engaged bringing the entire system back to its initial state.

All the pulses that lead out to the pump delivery system pass through a relay switch which allows the operator to switch the pump manually to the opposite voltage signal being given off by the pulse

generator, or it allows the pump to receive the voltage signal exactly as it comes out of the pulse generator. A relay unit is connected before the pump to convert the +5 V output pulse directed to the pump from the auto pump system into a 110 v AC power source to start the pump which operates only on this AC voltage.

CHAPTER THREE

RESULTS AND DISCUSSION

As mentioned previously, two types of pump delivery systems were employed: one a syringe pump and the other a peristaltic pump. Both of these were calibrated by using the manual control mode of the automatic pump unit. This calibration determined that the syringe pump used in this particular test is capable of 0.6% precision while the peristaltic pump used in the test is capable of 1.0% precision. The advantage of the syringe pump is its greater precision and its ability to deliver the sample volumes of stock solution in a shorter time, which makes the differential circuit in the decision unit of the automatic pump system more sensitive due to the large change of potential being detected. The disadvantage is the necessity of refilling the syringe manually for each set of different volumes and the problem of mounting the syringe pump properly. On the other hand, the advantage of the peristaltic pump is that there is one stock solution reservoir which supplies the pump continously with stock solution without the need for frequent filling, and there is the convenience of having the pump ready instantaneously for actual testing purposes. However, the disadvantage is that even at its maximum speed, the pump still delivers solution more slowly than the syringe pump, making the change of potential detected by the differentiator circuit smaller and, thus, reducing the sensitivity of the automatic pump unit.

A typical strip-chart recorder plot of an actual test with the syringe pump delivery system is shown in Figure 16. This plot displays the potential scan of the reaction cell containing a fluoride ion-selective electrode and an appropriate reference electrode for five different fluoride ion concentrations at a temperature of 25°C. Table 5 lists the concentrations of fluoride ion being analyzed and the corresponding potential for each plateau indicated on the plot in Figure 15. The potential is the recorded voltage output from the voltmeter amplified through the amplifier-filter unit.

Table 5

Analysis of Strip-Chart Recorder Plot of Potential versus Fluoride Ion Concentration

Plateau	F^- Conc. (M)	Potential (volts)
1	9.80×10^{-4}	+0.532
2	2.63×10^{-3}	+0.048
3	5.61×10^{-3}	-0.315
4	1.12×10^{-2}	-0.641
5	2.16×10^{-2}	-0.943

It must be stated that these concentrations are not the limit that can be evaluated by this automatic pump system; they are merely used to illustrate the capability of this system. When the ion-selective electrode responds completely to the concentration of the solution in the reaction cell, a plateau is recorded on the plot indicating the equilibrium response of the electrode. As demonstrated in the plot, the equilibrium plateau is followed by another stock solution addition, indicated by a change

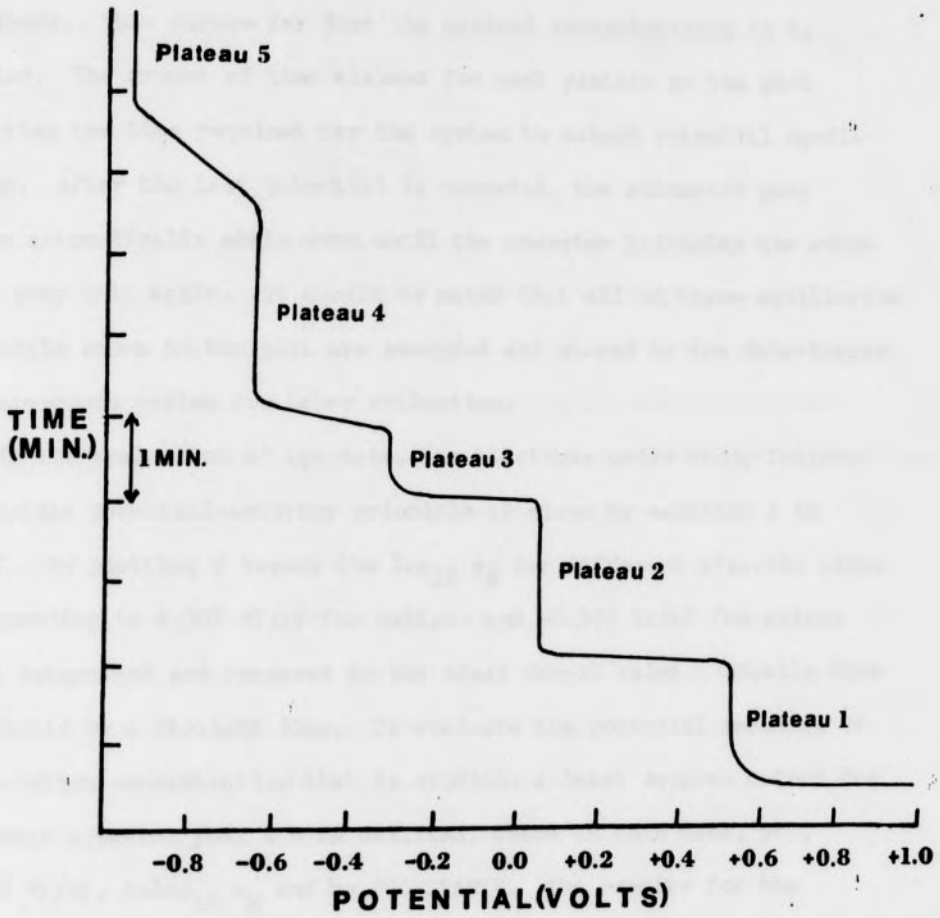


Figure 16. Illustration of a strip-chart recorder scan of fluoride electrode response towards five different fluoride concentrations using the automatic pump unit working with a syringe pump delivery system.

in slope, until an equilibrium response is once again detected by the electrode. This occurs for just the desired concentrations to be studied. The amount of time elapsed for each plateau on the plot indicates the time required for the system to detect potential equilibrium. After the last potential is recorded, the automatic pump system automatically shuts down until the operator initiates the automatic pump unit again. It should be noted that all of these equilibrium potentials shown in the plot are recorded and stored by the data-logger and tape-punch system for later evaluation.

In the evaluation of the data, the electrode under study follows a Nernstian potential-activity principle as given by equation 1 in Part I. By plotting E versus the $\log_{10} a_M$ for different a's, the slope corresponding to 2.303 RT/nF for cations and -2.303 RT/nF for anions can be determined and compared to the ideal Nernst value. Ideally this plot should be a straight line. To evaluate the potential readings of each solution concentration that is studied, a least squares method for the linear equation $y=mx + b$ is utilized, where in this case, $y=E$, $m=2.303$ RT/nF, $x=\log_{10} a_M$ and b= "CONSTANT". The results for the fluoride electrode using both the syringe and peristaltic pump showed good reproducibility and the individual data points correlated well with the slope of the E versus $\log_{10} a_M$ plot. Also, when the results of the automatic control mode were compared to those of the manual control with the peristaltic pump delivery system, which has a higher relative standard deviation of 1.0% compared to the syringe pump with only a relative standard deviation of 0.6%, very good agreement was obtained.

In general, the entire automatic pump system has proven to be efficient and reliable for the study of electrode response in solution analysis.

Summary

In conclusion, the thiamine liquid membrane electrode employing the thiamine-bromothymol blue salt in the 1:1 chloroform-octanol-1 ion-exchanger solution is suitable and convenient for limited clinical use of thiamine determination in certain commercially available vitamin preparations with no interference problems except for high concentrations of sodium ion (above 0.04 M). This electrode is pH dependent with fair response starting at 1×10^{-3} M thiamine concentration in a pH 6.0 buffer sample solution and has a high degree of reproducibility.

An automatic system for use with ion-selective electrode analysis has been successfully developed for controlling the addition of known incremental volumes of solutions and for recording the potential of the ion-selective electrode cell after solution addition.

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APPENDIX A

Fortran IV Program SALTBI

```
C PROGRAM SALTBI
      DIMENSION ALML(50),P(50),SALTC(50),I(50),ACTCOF(50),ACTIV(50),LAC
     +TIV(50)
      DIMENSION DATA(9)
      DIMENSION CN(50),BUFFCP(50),BUFFCR(50),I1(50),I2(50),I3(50)
      REAL I,I1,I2,I3,LACTIV,INVOL,INT
      INPUT=5
      JOUT=6
      JINPUT=7
      READ(INPUT,5)Z,F,F1,F2,F3
    5 FORMAT(5E13.4)
      READ(INPUT,5)CS,BCP,BCR,INVOL,STKCON
    3 WRITE(JOUT,6)
    6 FORMAT(/' ENTER # DATA VALUES'//)
      CALL FREE(DATA,1)
      N=DATA(1)
      WRITE(JOUT,8)Z,F,F1,F2,F3
    8 FORMAT(/' Z,F,F1,F2 & F3 ARE:'/5E13.4)
      WRITE(JOUT,9)CS,BCP,BCR,INVOL,STKCON
    9 FORMAT(/' STK SALT CONC,INVOL BUFF PROD,INVOL BUFF REAGT,INVOL &
     +STKCONC ARE:'/5E13.4)
      WRITE(JOUT,10)N
   10 FORMAT(' N IS:'/I5)
      WRITE(JOUT,12)
```

```
12 FORMAT(/8X,'TOTML',13X,'P'//)
   DO 13 K=1,N
   READ(INPUT,15)ALML(K),P(K)
15 FORMAT(2E15.4)
13 WRITE(JOUT,15)ALML(K),P(K)
   WRITE(JOUT,25)
25 FORMAT(/7X,'SALTC',16X,'I',18X,'ACTCOF')
   WRITE(JOUT,26)
26 FORMAT(7X,'ACTIV',13X,'LACTIV',21X,'P'//)
   DO 20 J=1,N
   SALTC(J)=ALML(J)*STKCON/(INVOL+ALML(J))
   CN(J)=(ALML(J)*CS)/(INVOL+ALML(J))
   BUFFCP(J)=(INVOL*BCP)/(ALML(J)+INVOL)
   BUFFCR(J)=(INVOL*BCR)/(ALML(J)+INVOL)
   I1(J)=F1*CN(J)
   I2(J)=F2*BUFFCP(J)
   I3(J)=F3*BUFFCR(J)
   I(J)=F*SALTC(J)+I1(J)+I2(J)+I3(J)
   ACTCOF(J)=10.00**(-.5115*Z**2*(SQRT(I(J))/(1.0+SQRT(I(J)))-.3*I(J
  +)))
   ACTIV(J)=SALTC(J)*ACTCOF(J)
   LACTIV(J)=ALOG10(ACTIV(J))
   WRITE(JOUT,35)SALTC(J),I(J),ACTCOF(J)
35 FORMAT(E15.4,E18.4,E25.4)
20 WRITE(JOUT,36)ACTIV(J),LACTIV(J),P(J)
36 FORMAT(E15.4,E18.4,E25.4//)
   CALL LINFIT(LACTIV,P,N,INT,SINT,SLOPE,SSLOPE,R)
   WRITE(JOUT,904)
904 FORMAT(//2X,'SLOPE',5X,'STD SLOPE',5X,'INT',5X,'STD INT',5X,'CORR.
  +COEFF.(R)'//)
   WRITE(JOUT,905)SLOPE,SSLOPE,INT,SINT,R
905 FORMAT(F8.2,F11.2,F13.2,F7.2,F15.7)
   WRITE(JOUT,50)
 50 FORMAT(' DONE? YES(1),NO(0)'/)
   CALL FREE(DATA,1)
   L=DATA(1)
```

```
      IF(L.EQ.1)GO TO 70
      GO TO 3
   70 CONTINUE
      STOP
      END
"
```

APPENDIX B

Fortran IV Program SALTIV

```
C PROGRAM SALTIV
      DIMENSION ALML(50),P(50),SALTC(50),I(50),ACTCOF(50),ACTIV(50),LAC
     +TIV(50)
      DIMENSION DATA(9)
      DIMENSION CN(50),BUFFCP(50),BUFFCR(50),I1(50),I2(50),I3(50)
      DIMENSION CS4(50),CS5(50),CS6(50),CS7(50),CS8(50)
      DIMENSION I4(50),I5(50),I6(50),I7(50),I8(50)
      REAL I,I1,I2,I3,LAGTIV,INVOL,INT
      REAL I4,I5,I6,I7,I8
      INPUT=5
      JOUT=6
      JINPUT=7
      READ(INPUT,5)Z,F,F1,F2,F3
    5 FORMAT(5E13.4)
      READ(INPUT,5)F4,F5,F6,F7,F8
      READ(INPUT,5)CS,BCP,BCR,INVOL,STKCON
      READ(INPUT,5)S4,S5,S6,S7,S8
    3 WRITE(JOUT,6)
    6 FORMAT(/' ENTER # DATA VALUES'//)
      CALL FREE(DATA,1)
      N=DATA(1)
      WRITE(JOUT,8)Z,F,F1,F2,F3
    8 FORMAT(/' Z,F,F1,F2 & F3 ARE:'/5E13.4)
      WRITE(JOUT,7)F4,F5,F6,F7,F8
```

```
 7 FORMAT(/' F4,F5,F6,F7 & F8 ARE:'/5E13.4)
   WRITE(JOUT,9)CS,BCP,BCR,INVOL,STKCON
 9 FORMAT(/' STK SALT CONC,INVOL BUFF PROD,INVOL BUFF REAGT,INVOL &
  +STKCONC ARE:'/5E13.4)
   WRITE(JOUT,11)S4,S5,S6,S7,S8
11 FORMAT(/' S4,S5,S6,S7 & S8 ARE:'/5E13.4)
   WRITE(JOUT,10)N
10 FORMAT(' N IS:'/I5)
   WRITE(JOUT,12)
12 FORMAT(/8X,'TOTML',13X,'P'//)
   DO 13 K=1,N
   READ(INPUT,15)ALML(K),P(K)
15 FORMAT(2E15.4)
13 WRITE(JOUT,15)ALML(K),P(K)
   WRITE(JOUT,25)
25 FORMAT(/7X,'SALTC',16X,'I',18X,'ACTCOF')
   WRITE(JOUT,26)
26 FORMAT(7X,'ACTIV',13X,'LACTIV',21X,'P'//)
   DO 20 J=1,N
   SALTC(J)=ALML(J)*STKCON/(INVOL+ALML(J))
   CN(J)=(ALML(J)*CS)/(INVOL+ALML(J))
   BUFFCP(J)=(INVOL*BCP)/(ALML(J)+INVOL)
   BUFFCR(J)=(INVOL*BCR)/(ALML(J)+INVOL)
   CS4(J)=(INVOL*S4)/(ALML(J)+INVOL)
   CS5(J)=(INVOL*S5)/(ALML(J)+INVOL)
   CS6(J)=(INVOL*S6)/(ALML(J)+INVOL)
   CS7(J)=(INVOL*S7)/(ALML(J)+INVOL)
   CS8(J)=(INVOL*S8)/(ALML(J)+INVOL)
   I1(J)=F1*CN(J)
   I2(J)=F2*BUFFCP(J)
   I3(J)=F3*BUFFCR(J)
   I4(J)=F4*CS4(J)
   I5(J)=F5*CS5(J)
   I6(J)=F6*CS6(J)
   I7(J)=F7*CS7(J)
   I8(J)=F8*CS8(J)
```

```
      I(J)=F*SALTC(J)+I1(J)+I2(J)+I3(J)+I4(J)+I5(J)+I6(J)+I7(J)+I8(J)
      ACTCOF(J)=10.00**(-.5115*Z**2*(SQRT(I(J))/(1.0+SQRT(I(J)))-.3*I(J
     +)))
      ACTIV(J)=SALTC(J)*ACTCOF(J)
      LACTIV(J)=ALOG10(ACTIV(J))
      WRITE(JOUT,35)SALTC(J),I(J),ACTCOF(J)
   35 FORMAT(E15.4,E18.4,E25.4)
   20 WRITE(JOUT,36)ACTIV(J),LACTIV(J),P(J)
   36 FORMAT(E15.4,E18.4,E25.4//)
      CALL LINFIT(LACTIV,P,N,INT,SINT,SLOPE,SSLOPE,R)
      WRITE(JOUT,904)
  904 FORMAT(//2X,'SLOPE',5X,'STD SLOPE',5X,'INT',5X,'STD INT',5X,'CORR.
     +COEFF.(R)'//)
      WRITE(JOUT,905)SLOPE,SSLOPE,INT,SINT,R
  905 FORMAT(F8.2,F11.2,F13.2,F7.2,F15.7)
      WRITE(JOUT,50)
   50 FORMAT(' DONE? YES(1),NO(0)'/)
      CALL FREE(DATA,1)
      L=DATA(1)
      IF(L.EQ.1)GO TO 70
      GO TO 3
   70 CONTINUE
      STOP
      END
**
```

APPENDIX C

Fortran IV Subroutine Program FREE

```
      SUBROUTINE FREE(X,NV)
      INTEGER SI
      DIMENSION X(NV),NA(80),PT(10)
      SI=7
      IZ=80
      NCP=1
      NVR=1
      PT(1)=1
      DO 10 NC=2,10
   10 PT(NC)=PT(NC-1)*10
      NOPX=1
      WRITE(SI,403)
   20 READ(SI,32) NA
      DO 11 ICNT=1,80
   11 IF(NA(ICNT).LT.0)NA(ICNT)=1+NA(ICNT)+32767
      NANC=NA(1)
      IF(NANC-17184) 40,30,40
   30 WRITE(6,32) NA(2),NA
   32 FORMAT(81A1)
      GO TO 20
   40 DO 68 NC=1,IZ
      NANC=NA(NC)
      IF (NANC- 8224) 50,68,50
   50 IF(NANC-14624) 51,51,56
```

```
 51   IF(NANC-12320) 52,68,68
 52   IF(NANC-11552) 53,68,53
 53   IF(NANC-11808) 54,68,54
 54   IF(NANC-11040) 55,68,55
 55   IF(NANC- 9760) 56,60,56
 56   IF(NANC-17696) 61,57,61
 57   IF(NA(NC+1)-11040) 58,68,58
 58   IF(NA(NC+1)- 9760) 59,68,59
 59   IF(NA(NC+1)-11552) 61,68,61
 60   NA(NC)=11040
      GO TO 68
 61   NA(NC)=8224
 68   CONTINUE
 70   NC=0
 80   NC=NC+NCP
      IF(NC-IZ) 90,90,20
 90   NANC=NA(NC)
      IF(NANC- 8224) 100,80,100
100   ND=0
      NDD=1
      NDDF=0
      NVAL=0
      NVAL2=0
      NC2=1
      NOPXL=NOPX
      NOPX=1
      NSG=+1
      IF(NANC-11552) 130,120,130
120   NSG=-NSG
      GO TO 200
130   IF(NANC-11040) 190,200,190
190   IF(NANC- 9760) 220,200,220
200   NC=NC+NCP
      IF(NC-IZ) 202,202,340
202   NANC=NA(NC)
220   IF (NANC-11808) 240,230,240
```

```
 230  NDDF=1
      GO TO 200
 240  IF(NANC-17696) 260,320,260
 260  IF(NANC- 8224) 270,340,270
 270  I=(NANC-12320)/256
      IF (I) 399,271,271
271   IF (10-I) 399,272,272
272   ND=ND+1
      NDD=NDD+NDDF
      IF(ND-5) 275,285,285
 275  NVAL=NVAL*10+I
      GO TO 200
 285  NVAL2=NVAL2*10+I
      NC2=NC2+1
      GO TO 200
 320  NOPX=2
 340  X(NVR)=((NVAL*PT(NC2)+NVAL2)/PT(NDD))*NSG
      GO TO (360,350),NOPXL
 350  X(NVR-1)=X(NVR-1)*10.0**X(NVR)
      NVR=NVR-1
 360  GO TO(410,480),NOPX
 410  IF(NDDF) 440,430,440
 430  IF(NVAL-9999) 440,450,440
 440  IF(NVR-NV) 480,460,460
 450  X(NVR)=0
      NV=NVR-1
 460  RETURN
 480  NVR=NVR+1
      GOTO 80
 399  WRITE(6,401) NA
 401  FORMAT(/' ***INVALID NUMBER(S) ON CARD BELOW***'/1X,80A1)
      WRITE(6,402)
 402  FORMAT(/' $$$PROGRAM STOPPED$$$')
 403  FORMAT(/)
      STOP
      END
**
```

APPENDIX D

Fortran IV Subroutine Program LINFIT

```
      SUBROUTINE LINFIT(X,Y,NPTS,B,SIGMAB,A,SIGMAA,R)
C     LEAST SQUARE FIT TO Y = A*X + B                                    1
C     PROGRAM WAS ADAPTED FORM ONE IN TEXT BY BEVINGTON                  2
      DIMENSION X(NPTS),Y(NPTS)                                          3
      SUM=NPTS
      SUMX=0.                                                            5
      SUMY=0.                                                            6
      SUMX2=0.                                                           7
      SUMXY=0.                                                           8
      SUMY2=0.                                                           9
      DO 50 I=1,NPTS                                                    10
      XI=X(I)                                                           11
      YI=Y(I)                                                           12
      SUMX=SUMX+XI                                                      13
      SUMY=SUMY+YI                                                      14
      SUMX2=SUMX2+XI*XI                                                 15
      SUMXY=SUMXY+XI*YI                                                 16
      SUMY2=SUMY2+YI*YI                                                 17
50    CONTINUE                                                          18
      DELTA=SUM*SUMX2-SUMX*SUMX                                         19
      B=(SUMX2*SUMY-SUMX*SUMXY)/DELTA                                   20
      A=(SUMXY*SUM-SUMX*SUMY)/DELTA                                     21
      C=NPTS-2                                                          22
      VARNCE=(SUMY2+B*B*SUM+A*A*SUMX2-2.*(B*SUMY+A*SUMXY-B*A*SUMX))/C   23
                                                                        24
```

```
      SIGMAB=SQRT(VARNCE*SUMX2/DELTA)
      SIGMAA=SQRT(VARNCE*SUM/DELTA)                                          25
      R=(SUM*SUMXY-SUMX*SUMY)/SQRT(DELTA*(SUM*SUMY2-SUMY*SUMY))              26
      RETURN                                                                 27
      END
"
```