ION SELECTIVE POLYMERIC MEMBRANES AS CHEMICALLY SELECTIVE

COULOMETRIC ELECTRODES

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ION SELECTIVE POLYMERIC MEMBRANES AS CHEMICALLY SELECTIVE COULOMETRIC ELECTRODES

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Vishnupriya Bhakthavatsalam

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Vishnupriya Bhakthavatsalam, daughter of Late. D. Bhakthavatsalam and B. Sugumari, was born on May 13, 1976 in Vellore, TN, India. After finishing her Bachelors in Science in Chemistry at Madras university, India, in May 1993, she entered the Anna university, India to obtain Masters in Science in Applied Chemistry in May, 1995 And graduated with the degree of Masters in Technology in Polymer science and Engineering from the same university in May, 1997. She worked as an associate scientist in Centre for Energy and Electrochemical research of SPIC India Ltd. before pursuing Ph.D. program in the Department of Chemistry and Biochemistry, Auburn University in Fall 2001.

DISSERTATION ABSTRACT

ION SELECTIVE POLYMERIC MEMBRANES AS

CHEMICALLY SELECTIVE COULOMETRIC ELECTRODES

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Potentiometric and optical ion sensors use highly selective lipophilic ionophores as a special additive in a plasticized PVC membrane have been thoroughly studied for application as a coulometric electrode. This dissertation presents theory, experimental evidence and analysis of membranes optimized to suit the needs of a coulometric electrode. These electrodes function on the basis of ion transport from the viscous polymeric phase into the sample by means of current control. Selective coulometric release of non-electroactive ionic reagent is carried out using an ion-selective polymeric membrane. This is an elegant method for establishing calibration-free titration techniques. The ion selective membrane has been applied as a coulometric electrode for several well-known and useful complexometric and precipitation titrations. A coulometric technique involving constant current pulses and ramping current pulses were

utilized with either potentiometric or pulse galvanostatic detection in conjunction. A small volume of 1mL was used as a sample. The inner membrane phase boundary is polarizable unlike the metal-based coulometric electrodes. Experiments were carried out with PVC membranes containing a lipophilic salt in a high concentration of 10wt%. Chronopotentiometry has been utilized to characterize the ion selective membranes for their polarizability under constant current coulometry. Anodic electrolysis current across the membrane results in coulometric release of cations. The high selectivity as a result of lipophilic ionophore in the membrane results in good coulometric efficiency, even in the presence of interfering ions in the background.

This process completely obeys Faraday's law until before the region of concentration polarization. This polarization behaviour depends on the nature and the activity of sample anions and cations and on the magnitude of current applied with the duration of applications taken into consideration. Knowledge of this polarization behaviour is essential for design and applications of coulometric electrodes. A simple model is put forward based on the chronopotentiometric experiments. This coulometric technique is utilized to release calcium from the calcium selective membrane electrode to titrate EDTA in the complexometric direct and back titrations. This is especially useful in the estimation of nickel concentration.

The coulometric protamine selective membrane has been utilized to release protamine and titrated against anticoagulant heparin in a clinically relevant concentration range as an example of coulometric non-electroactive polyion release The pulse galvanostatic PVC/DOS based membranes were also developed for whole blood protamine sensing in view of this matrix being better for *in vivo* applications.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

Titration is a reliable and fairly simple method in analytical chemistry used widely in laboratories and in industrial processes. This analytical method is quantitative because of precision and accuracy. The four different classes of titrations are precipitation; complex formation, acid-base and redox titrations are still useful to this day. Even though, this is a preferred method, batch-wise titrations are time consuming and labor-intensive, they also exhibit several disadvantages such as the tedious processes of collecting and plotting the data for the titration curve as well as the standardization. Also, the time of analysis in titrimetry is comparatively long due to the large volume of analyte. In order to eliminate these problems, researchers were interested in the automation of the titration procedure.

The automation of batch titration systems was either partial[1, 2] or total. Even though, these automated volumetric titrators gave accuracy and precision, they were not suitable for in situ measurements of titrant additions. Since these systems required expensive instrumentation, continuous flow systems were suggested as a solution.[3,4] This lead to the development of the continuous titrator where a standard titrant is not required. Examples of several studies involving the development of flow and gradient titration techniques are found in the literature.[5]

Most of the common types of commercial automatic titrators are built to work on individual samples one at a time, and relatively complicated supplementary units are needed to promote the processes such as sample introduction, draining, and washing of the titration vessel. All of these techniques required a calibration step, which also prolonged the procedures. These titrations were also unable to operate at the micro scale.

This chapter first discusses attempts made by researchers to decrease the analyte volume during conventional titrations as compared to coulometric titrations. Special attention will be given to the comparisons with respect to small analyte volume, titrant consumption and faster analysis time. Later, the focus is on the basis and advantages of a chemically selective polymeric membrane being used as the coulometric electrode. It will further establish the superior nature of ion selective membrane based coulometry with wide choice of titrants that could be electro released.

1.1 Conventional burette titration-challenges involved in shrinking the sample size

Research in the direction of reducing the sample volumes and alternate ways of delivering a reagent has long been a topic of interest. J. A. Ramsay and R. H. J. Brown used two different approaches to overcome the sample size problem.[6]

In the conventional burette titration method, silver nitrate was added from a special burette with volumes as small as 0.2 μL. This procedure was proven to be useful in the potentiometric measurement of 1 μg of chloride. This method did not become popular for various reasons; such as less distinct end-point as the dilution is increased, and increases in volumetric error as the volumes of sample and titrant are reduced. Additionally, 5 minutes are still required to complete a titration.

However, with their unique second method, they were able to titrate a tiny volume of 0.5 x 10^{-3} μl by adding Ag^{+} ion coulometrically upon passing a current through a silver electrode in series with a condenser. The charge developed on the condenser gave a measure of the amount of chloride titrated. This method can measure 10^{-4} μg of chloride with an error of $< \pm 1$ %. The amount of the titrant needed to reach the endpoint gives an indirect measure of volume. The volume errors were minimized.

One way of removing the effect of dilution is to use titrant in small nL droplets with higher concentration. An automatic micro droplet titrator, that can be used in micro scale titrations with high degree of accuracy and precision was reported by A.W. Steele and *G.* M. Hieftje.[7] However, this method is not attractive since, calibrations are needed every hour and titrant is not determined directly. Also, the calibration of the instrument changes as a result of drift in the driving electronics, and the calibration is time consuming. In another approach[8] to miniaturize titration, diffusional micro titration of a small volume of sample was carried out with the reagents diffusing through an agar gel membrane with the end point visually detected.

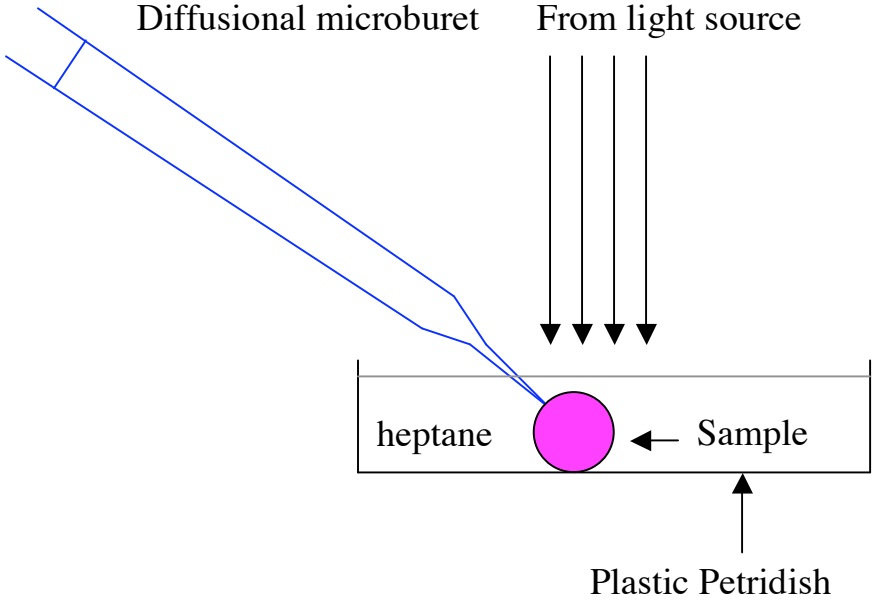


Figure 1.1 Diffusional micro titration of small volume of sample was carried out by reagents diffusing through the agar gel membrane. The reagent solution is usually backfilled.

Figure 1.1. depicts a diffusional burette with an agar gel membrane with a reagent backfilled. And this is used to deliver the titrant through its tip into the sample droplet. This method is useful for studying physiological processes in a single cell. This system works beautifully for acid-base titrations where the color change of the indicator dye is sharp, unlike the complexometric titrations with $CaCl_2$. The diffusional titration curve has scattered points and the first break point was blurred. Data was always smoothed. Though this method is elegant in handling small sample volume, the main disadvantage is that, the calibration was still required and the calibration graph still had a full–scale relative error of 6-8%.

Reduction in volume of the titrant using fused silica capillaries and mechanically driven syringes has been reported.[9] The time needed for a measurement is still about 5 minutes. Because of the several advantageous properties of fused-silica capillary tubes used for gas chromatography they were used for constructing micro burettes, micropipettes and micro-flow cells.[10] A small sample solution of less than 120 μl in a micro beaker (500 μl) was titrated against EDTA solution using a micro burette. The end point is read out spectrophotometrically from a change in color of the indicator as the solution was circulated through the micro-flow cell of a detector.

Otto S. Wolfbeis reported the development of an innovative opto-electronic titration unit.[11] This was used for observing acid-base titrations through an optical light-guide that was an economical alternative to other electrode-based titrators.

The titrator included, as a light source a blue light-emitting diode (LED), as well as an inexpensive photodiode as a detector that measured changes in the fluorescence. Several types of acids and bases have been titrated with good precision using the above technique..The main advantages include simplicity and wide applicability as demonstrated by the titration of aggressive acids, such as hydrofluoric acid, as well as the possibility of automation, and small sample volumes of about 0.5 ml. The average error reported for titration of hydrogen phosphate with hydrochloric acid was still high at ± 5%

1.2 Coulometry

Coulometry is a quantitative analytical method for measuring an unknown concentration of analyte in a small sample solution. Complete determination of the analyte is carried out using an electro-generated titrant released as a result of transformation from one oxidation state to another. Coulometry is a perfect measurement similar to gravimetry or titration, needs no chemical standards and is free of dilution effects. It is therefore beneficial for making absolute concentration estimations of standards. The analyte concentration in the sample is calculated from the reaction stoichiometry and the amount of charge needed to release enough reagent to react with all of the analyte.

Table. 1.1. Advantages of coulometric titrations over volumetric titrations

Requirements	Coulometry	Volumetry
Preparation and standardization of solutions	Not necessary	Required
Storage of the titrant	Not necessary	Required
Addition and determination of Micro quantities of analyte	Possible	Difficult
Determination of micro volumes	Yes	Difficult
Accuracy of titration	High	o.k.
Economy of the reagent	Maximum	Depends
Automation	Ideal	Possible

Coulometry uses a constant current source to deliver a predetermined amount of charge. One mole of electrons is equal to 96,485 coulombs of charge, and is called a Faraday. The main advantage of coulometric titrations is that they are calibration-free. So the process is easily automated. The elegance of using coulometry caught on with researchers. Schreiber et al., in the mid fifties tried the alternative of microburettes namely coulometric titration in microvolumes of acid –base and redox titrations.[12]

The volume used was typically 10uL and the concentration range was from 0.01 M to 0.0002 M. The method involved no significant error. Nagy et al. demonstrated that triangular-programmed coulometric titrations have several advantages over conventional titrations.[13-16] Comparison between coulometric and volumetric titrations is briefly discussed here in the following table 1.1. The flow titration combined with an electro-generated concentration gradient brings about sample automation, low noise and easy coupling of on-line sampling devices to monitor chemical and biotechnical processes.

Spohn developed micro-flow titration to quantify creatinine and ammonia in urine samples.[17] This method is precise, sensitive and can be made selective by the separation of the released ammonia into an acid acceptor stream using a gas diffusional membrane. Following this, coulometric titration of ammonia with hypobromite takes place. Hypobromite is formed after the electrogeneration of bromine in an electrolyte by a triangular-programmed current-time course while an amperometric flow detector is used in conjunction.

This advantageous method eliminates the calibration step. The microflow titration technique was exceptionally useful in the estimation of small quantities of extremely toxic substances such as nitrite, a common and toxic pollutant found in many natural and artificial water reservoirs[18] and highly toxic hydrazine in the sample [19]

A generator–collector mode titration using microband electrodes is a fast and straightforward analytical tool that was applied to many common titrations.[20] Actual process involves electro generation of the titrant using a constant current at the generator electrode. Detection of the end-point was carried out either amperometrically or potentiometrically. Current or potential due to the presence of the titrant at the collector electrode gives the concentration released. The fluxes of all reactive species are well balanced to indicate that the process does not require a separate titrant or absolute volume monitoring.

Miniaturization of the coulometric titrators further reduced the analysis time to seconds and increased the precision and sensitivity.[21,22] This coulometric nanotitrator (see Fig 1.2) is found to be useful for all four common types of nanotitrations: precipitation, complex-formation, redox and acid-base. This simple microfluidic device, composed of planar metal electrodes in a microchannel, yields very reproducible results with a linear dependence between the analyte concentration and the equivalence time for concentrations up to 10^{-2} M of analyte.

Recently, coulometry gained much attention with the introduction of a commercial product for fast determination of acidity/alkalinity of all kinds of samples in fewer seconds in a non-destructive manner.[23]

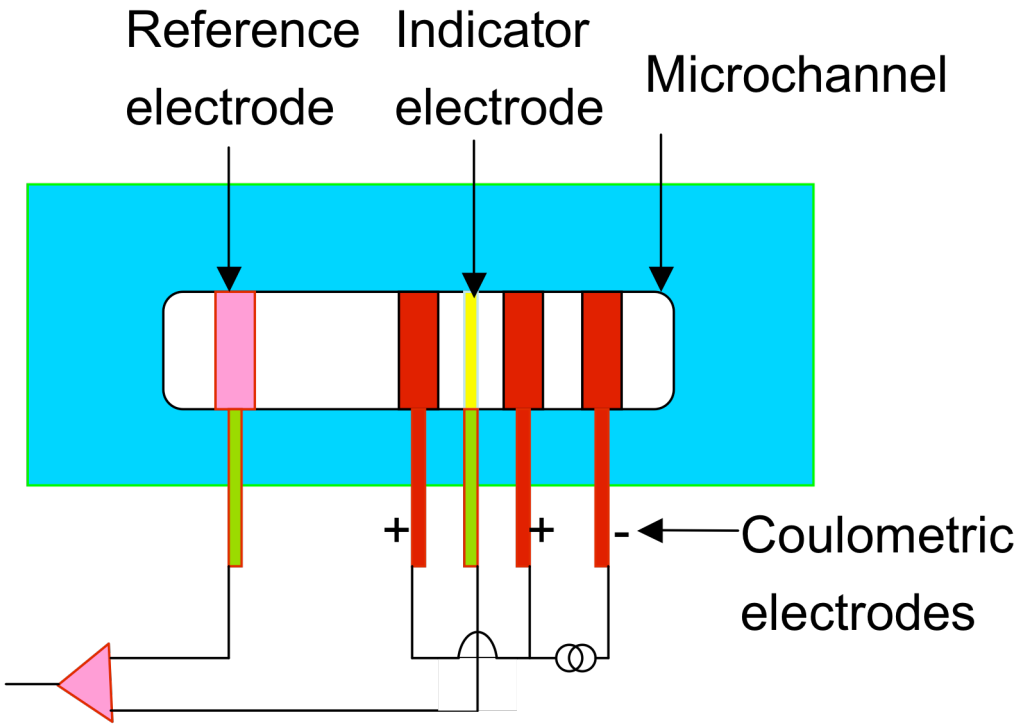


Figure 1.2 Schematic view of the coulometric nanotitrator with potentiometric detection. This simple device is composed of Pt or Ag planar electrodes located in a microchannel.

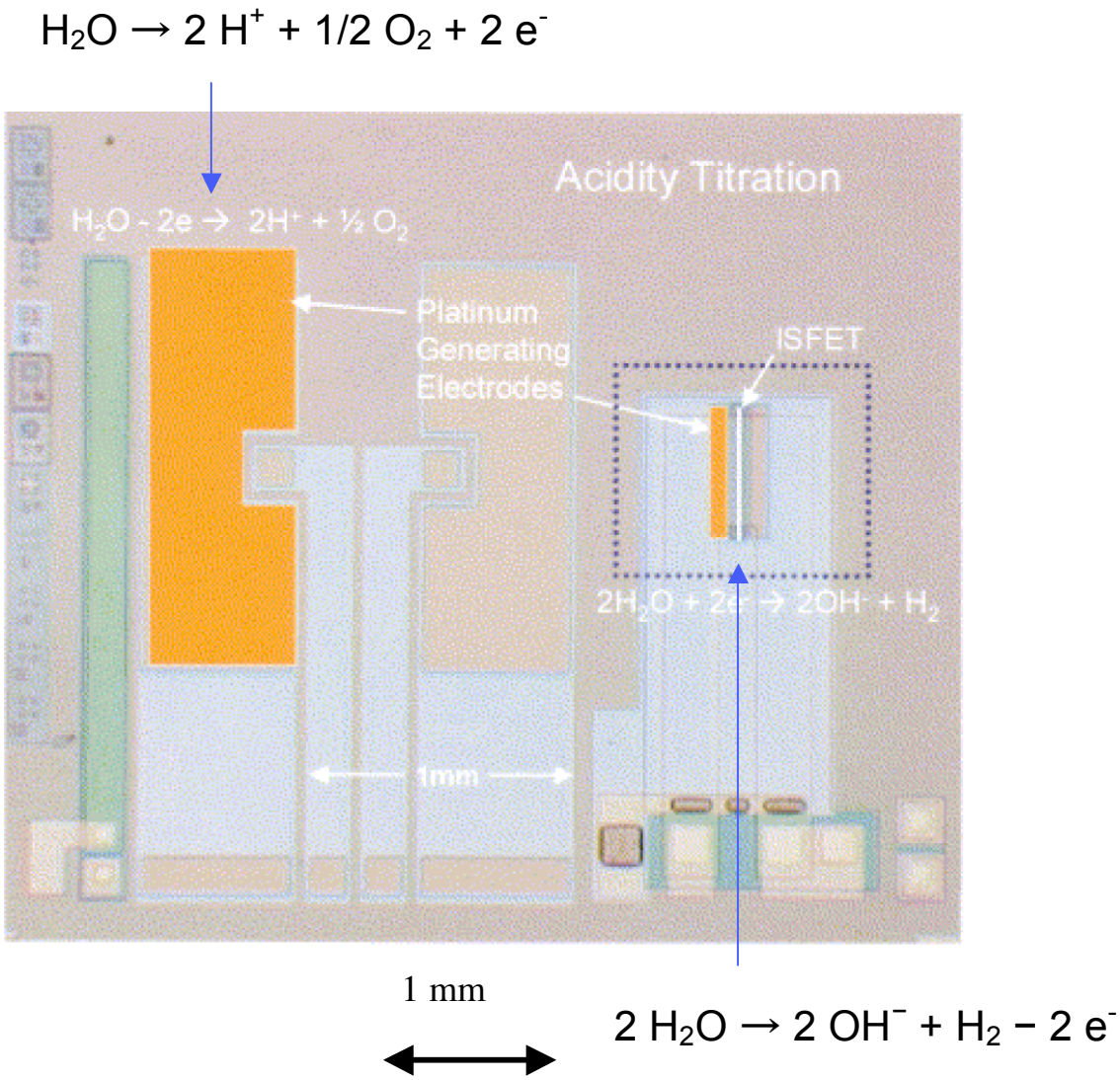


Figure 1.3 Chip lay-out of Orion's Flash titrator.

Fig.1.3. Illustrates the layout of the *FLASH* titration chip. The two main elements of the *FLASH* titrator are, a pH-sensitive ISFET gate and the platinum generating electrode next to it. This chip is placed at the end of 12-mm dipstick, similar to a conventional pH electrode. When the solution is quiet, measurements are carried out. *FLASH* Titration when compared to coulometric titration has an important difference. Here, the quantity of electric charge is not equated to the concentration released. Time is measured, not the charge. The main disadvantage of this technique is that it has to be calibrated and should be corrected for matrix effects as diffusion is the main process. This technique involves a laborious procedure as part of the calibration. This procedure is called the matrix factor adjustment and is application specific. It involves volumetrically determination of the total acidity/alkalinity of a sample of the product to be tested.

1.3 Principles of coulometry

The coulometric technique includes both constant current coulometric and constant potential coulometry. Constant potential coulometry involves holding the working electrode at the constant potential. Since, the potential of the working electrode has direct control on the completion of an electrolytic process, it is the preferred technique. Bard in his review showed that this method can be widely used in determination of actinide elements, tin, europium (III) and ytterbium (III) and also several organic acids.[24]

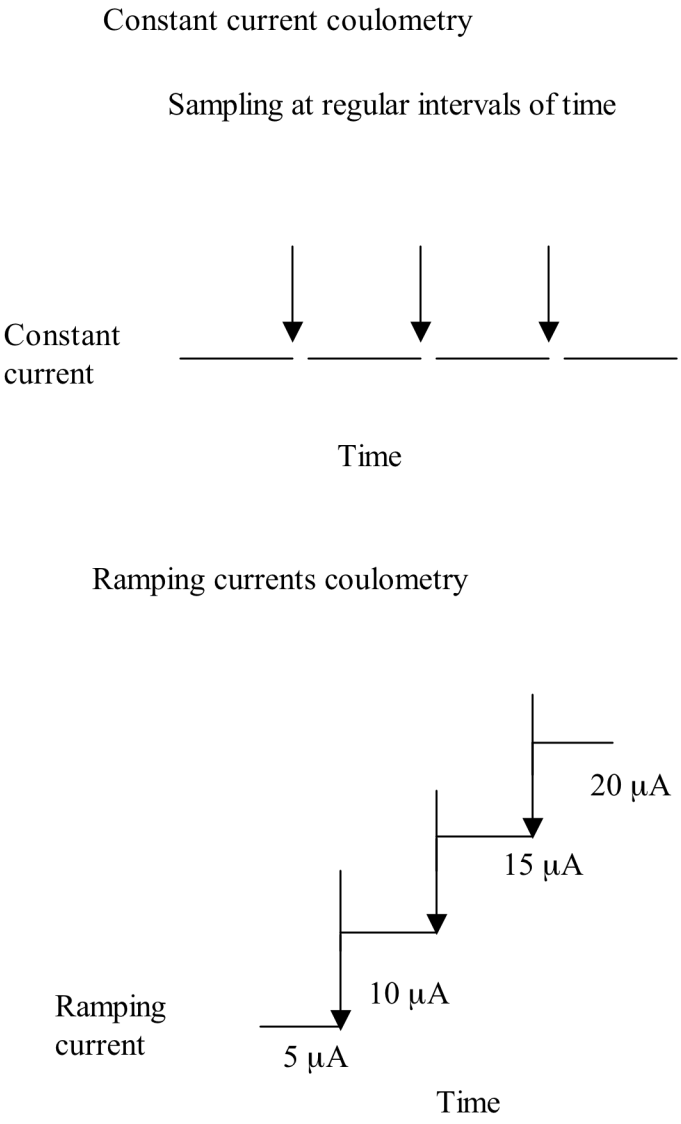


Figure 1.4. Schematic representation of constant current coulometry and ramping current coulometry

Improvement of the conventional constant potential coulometric technique was also reported by varying potentials but the reproducibility of the potentials is good only in the region where oxidation / reduction took place.[24] This has limited usage due to its necessity of a potentiostat capable of high output current and voltages. It is also limited by the need for a stable reference electrode in the electric field environment. The auxiliary electrode is placed in the high electric field in such a way so as to provide distribution of a uniform field. Usually, the auxiliary electrode is separated from the working electrode by a sintered glass.

Bard in his book[25] summarized the titrants electrochemically generated. Constant current coulometry is discussed in depth here. The working electrode is held at one particular constant current or stepped up in synchronization with time as shown in Fig. 1.4. The steps of current usually are of equal duration. At the end of every step of current, the sampling is done. Usually, amperometric or potentiometric sensors are used for sensing. The technique is robust and simple when compared with regular volumetric titrations.[26]

In constant current coulometry, the magnitude of current is equivalent to the titer of the reagent. The time of application of constant current is equivalent to the incremental small volume of the reagent added. The amount of coloumbs required to convert the chemical state is predetermined. The titrant addition from the generator electrode involves the flow of electrons as a result of chemical oxidation or reduction. “Titrating with electrons” is an apt description of it. There are several means for equivalence point determination.

The primary requisite of this coulometric technique is 100% current efficiency of titrant generation. Current efficiency = charge consumed / total charge. Current efficiency remains one hundred percent if the titrant generation is the only process taking place at the generating electrode. The current by which the generating electrode operates should be less than the limiting current of the electrode. The duration of current application should also be less so no depletion takes place at the electrode. If these conditions are violated, the result is an unwanted process leading to a decrease in current efficiency.

For a constant current coulometry, Faraday's 1st law gives the analyte concentration by dividing n by volume V in liters.

$$C = \frac{n}{V} = \frac{it}{zFV} \tag{1}$$

For constant current i, as Q = It, The number of moles of titrant n in the reaction obtained from charge Q divided by Faraday's constant F and the equivalent number z of electrons involved in a reaction. And t corresponds to the equivalence time. For a variable current, the number of coulombs is given by the following equation:

$\int_{t=0}^{t} idt$ where i is the variable current (2)

Endpoint determination in constant current coulometric titrations is similar to that in volumetric titrations using either endpoint indicators or by respective ion selective indicator electrodes. The applications of coulometric titrations continue to grow tremendously.

The design of the coulometric cell was very challenging for scientists, as the configuration should minimize the field effects of the generating electrode on the indicating electrodes in automatic titrations. The voltage or current applied between the electrodes usually creates interference with potentiometric or amperometric endpoint indication. Several researchers solved this problem by using visual endpoint detection.[27] We used the pulsing of current in conjunction with disconnecting the galvanostat completely from the measuring circuit, so it will not be in the ground loop of the galvanostat, as a solution to this problem. Another important consideration in field effects minimization is the way the electrodes are placed in the cell and the design of the coulometric cell itself.

Christian and Feldman developed an electrochemical cell designed to perform coulometric titrations in small volumes with potentiometric or amperometric end point detectors.[28] As shown in Fig 1.5. three platinum foil electrodes were mounted on platinum wires in the cell compartment. Two electrodes were held parallel on one side of the cell and the third electrode was held opposite to those two and not overlapping with them. A sintered glass frit separated the opposite sidearm compartments from the cell compartment. This arrangement of electrodes and along with the cell design should overcome the field effects and yield a sharp endpoint either with potentiometry or amperometry.

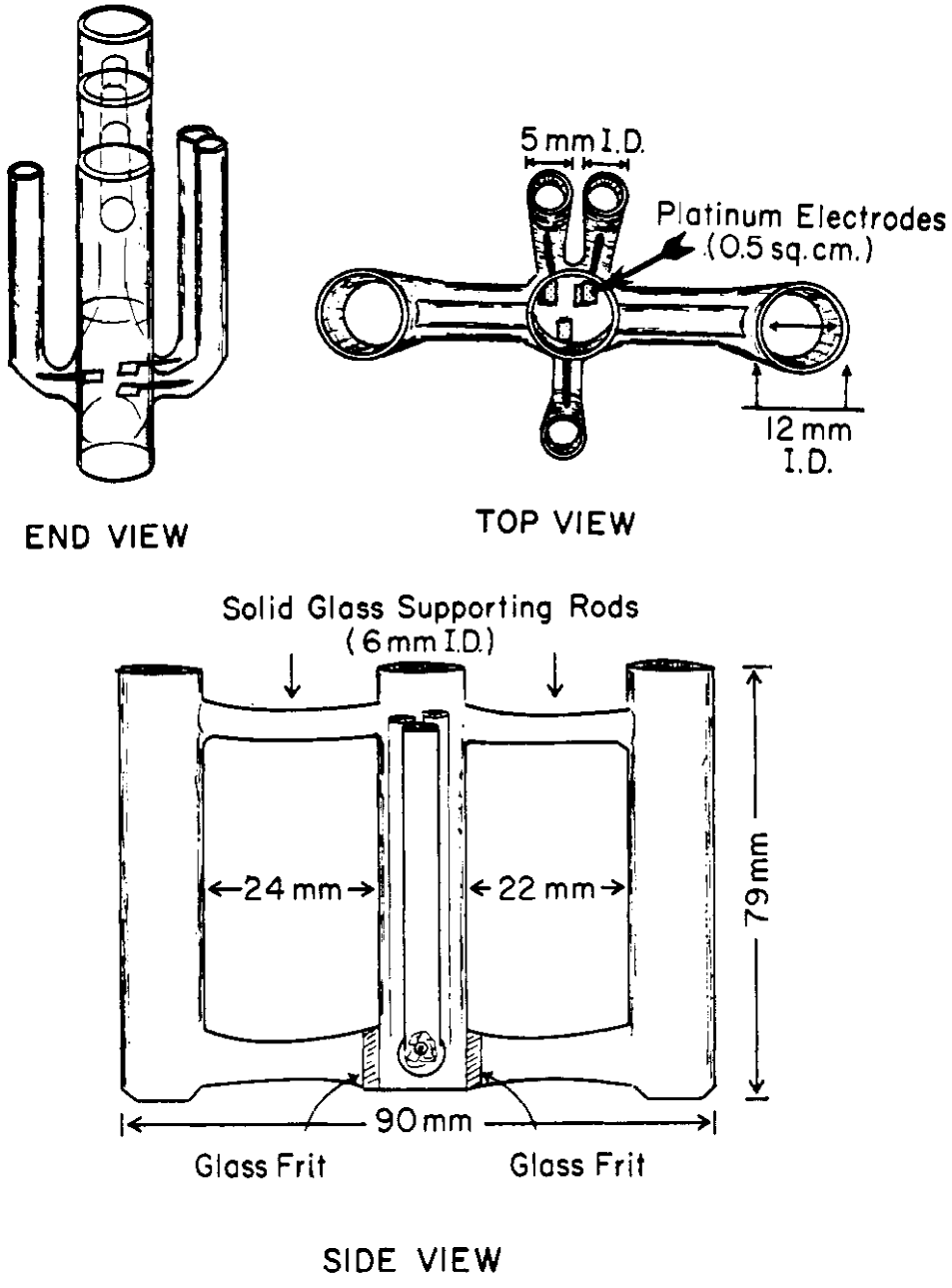


Figure 1.5. Different views of micro coulometric cell of 1mL volume designed to avoid field effects [28]

1.4 Special applications of coulometric titrations

Apart from the several titrants that could be electrogenerated, researchers were also interested in non- aqueous acid / base titrations and oxidation/reductions.[24,25] Table 1.2. gives information about the titrants that could be electrogenerated. For example aromatic amines were titrated coulometrically in acetonitrile solution under particular conditions. Aliphatic and heterocyclic amines have been determined by this technique in conjunction with an antioxidant. Accuracy and precision of determination were very good for every amine tested when hydroquinone was present in the solution.[29] No loss of current efficiency was observed, even though there were side reactions.

Other than determination of analytes, coulometric titrations were also employed by pharmaceutical industries in calibration-free drug determinations by galvanostatic bromine generation.[30] This method was adopted, as other pharmacopoeial methods are laborious in nature. Also, according to standard procedures, the use of an unstable reagent causes errors. Coulometric determination of the antioxidant capacity of tea by bromine electrogeneration was also carried out with good correlation to photometric titration.[31]

In an interesting application, the chloride ion concentration was measured coulometrically by the electrogeneration of silver ions and titrating chloride in rumen fluid to measure an obstructive gastrointestinal disease in ruminants and camelids.[32]

Table 1.2. List of typical titrants that coulometrically generated either directly or through a precursor via redox reaction at the electrode. [25]

Typical Electro generated titrants			
	Electrogenerated Titrant	Generating Electrode	Reagent Precursor
Oxidants	Br_2 I_2 Cl_2	Pt Pt Pt	Br^- I^- Cl^-
Reductants	Fe(II) Cu(I) Sn(II) U(V),U(IV)	Pt Pt Au Pt	Fe(III) Cu(II) Sn(IV) UO_2SO_4
Precipitation and Complexation agents	Ag(I) Hg(I) EDTA	Ag Hg(Ag) Hg(Ag)	Ag Hg Hg^-EDTA^{2-}
Acids and Bases	OH^- H^+	Pt Pt	H_2O H_2O

On comparing the results obtained by coulometry with potentiometry, Cebra et al., observed less interference. A novel method for the determination of acetylcholine by enzymatic hydrolysis of acetylcholine in a flow-through analytical reactor column, coupled with the detection using triangle programmed coulometric flow titration is reported.[33] This method was reported to be precise in determination of acetylcholine in small concentration ranges with the detection limit of acetylcholine 8 x 10^{-5} M as compared with the other methods using changes in pH. This could be used for indirect determination of acetyl cholinesterase inhibitors. A simple and rapid method to estimate penicillin compounds in large samples was developed.

An enzyme-catalyzed reaction of penicillin compounds in a sample pretreatment step gives pencilloic acid as one of the products. An enzyme reactor is built into the sample line of the penicilloic acid and the acid is determined by a flow-through coulometric triangle programmed acid-base titration.[34]A fast and reliable coulometric titration assay of antihistamines such as fenoterol hydrobromide, orciprenaline sulphate and terbutaline sulphate against the electrogenerated chlorine is investigated.[35]

Other compounds and drugs such as nizatidine and sulfamethoxypyrazine were also estimated by coulometric titration.[36,37] The concentration of reducing sugars before and after the incubation of the serum sample with starch substrate was determined by coulometric titration with electrogenerated bromine. Above step is the main part of the saccharogenic method for serum amylase estimation in a 100 μl volume.[38]

1.5 Ion exchange membranes as coulometric electrodes

Non-electroactive titrants, which cannot be generated using metal-based coulometric electrodes, can be electro generated using an ion exchange membrane. This opened a wide spectrum of reagents available for coulometric titrations. However, the passage of ions through ion exchange membranes was not 100% current efficient.[39] The Feldberg group has reported the use of anion and/or cation exchange membranes for the separation of the anode and cathode compartments in coulometric acid-base titrations.[40]

However, at extreme pH values, they lose their exchange capacity which reduces the total membrane capacity until the titration end point is reached.[41] After this work, the scientific literature seems to have no other follow up on ion-exchange membrane based coulometry for a while. This may be due to the fact that scientists needed very selective membranes instead of ion exchange membranes. A less selective membrane gave rise to less efficient transport due to spontaneous ion exchange occurring at the membrane-sample interface.

Then, Russian scientists demonstrated a similar type of the work with ion exchange membrane. These investigators were able to overcome some problems mentioned earlier, by careful selection of both the cation and the anion exchange membrane so as to allow only the ion-titrant to enter into the sample, hindering the back-diffusion of the titrated ion. When selecting an ion-exchange membrane, care also was taken to choose a membrane that prevents H^+ or OH^- ions interference as a result of generation as by products to the electrode cells, from diffusing either into the titrant solution or into the sample to be titrated.[27]

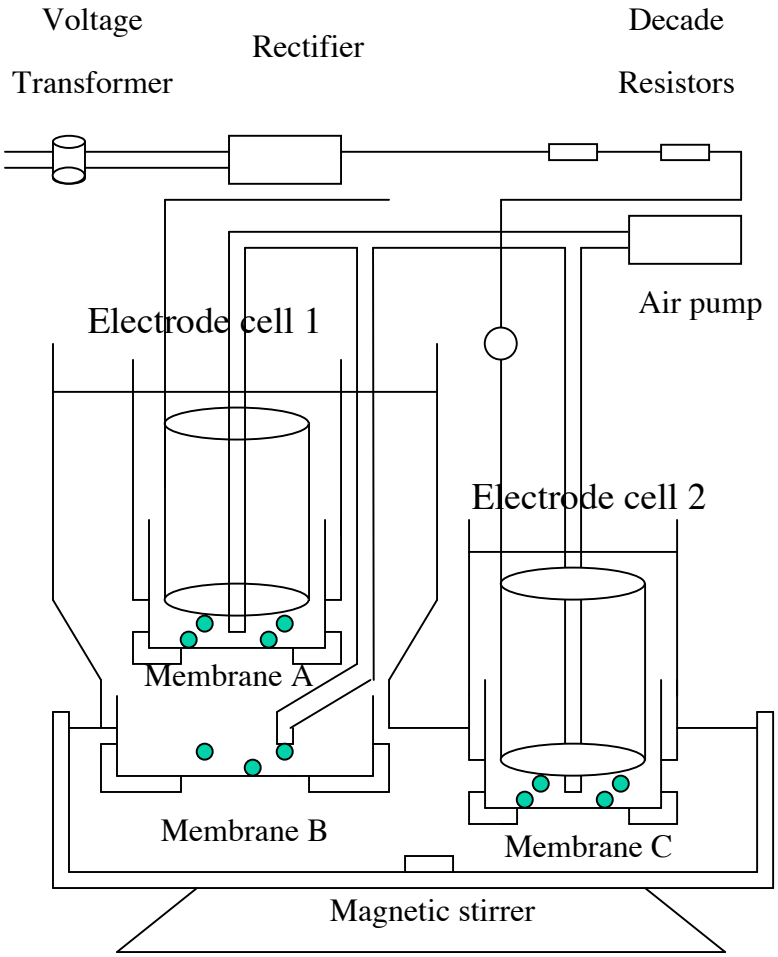


Figure 1.6. Novel coulometric titration cell employing ion-exchange membrane is shown. Here an electrodialysis procedure was adapted to release the titrant and was applied for all titrations with visual end-point detection. [27]

Grutsch et al., reported a technique to electro generate vanadyl, lithium, thorium, phosphate, and many other ions using ion exchange membranes.[42] However, the coulometric efficiency was less than 100% due to a less than desirable permselectivity of these membranes. The membrane-based coulometric electrodes were remarkable as no other electrode material is available to generate vanadyl, lithium, thorium, phosphate ions.

Coulometric electrodes based on metal and ion exchange membranes are seldom used as a routine quantitative analysis technique like titrimetry, except for water determination in organic matrices (Karl-Fischer titration). This may be due to the greater complexity of the electrochemical reactions and also due to the limited selectivity. Several attempts have been made in the past to alleviate problems on account of the selectivity of the coulometric electrodes.

Schumacher and Hackmann reported the development of a pulse coulometer for coulometric reagent-generation from permselective glass membranes of high resistance. Pb^{2+} was generated from Pb chalcogenides to determine sulfur in water and oil samples by titration involving $PbSO_4$ precipitation titration in mixed solvents.[43] Lead (II) ions were released from Pb chalcogenides without a direct redox change of these ions. Using modified coulometric techniques, the n-PbSe should be suited for Pb^{2+} generation. They reported that SO_4^{2-} contents of 35-130 mg/L could be determined in water samples. The relative standard deviations reported were 1.6-2.5%, as compared to 3.0-4.5% in the gravimetric titration.

In an innovative attempt, a perm-selective membrane of europium-doped lanthanum fluoride was used to electrochemically generate fluoride, and a coulometric efficiency of 99.2% was reported.

This was the first ever venture to electrogenerate ions using a selective membrane.[44] Since ISE was in its infancy during this work, silver ions were transported from a reservoir using the solid silver sulfide membrane. This procedure can be adapted in solutions having strong oxidants, where the use of a silver wire anode for electro generation of silver ions would be impossible. The principle involved here is still a redox process of the silver/silver (I) couple.[45]

However, the selectivity is largely dependant on the sulfide solubility of the involved salt and hence, is unlike other simpler redox systems. Kihara et al., reported a novel coulometric ion transport across the aqueous/organic interface, as this is important not only in the cases of redox species but also for materials that are not easily oxidizable.[46] A flow cell was made using a porous teflon tube with an aqueous|organic solution (W|O) interface to which a potential difference, E, was applied to transfer ions. The figure 1.7. shows that the cell had a silver wire fitted into the teflon tubing, with a platinum wire placed outside the tube, O into which the tube was completely immersed and a reference electrode, RE, in O.

The transfer of K^+ from water to 1,2-dichloroethane is facilitated by selective complexing of K^+ to dibenzo-18-crown-6. The organic phase is allowed to flow through a narrow gap between the tube and the silver wire. A potential was applied by using the silver wire and the RE, and the current obtained as a result of the interfacial K^+ transport was measured between the silver and platinum wires. The K^+ ion transfer is completed within 40 s with a coulometric efficiency of 99% for a determination range of 2×10^{-4} to 2×10^{-3} mol dm ^{3} with an electrolysis time of 30 min. This is also found to be useful in coulometric determination of Mg^{2+} or Ca^{2+}, and hence, could be used as a detector in flow analysis.

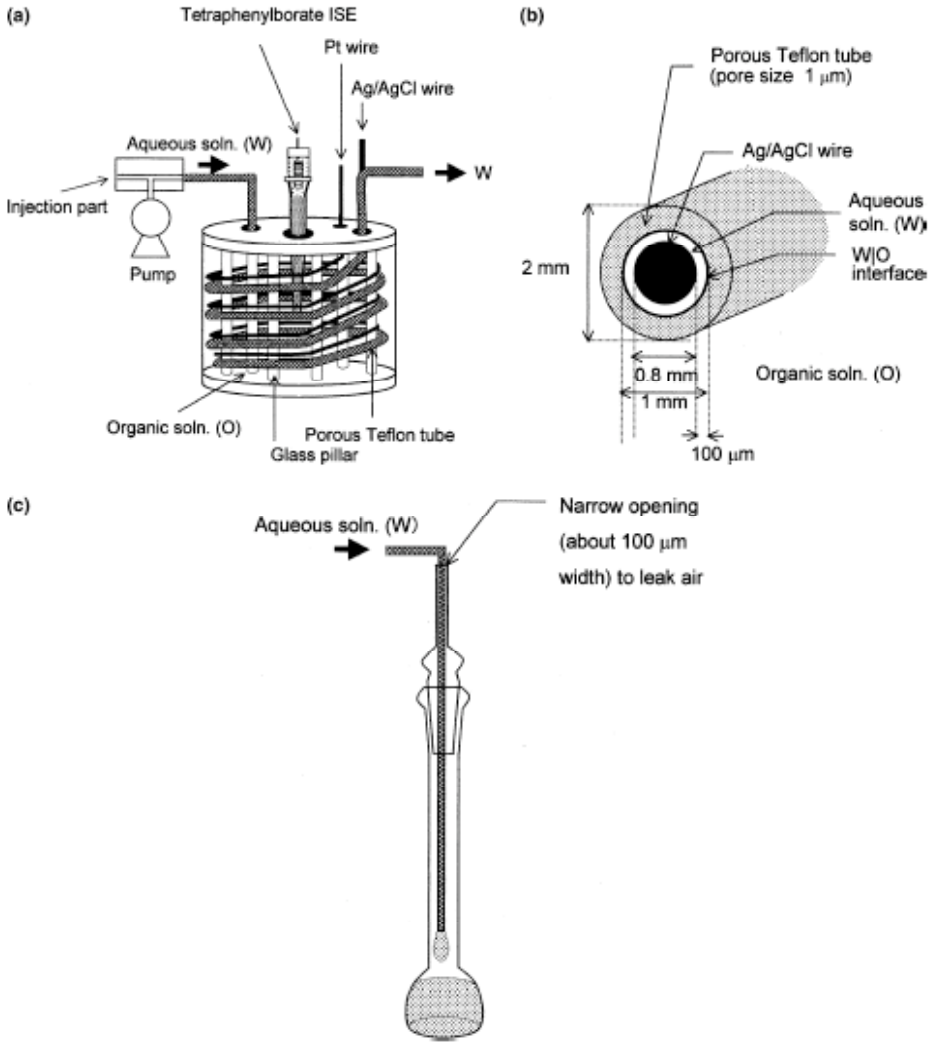


Figure 1.7. Constant potential coulometric extraction ions from sample in to the organic phase using a column cell made out of teflon tube with a selective ionophore [46]

Coulometry in chemical analysis has always been a highly attractive approach because it offers the promise of calibration-free measurements. Glucose estimation in blood was carried out by oxidation of glucose in the sample using an enzyme, resulting in peroxide being titrated with electrogenerated hypobromite.[47]Imparting selectivity to coulometric electrodes by using ionophore based ion-selective polymeric membranes to electro generate titrant makes coulometry more efficient in real time analysis in the presence of interferents.

This was employed very successfully in complexometric and precipitation titrations to estimate very dilute quantities of analytes. This method is also calibration free.[48] In the next part of this section the motivation for using ion selective membranes as a coulometric electrode is explored. The scientific literature contains reports by several researchers who made landmark discoveries in using electrochemical techniques to study the electro-assisted transfer of ions across the polymer-sample interface. Some of their work is briefly discussed here.

1.6 Ion selective polymeric membrane based ISEs

Ion -selective electrodes are currently one of the most extensively used methods in areas of applications including industrial process control, biomedical screening, pollution monitoring and clinical laboratories. Several scientists have described the use of ion-selective electrodes in continuous monitoring.[49-51] Ionophore based ISEs are made of plasticized polymeric PVC matrix along with ion exchanger sites and a lipophilic ionophore.

These membranes are placed between two aqueous solutions, one being the sample and the other one being inner filling solution having an internal reference element immersed in it. The analyte ion to be sensed enters the organic membrane phase by selective complexing with the ionophore. There are plethoras of ionophores[52] available for clinically relevant ions. The measured membrane potential is proportional to the free ion activity in the sample. Fig.1.8 shows a schematic representation of a typical potentiometric setup having an ion-selective membrane electrode. An ISE is considered to be a galvanic half-cell having an ion selective membrane with an internal Ag/AgCl reference element.

The sample has an external reference against which the ISEs potential is measured. The electrochemical cell is represented as follows

Ag,AgCl;KCl(sat) | Salt bridge || Sample | Internal solution
| AgCl, Ag.

The total electrical potential differences at the two terminals of the cell are given by the individual potential differences arising at the solid-solid, solid-liquid and liquid-liquid. interfaces. The sum of all theses terms are given as

$$E_{Cell} = E_{(Ext,\ Ref)} - E_{(Int,\ Ref)} + E_D + E_J + E_{PB1} + E_{PB} \qquad (2)$$

E_J is the liquid junction potential and is in general negligible and (E_J~0) and E_D is the diffusional potential. Ideally, in zero current potentiometry, the EMF is a measure of activities of the contacting aqueous solutions on either side.

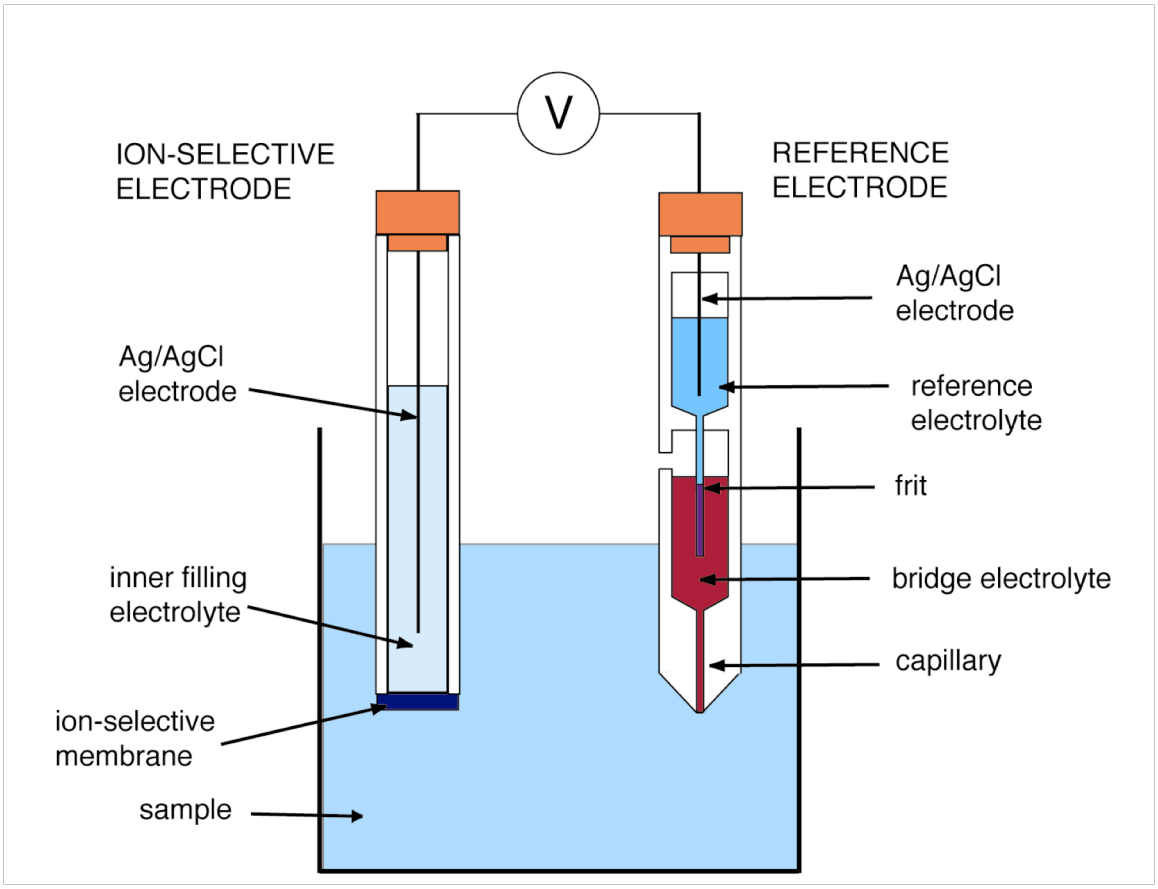


Figure 1.8. Electrochemical cell used for potentiometric measurements

The phase boundary equilibrium potential response with the analyte ion from the sample is proportional to logarithmic analyte activity following a Nernstian like equation for ion M+,

$$E = E_M^{\circ} + \frac{RT}{Z_M F} \ln a_M^{+} \quad (3)$$

Where E is the potential of the cell, $E°_M$ is the cell constant which corresponds to the combination of contributions from the inner phase boundary potential of the membrane plus the inner and as well as the outer reference electrodes; Z_M is the charge of the analyte ion; a_M^+ is the analyte activity in the sample solution. R is the gas constant, T is the absolute temperature and F is the Faraday's constant. In this case only the analyte ion interacts with the membrane without changing its composition. This relationship between EMF and activity of analyte ion makes it an important analytical technique. Fig.1.9. is a schematic diagram of the response function of an ISE. [52]

The lower detection limit of an electrode is determined by the interfering ions in the background and ions leaching out of membrane. The upper detection limit is related to the extraction of ions of opposite charge. The membrane should be impermeable to oppositely charged ions i.e. permselectivity condition should be maintained with the cation exchanger, which is essential to this behavior. If an interfering ion is present along with the analyte ion, which can also interacts with the membrane by ion exchange, then the ISE responds more to this interferent ion depending upon its concentration. The Nikolsky-Eisenman equation describes the influence of this interfering ion of same charge number on the ISE potential response.[53]

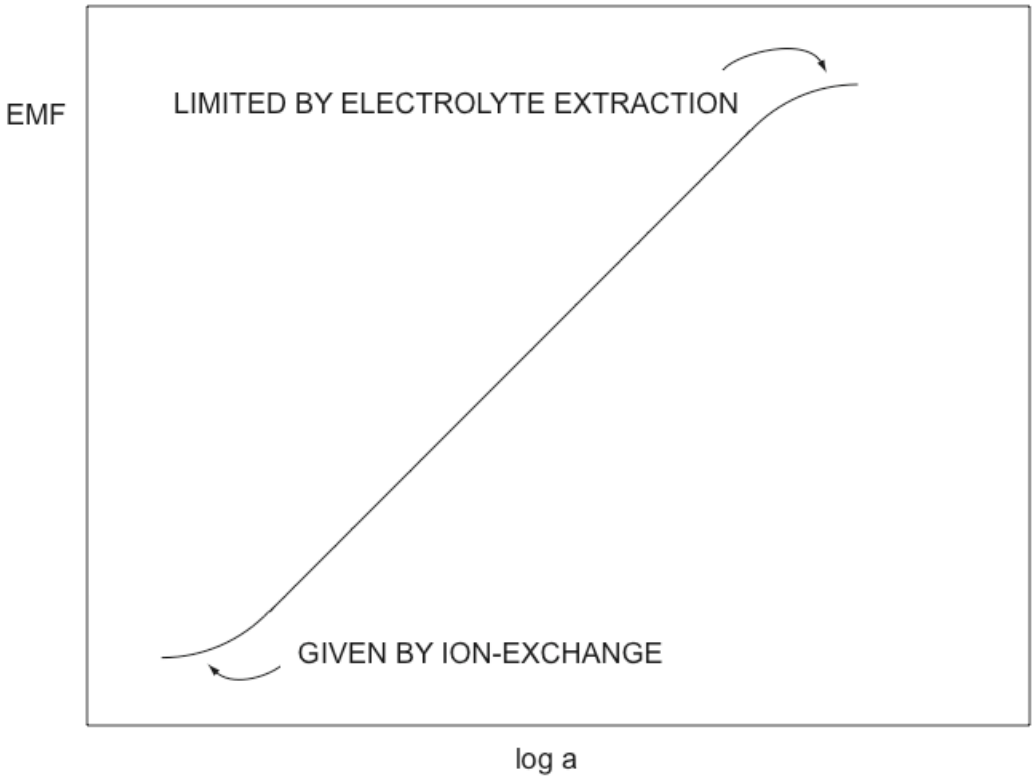


Figure 1.9. Schematic representation of processes that dictate upper and lower limits of the response function of an ISE according to IUPAC [100]

$K^{pot}_{J,K}$ is the potentiometric selectivity coefficient of the ion K with respect to the ion J by which an ISE is characterized for its selectivity.

$$EMF = E^{\circ}_{J} + \frac{2.303RT}{z_J F}\log\left[a_J + \sum K_{J,K}^{Pot} a_K^{\frac{z_J}{z_K}}\right] \quad (4)$$

The above equation takes into consideration the influence of interfering ions provided that they have the same charge as the primary ion. However, this equation is still valid for the condition that the resulting potential is entirely dominated by primary ion of interest. Recently, a new equation was developed which related the difference in potentials of individual ions for both primary and interfering ions to the selectivity coefficient as:[54.55]

$$\log K_{J,K}^{pot} = \frac{(E^{\circ}_{J} - E^{\circ}_{K})z_J F}{2.303RT} \quad (5)$$

$E_J°$ and $E_K°$ are the individual potentials obtained by extrapolating their respective calibration curves of ions J and K at 1M sample concentration, z_J. Conventional ISEs are based on the equilibrium partitioning of analyte ions at the phase boundary of a sample and an ISE membrane. But, the potentiometric polyion sensors function based on the principle of non-equilibrium steady state.

For the polyanion heparin, the electrode membrane has an exchanger with chloride as counter anion, which exchanges spontaneously with heparin yielding high slopes.[56] Fig.1.10 shows a typical response of an ISE to polyion heparin. Hence, this sensor has to be calibrated differently compared to an ISE as the membrane-sample interface is repeatedly renewed. [57]

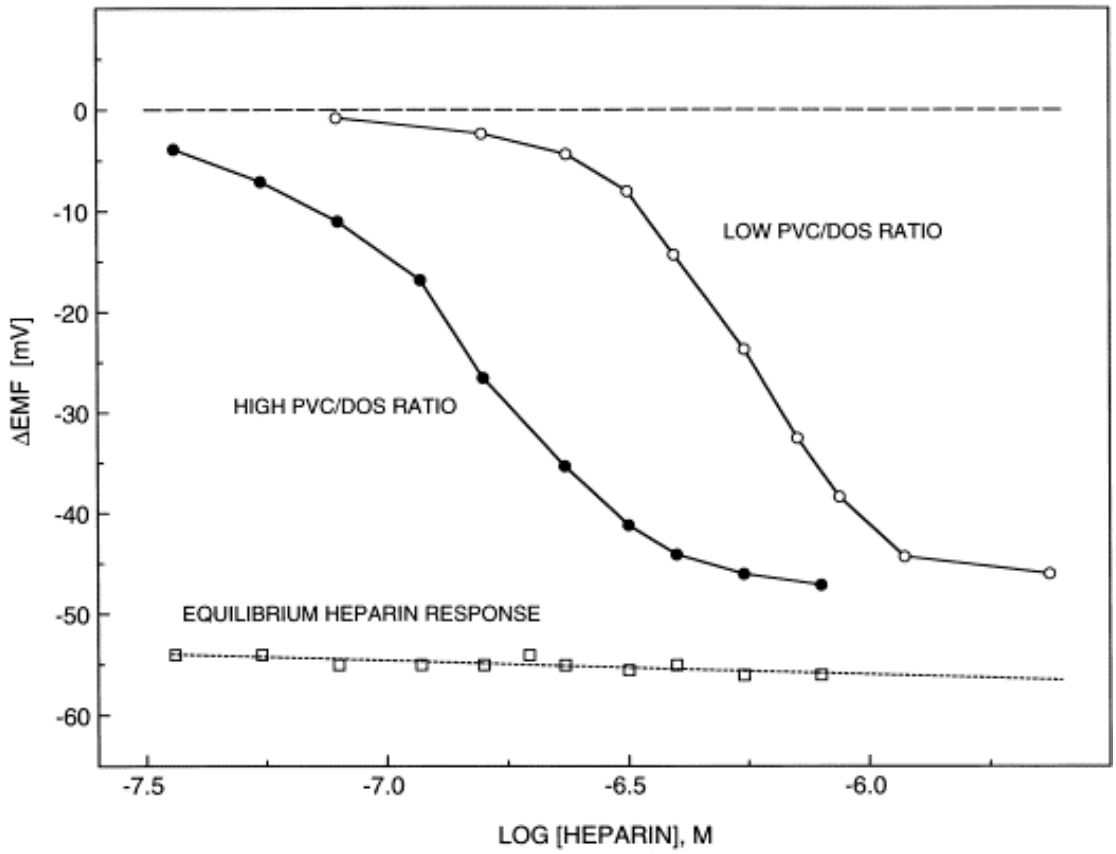


Figure 1.10. Responses of the polyanion heparin sensor based on non-equilibrium steady state extraction principle. [56]

These sensors were found to be irreversible and very recently this problem of irreversibility was overcome by a stripping and renewing method using galvanostatic current control across the ISE membrane with a lipophilic salt.[58]

The passing of current or voltage across the PVC membrane is not entirely new. As we can see from the literature, the electrical and transport characteristics dictating the response behavior of ISEs were studied by electro dialysis experiments.[59,60] These experiments were carried out with stacks of membrane slices (40-50 μm thick each). Following the experiment, the membrane slices have been separated and the concentrations of the free and complexed ionophore were determined.

Impedance[61-63] and conductivity measurements[64] were used to understand and describe the transport mechanism of the neutral-carrier based ion selective membrane. Horvai and Buck extensively characterized fixed-site ion selective membranes using a.c impedance by varying the bathing concentrations, the time of membrane soaking in the bathing electrolytes and by the use of different plasticizers. In a pioneering work, Armstrong was able to show how impedance measurements could be used to map both the bulk and interfacial charge-transfer of valinomycin-based PVC matrix potassium selective membranes in contact with aqueous solutions.

Calculation of diffusion coefficients of the neutral carrier in ion-selective membranes were based on chronoamperometric transient responses obtained as a result of externally applied potentials.[65-68]

An ionophore concentration profile under a voltage step was completely researched. Current-voltage curves obtained gave an idea about field induced extraction that was confirmed with the evidence from the impedance data. A number of studies have been carried out to explore the electro-assisted transfer of metal ions complexed with a hydrophobic ionophore at the liquid/liquid interfaces (interface between two immiscible electrolyte solutions (ITIES)). Here, the classical solvent extraction was combined with an electrochemical technique, so that metal ion separation at the water/1,2-dichloroethane (DCE) and water/nitrobenzene (NB) interfaces has been improved in the last 10 years [69-77] This separation has been useful as a recovery process in the field of waste containing metal ions.

Tetramethylammonium transfer between water and 1,2-dichloroethane (1,2-DCE) using a microhole and micropipette tip-supported interface was studied. ITIES was also utilized in studies of the kinetics of ion and assisted ion transfer reactions. This motivated several researchers to explore voltammetry at the interface of aqueous and nitrobenzene jellified with PVC.[78-81] Later, cyclic voltammetry, which was widely studied in liquid/ liquid interfaces, was applied as a technique to explore ion transfer at the solvent polymeric membranes used as ion selective electrodes.[82,83] Jadhav et al., also explored important response features of voltammetric and amperometric ion selective membranes with two polarizing interfaces. Ion transfer mechanisms for voltammetric sensor membranes were studied with and without ion exchanging capabilitites.

With the membrane containing no ion exchanger an external potential was used as an ion exchanger to extract ions into the membrane. After an accumulation potential, A stripping potential was employed to remove the accumulated ions. Dummy membranes containing a lipophilic salt additive were examined with symmetric and asymmetric bathing electrolytes.[8] All these electrochemical techniques, which studied the ion fluxes as a result of electro assisted transport, improved the chances of reaching lower detection limits in ion selective electrodes.

Researchers showed that lower detection limits are achieved not only by the use of selective ionophore, but also depended upon the elimination of greater ion flux resulting from the use of a traditional inner filling solution to the sample. Pretsch and coworkers[85,86] used buffered inner filling solution to prevent leaching of primary ions from the membrane/sample interface, thereby improving the detection limits to the sub nano-molar range.

Erno Lindner and colleagues showed another way to modulate Tran membrane ion fluxes (see Fig. 1.12) and eliminate primary ion flux across the membrane by applying nano-amperes of direct current.[87] The current is passed to remove the leaching ions that increase the ionic concentrations in the interface and also current was also applied to eliminate ion depletion at the interface. Hence, the micromolar and sub-micromolar sample activites ion gradient remains flat. With this phenomenal invention, Nernstian response up to $3x10^{-12}$ M was achieved.

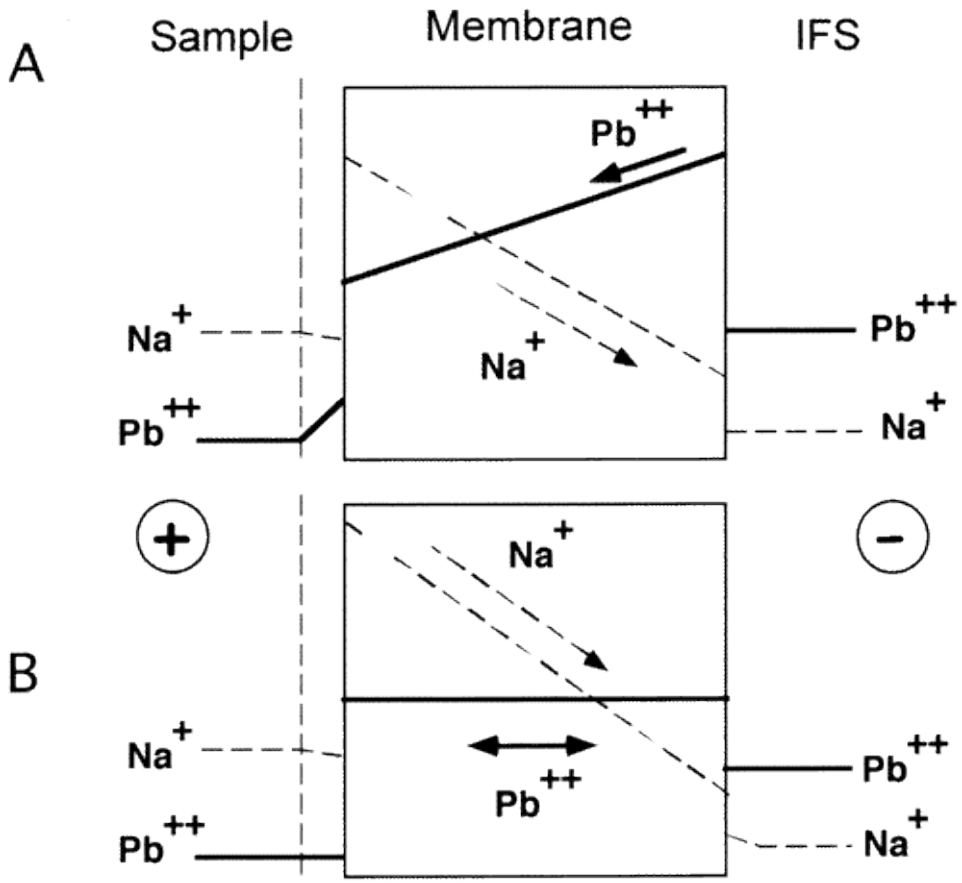
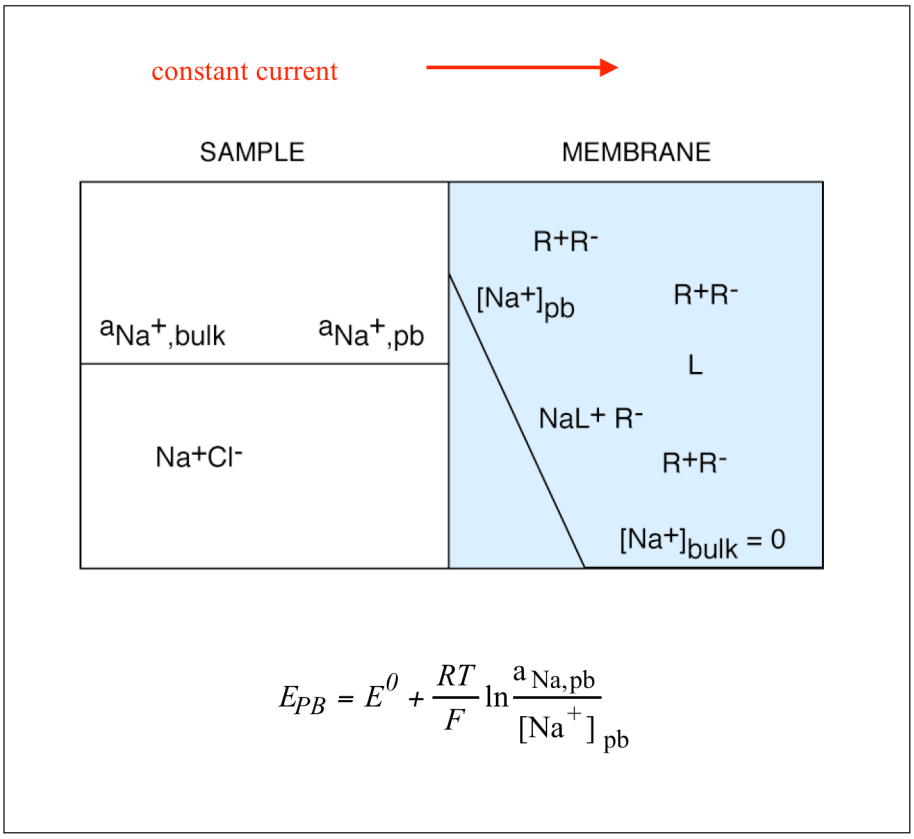


Figure 1.11. Fine tuning of fluxes by application of an external current to improve detection limits of ISEs [97]

In another method that employs an external current, Shvarev et al., described a pulsed galvanostatic technique to investigate ion-selective electrodes having no essential ion-exchanger properties. In this technique, after the application of current pulse, a longer stripping baseline potential pulse is applied to regenerate the phase boundary region of the ion-selective membrane. The applied current acts as an ion exchanger completely controlling the magnitude and sign of the ion flux into the membrane. This approach was shown to lead to the optimization of ion concentration gradients in the membrane, by using an easier and straightforward instrumental control.[89-90]

The disturbance of an electrochemically imposed ion flux across the interface (see Fig. 1.12) is an elegant method now utilized as a technique to study reversibly the adsorption of the neutral surfactant Brij 35 at a liquid/liquid interface. This galvanostatic current control is an innovative tool for monitoring surface confined reactions at such liquid and polymer interfaces.[90]

With the above galvanostatic current control the ion-selective electrodes yielded a 10-20-fold increase in sensitivity for calcium measurements as compared to the regular Nernst slopes of 30-mV potential change for a 10-fold activity change measured at room temperature.[91] This is useful to measure concentration ranges in physiological liquids. Super-Nernstian responses of more than 700 mV/decade were reproducible and improve the accuracy and precision of measurements in clinical analysis.



$$E_{PB} = E^{0} + \frac{RT}{F} \ln \frac{a_{Na,pb}}{[Na^{+}]_{pb}}$$

Figure 1.12. Principle of pulse galvanostatic sensor under current control.

Morf [92-94] attempted to provide a theoretical explanation for the effects of externally applied current on the transmembrane ion fluxes and on the potential response of ISE membranes.[95] The main transport mechanism under current was identified as a current-induced migration of ions, which is overemphasized than the zero current diffusional fluxes that usually influence the detection limits of ISEs. Recently, chronoamperometric transients were used to estimate the ionophore loss from fixed-site, solvent polymeric membranes.[96,97] The effect of applied voltage on the current time transients of a valinomycin-based potassium electrode loaded with varying concentrations KTpClPB was studied by Lindner's group. They also tried to characterize the perm-selective properties of the ion selective membrane under large applied potentials. They attempted to calculate the diffusion coefficient of the cation exchanger TPB^-, and suggested that the flux of $Kval^+$ and TPB^- is dependent on the slower moving TPB-. Lindner and coworkers[98] tried to monitor the fine-tuning of fluxes using an external current across the ion selective membrane by coupling a scanning electrochemical microscopy and a very sensitive differential pulse voltammetry. They applied SECM to image concentration profiles.

1.7 Selective Coulometry using PVC based ion selective membranes

All the studies[59-98] mentioned earlier in this chapter have explored the possibilities of extracting ions selectively using either potential or current control across the interface of plasticized polymeric ion selective membranes.

These examples showed that ion selective membranes have been used for electro transport. In this work, the ion selective membrane is preferred for three main reasons first is due to its selectivity, which gives electro-transport through the membrane an edge over other coulometric electrodes. The resistance of this polymeric membrane typically less than 5 kohms after it was reduced with the addition of a lipophilic ion-exchanger. The current is added as a short pulse and the ions were coulometrically released.

The released ions were measured using ion selective electrodes. A second advantage of using ion selective membranes as coulometric electrodes is that the current efficiency is high in real samples containing interferences due to their selectivity. There is also a final important reason why coulometric electrodes should be made out of ion selective membranes; the available wide spectra of organic, inorganic, proteins and charged drugs for which ISEs exist. These could be used to selectively release the ions mentioned in table 1.3.

Though metal electrodes are the only ones to be employed in coulometry to make commercial devices, their selectivity is far from desirable and not many titrants could be electro released. Ion selective membranes with a wide palette of selective ionophores are definitely superior.[95] The robustness in discrimination from interferents makes these ion selective membranes the natural choice for the next generation of coulometric devices.

Table 1.3. Analytes for which Carrier Based Ion-selective Electrodes are available.[95]

Anayte group	Ion-selective electrodes
Organic anions	H^+, Li^+, Na^+, K^+, Rb^+, Cs^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}, Mo(IV), Fe(III), Cu^{2+}, Ag^+, Zn^{2+}, Cd^{2+}, Hg^{2+}, Tl^+, Bi^{3+}, Pb^{2+}, U(IV), Sm(III), $NH4^+$
Organic cations	CO_3^{2-}, HCO^{3-}, SCN^-, NO_2^-, OH^-, Phosphate, sulfite, SO_4^{2-}, Cl^-, SeO_3^{2-}, I^-
Inorganic cations	Salicylate, phthalate, maleate, 2-hydroxy benzhydroxamate, nucleotides, heparin
Inorganic anions	1-phenylethylamine, 1-(1-naphthyl)-ethylamine , ephedrine, norephedrine, pseudoephedrine, amphetamine, propranolol, amino acid methyl esters, α-amino-ε-caprolactum , amino acid amides, benzyl amine, alkyl amine, dopamine, mexiletine, local anaesthetics (procaine, prilocaine, lidocaine, bupivacaine, lignocaine), diquat and paraquat (herbicides), tetramethyl and tetraethyl ammonium, guanidine, metformin, phenformin, creatinin, protamine.

The ion selective membranes as coulometric electrodes were explored as examples of their usefulness as a precise and quantitative analytical technique in complexometric and precipitation titrations in the subsequent chapter. The further chapters also involve emphasis on the optimization of conditions for the polarizable membrane based coulometric electrodes. Experiments were also performed to electro release polyions such as protamine and titrate heparin, proving this technique to be useful as a clinical method.

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CHAPTER 2

SELECTIVE COULOMETRIC RELEASE OF IONS FROM ION SELECTIVE POLYMERIC MEMBRANES FOR CALIBRATION-FREE TITRATIONS

2.1. Introduction

Coulometry in chemical analysis has always been a highly attractive approach because it offers the promise of calibration-free measurements. For instance, calibration-free enzyme-based glucose biosensors for blood measurements have been realized by exhaustively oxidizing all available glucose in a confined sample.[1] The coulometric detection of ions has recently been reported by Kihara, by selectively and quantitatively extracting them under potential control into an organic phase doped with a selective receptor for that ion.[2] Coulometry is also very attractive for reagent generation and delivery, especially in chemical titrations.

Traditional volumetric titrations require standardized stock solutions, and the accuracy of the volumetric delivery becomes difficult to achieve with decreasing sample volume.Recent efforts in miniaturizing fluidic delivery of reagents include the use of fused silica capillaries and mechanically driven syringes,[3,4] and a diffusional titration approach, with reagent delivery from a plug of a gelled membrane back-filled with reagent into drop sized samples.[5] The coulometric electro-generation of reagents is an elegant, calibration free alternative to miniature titrators.

A coulometric nano-titrator with fast response integrated into a flow-through automatic analyzer consisted of two coulometric metal-based electrodes for reagent generation and a potentiometric detection electrode.[6] This type of work resulted in a commercial product, including the so-called Orion FLASH titrator™ by Thermo Electron.[7] Potentiometric titration using dual microband electrodes in generator-collector mode has been reported very recently.[8] The main advantage of the above method is the electrogeneration of titrant to determine the endpoint by a single scan with the ramp current method. Another area where calibration free coulometric titrators have been of interest is for the determination of drugs in pharmaceutical preparations by galvanostatic bromine generation, for instance.[9]

There are two key challenges associated with coulometric reagent delivery: a limited selectivity and a limited choice of reagents that can be released in such a manner. Redox reactions at metal electrodes are normally not perfectly selective, especially in complex and unknown samples. Besides acid-based titrations,[10] such principles have also been used for the generation of chemically reactive reagents such as bromine,[9]but the possibilities are limited.[11]

Besides the triggering of a direct redox reaction by applying suitable potentials or currents, coulometry can also be applied for non-redoxactive processes as long as the reagents carry a well defined charge. In such cases, a suitable applied current generates a flux of such ions whose magnitude can be accurately calculated. Of course, the selectivity of this process depends on the materials from which the ions are released.

Simple hydrophilic ion-exchange membranes have been used for such purposes,[12,13] but the selectivity of such materials is normally not well controlled and spontaneous exchange with competing ions may occur in contact with complex samples. Ion conductors such as silver sulfide deposited onto silver wires were used to generate silver ions.[14] Although this principle still relies on a redox process of the silver/silver(I) couple, the selectivity is largely dictated by the sulfide solubility of the involved salt and therefore uniquely different from simpler redox systems. More recently, lead(II) ions were released coulometrically from chalcogenide glasses for the purpose of determining sulfate ions.[15] The selectivity of the process was dictated by the properties of the glass membrane, and the lead ions were released without involving a direct redox process for these ions itself.

When coulometric reagent generation was first starting to emerge, ionophore-based ion-selective membranes were not yet a mature technology. Today, they are widely established for the selective chemical detection of ionic analytes, and a chemical receptor (ionophore) in the membrane is normally used to tune the selectivity to a wide extent. Such membranes contain a lipophilic ion-exchanger and their counterions are the analyte ions to be determined.

In recent years, the selectivity of such sensors has been more fully understood;[16] protocols determining unbiased selectivity coefficients have been introduced, shown to be extremely high in some cases;[17] and their detection limits have been lowered to ultra-trace levels for a number of examples by carefully minimizing undesired zero-current ion-fluxes across the membrane.[18,19]

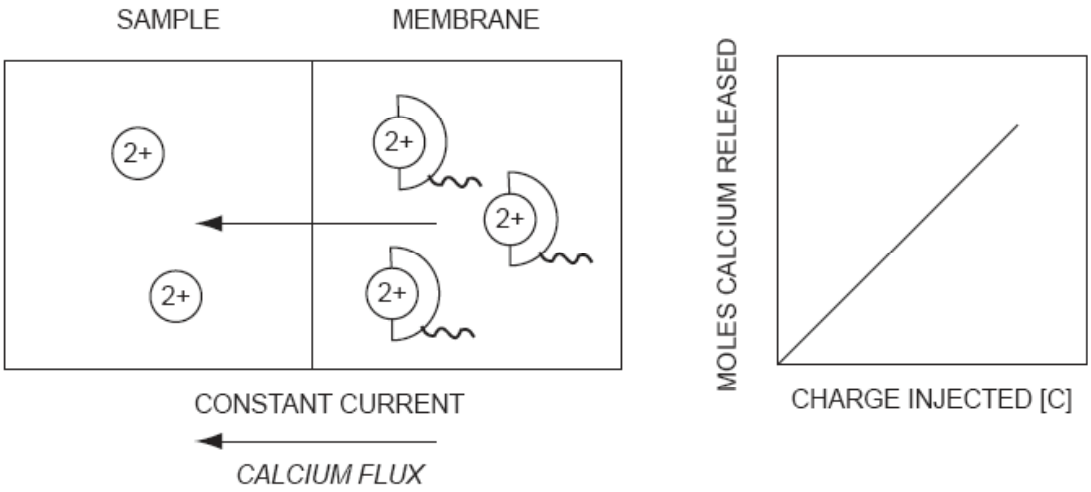

Figure 2.1. Scheme: a constant current pulse applied across a calcium-selective polymeric membrane results in the injection of a fixed amount of calcium into the sample. This is here used for chemically selective coulometry.

To my knowledge, I report here for the first time on the selective coulometric release of ionic reagents from ionophore-based solvent polymeric ion-selective membranes for the purpose of conducting calibration-free titrations.If a suitable current pulse of fixed duration and amplitude across such an ion-selective membrane is applied, a well-defined flux of ions in direction of the sample may be observed (see Figure 2.1). So far, such types of experiments have already been performed, for instance, to counter undesired spontaneous ion fluxes and to lower the detection limit of the electrode.[20]

More recently, applied current pulses leading to defined ion fluxes into and from the membrane have been used to develop the concept of instrumentally controlled ion-selective electrodes.[21] This has brought ion-selective electrodes closer to the realm of voltammetric sensors. In this chapter, calcium-selective membrane electrodes are explored as an initial model system for the coulometric release of calcium and barium ions, coupled with potentiometric detection using ion-selective membranes with nanomolar detection limits.

2.2 Experimental

Reagents

High molecular weight poly(vinyl chloride) (PVC), the plasticizers 2-nitrophenyl-octyl ether (o-NPOE) and bis(2-ethylhexyl) sebacate (DOS), the ionophore Calcium (IV) N, N-dicyclohexyl-N',N'-dioctadecyl-3-oxapentanediamide (ETH 5234),

the lipophilic cation-exchanger salt sodium-tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), the inert lipophilic salt tetradodecylammonium-tetrakis(4-chlorophenyl)borate (ETH 500), tetrahydrofuran (THF) and all salts were purchased from Fluka Chemical Corp. (Milwaukee, WI) in Selectophore quality or puriss quality. Aqueous solutions were prepared by dissolving the appropriate salts in nanopure-deionized water (18.2 MΩ cm).

Ion selective membrane preparation

The calcium selective membrane components consisted of 13 mmol kg^{-1} Ca(IV) ionophore, 4.5 mmol kg^{-1} NaTFPB and 10 mmol kg^{-1} of inert lipophilic salt ETH 500 together with o-NPOE and PVC (in a 2:1 mass ratio) with a total mass of 140 mg, dissolved in 1.5 mL of THF and pouring into glass ring (22 mm i.d.) affixed to microscope glass slide. The solvent THF was allowed to evaporate overnight to give a membrane of 200 mm approximate thickness.

Electrodes

Ion-selective electrodes for the detection of the released calcium ions were prepared by punching a series of 3 mm discs from the parent membrane and gluing to plasticized PVC tube with PVC glue, which was mechanically affixed to a 1000 μL pipette tip. The detecting calcium electrodes were conditioned for 1 day in 10^{-3} M $CaCl_2$ followed by conditioning in 10^{-5} M of NaCl and HNO_3 each for one additional day. The inner filling solution contained $CaCl_2$ 10^{-3} M with 0.05 M Na_2EDTA at a pH of 5.8 adjusted by 0.01 M NaOH to give a calculated calcium activity of 3 x 10^{-8} M.

The calcium selective coulometric electrodes were prepared by punching a series of 9 mm discs from the parent membrane and gluing to wide plasticized PVC tube with PVC glue, which was mechanically attached to a rigid Teflon tube. These electrodes were conditioned in 10^{-2} M $CaCl_2$ overnight. The inner filling solution was identical to that of the conditioning solution. The calcium selective electrodes characterized for the selectivity were conditioned in a 0.01 M NaCl solution identical to the inner filling solution over the night. Selectivity characterization required no prior exposure to the primary ion calcium before the measurement. Standard deviations were obtained based on the measurements of sets of at least three replicate membrane disks from the same parent membrane.

Experimental setup for coulometry

Due to the simplicity of instrumentation for constant current coulometry, we built the apparatus from a galvanostat as the constant current source and the electrolysis cell, including end-point detection system. Traditional constant current coulometry consists of a 2-electrode set-up with the coulometric electrode as the anode in the case of cations. A high surface area coiled Pt wire was used as a counter electrode. The experiments were performed with an AFCBP1 Bipotentiostat (Pine Inst., Grove City, PA). The detection circuit consisted of an ISE and a reference electrode isolated from the current generating galvanostat ground loop. In our setup ISE potentials were recorded with a battery-powered high impedance amplifier (AD820, Analog Devices)

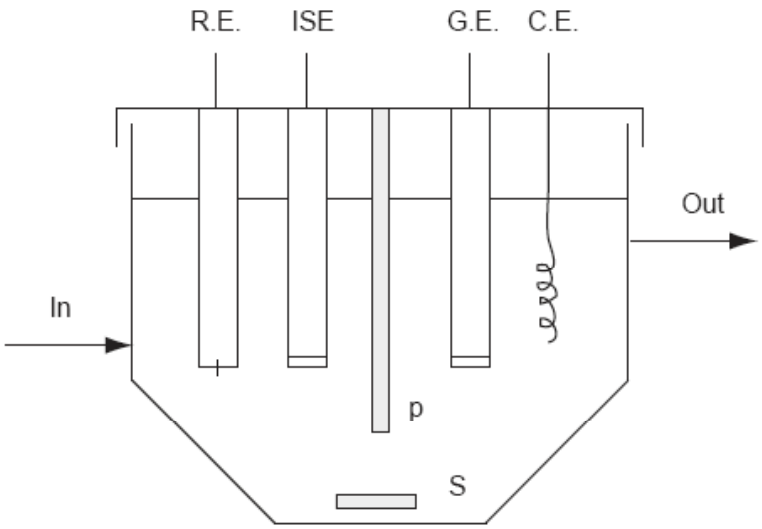

Figure. 2.2. Schematic representation of the coulometric cell with the generating ion-selective membrane anode (G.E.), a Pt counter electrode (C.E.) separated by a polypropylene separator (p) from the calcium ion-selective electrode (ISE) and a reference electrode (R.E.) S is a stirring bar; In is the solution inlet and Out the outlet, ion selective membrane based generating and Pt wire counter electrode on the other side

connected to the data acquisition system (AduC824, Analog Devices) controlled by handheld computer (Palm III) at room temperature. The external reference electrode consisted of a double-junction Ag/AgCl electrode with a 1 M LiOAc bridge electrolyte and 3M KCl as reference electrolyte.All EMF values were corrected for the liquid junction potential using the Henderson equation. The activity coefficients were calculated according to Debye-Huckel approximations.[22]

Coulometric cell

The electrochemical cell was made out of Teflon. The cell was separated into two compartments by a flexible polypropylene separator. It contained a wide opening on the top to fit all four electrodes and included ISE and reference electrodes on one side with (see Figure 2.2). The electrolysis cell was fitted with a solution inlet and outlet for the replacement of the sample with fresh solution by means of a syringe when calibration of each with 0.5 cm i.d. The wide opening on the top has a 3 cm diameter; the total height of the cell was 1.8 cm. The diameter of the flat bottom of the cell was 1.2 cm.the sensor was carried out. The volume of 1mL was measured exactly by pipetting out the sample into the cell after arranging the electrodes and closing the inlet and outlet valves.

Titration curves were calculated according to established procedures, taking into account the following EDTA complex formation constants for the considered metal ions: log K_f(Ca–EDTA) = 10.69, log K_f (Ni-EDTA) = 18.62.[23]

The sample pH of 10 and 11.6 for the two experiments were used to calculate the effective complex formation constant with $\alpha_y^{4-} = 0.36$ and $\alpha_y^{4-} = 0.93$.[23] End points were obtained according to established procedures from the second derivative plot of $[\Delta(\Delta pM^{2+}/\Delta t)] / \Delta t$ versus t. The end point is the t value when the curve crosses the abscissa.

2.3 Results and discussion

The coulometric release of calcium ions was here explored with an ionophore-based polymeric membrane calcium-selective electrode. In addition to a high concentration of inert lipophilic electrolyte to reduce the membrane resistance to about 7 kOhms, the membrane was formulated according to standard procedures with the ionophore ETH 5234 and an appropriate cation-exchanger, and backfilled with a relatively concentrated calcium chloride solution.

The ionophore ETH 5234 is known to be very selective to calcium.[24] So-called unbiased selectivity coefficients[17] of ETH 5234 based coulometric electrodes towards discriminated ions were measured and are reported in Table 2.1. These were obtained from three identical Ca^{2+} selective electrodes conditioned overnight in 0.01 M NaCl. The obtained response slopes were all near-Nernstian, suggesting that the selectivity coefficients shown are close to their unbiased values. From the small values of log $K_{Ca,J}^{pot}$ obtained for all the discriminated ions, it is evident that the membranes exhibited a high selectivity over all these potential

Table 2.1. Experimental selectivity coefficients log $K_{Ca,J}^{POT}$ for the PVC/o-NPOE releasing membrane containing Ca (IV) ionophore

Ion J	Slopes of ion J [mV dec^{-1}]	log $K_{Ca,J}^{pot}$
Na^+	46.1±2	-7.13±0.1
K^+	43.8±3	-9.32±0.2
Mg^{2+}	29. 9±1	-9.47±0.1
Ba^{2+}	27.4 ±3	-3.54±0.1
Ca^{2+}	29.7±1	0

interferences. If the membrane is not selective to the ion it releases, interfering ion from the sample may spontaneously ion-exchange into the membrane and limit the accuracy of the method. In order to facilitate the potentiometric detection of the sample calcium activity with an ion-selective electrode, cross-talk between the two electrochemical cells needed to be eliminated. The ion-selective electrode and reference electrode formed a circuit that was physically isolated from the ground loop of the galvanostat. This allowed the calcium gradients within the releasing membrane to re-establish to some extent, and hence relatively large current densities on the order of 31 x 10^{-6} A cm^{-2} could be applied. In the setup tested here, the sample volume and hence the releasing electrode area were 1 mL and 0.65 cm^2, respectively, and hence did not yet constitute a miniaturized setup.

2.3.1 Basic characterizaton of coulometric calcium release

Equi-duration steps of constant current were applied to coulometric electrodes in order to electro-release calcium into an electrolyte solution, the activity of which was observed with a calcium-selective electrode after each release step. The calcium sensing membrane was calibrated before the release experiment. It showed a typical response curve of the calcium electrode and an improved lower detection limit relative to classical ISEs.[25] The calibration was carried out in a sample solution containing 10^{-5} M NaCl as a background electrolyte.

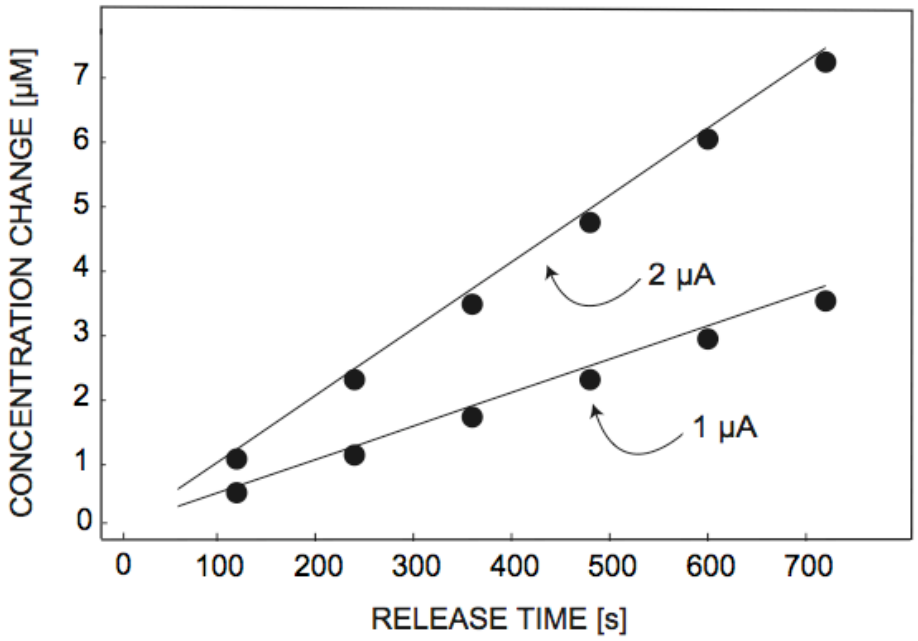

Figure. 2.3. Typical concentration, in moles of calcium, observed by coulometric release from the inner filling solution into the sample across the membrane by passing constant current pulses of 1 and 2 μA respectively. Individual release step durations were 120 s in a background of 5 x 10^{-7} M calcium chloride. The solid lines are theoretical predictions calculated with Faraday's law.

The potentiometric response of the sensor was found to be linear over the range of 10^{-9} to 10^{-5} M of calcium activity with a Nernstian electrode slope of 30 mV/decade. The potential response of these sensors was stable in the measured concentration range and exhibited high reproducibility. Figure 2. 3 shows a plot of calcium concentration increase from the calcium selective coulometric membrane electrode at constant currents of 1 and 2 mA, respectively, in a background of 5 x 10^{-7} M calcium chloride in a 1 mL sample volume. This calcium background was chosen here to avoid the occurrence of a sub-Nernstian slope for the potentiometric sensor calibration curve at low calcium activities. Individual release step durations were chosen as 120 s. The experimental points were in good agreement with theoretical predictions.

A release experiment of calcium in a higher background concentration of 0.01 M KCl gave the same results (data not shown). Figure 2.4 presents data on the coulometric release of calcium from ion selective coulometric membrane electrode with sequentially increasing current amplitudes into a background of 5 x 10^{-7} M calcium chloride in a 1-mL sample volume. Again, experimental data showed very good correspondence with theory, indicating that the applied current densities did not diminish coulometric efficiency under these conditions.

2.3.2 Direct EDTA titrations

The titration of EDTA with calcium was chosen as a typical example for a coulometric complexometric titration. Shown in Figure 2.5 are data from complexometric titrations of three different concentrations of EDTA with calcium.

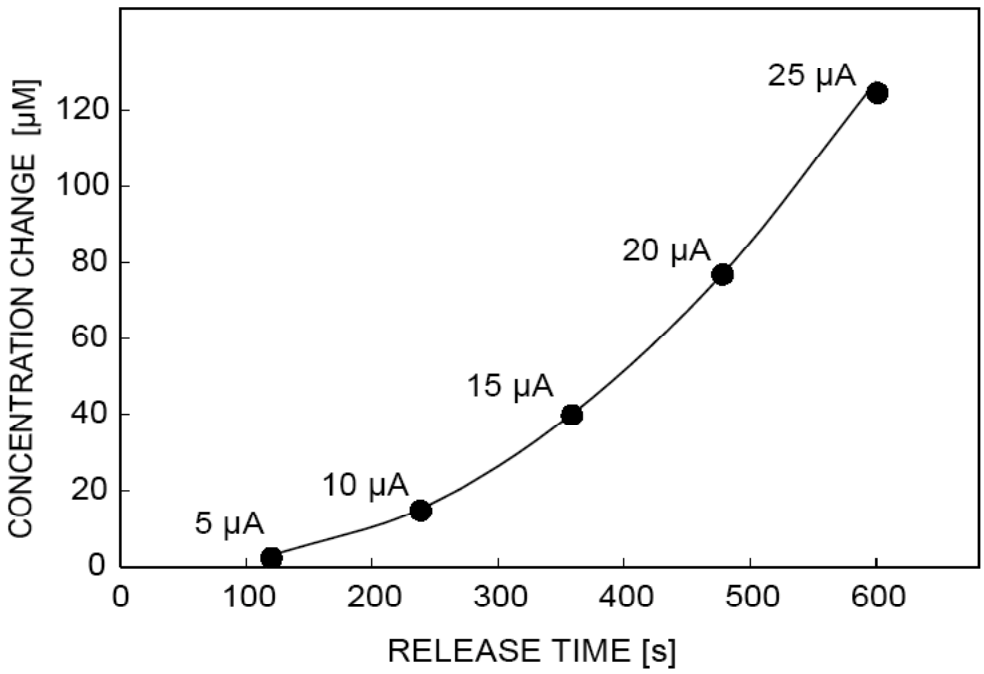

Figure.2.4. Coulometric release of calcium at increasing anodic current amplitudes. An individual release step duration of 120 s in a background of 5 x 10^{-7} M calcium chloride were used. The solid lines are theoretical predictions calculated with Faraday's law.

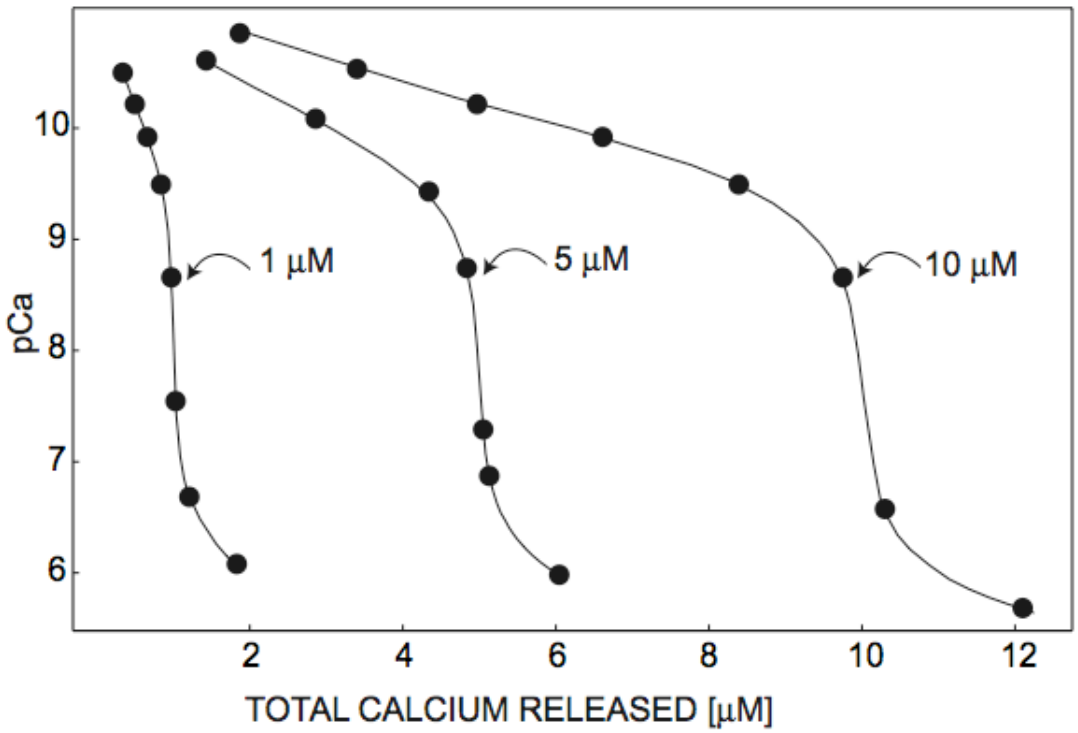

Figure. 2. 5. Coulometric titrations of 1, 5 and 10 µM EDTA with calcium. The endpoints shift to the right with increasing concentrations of EDTA. The sample was buffered to pH 10 and titrations were carried out at a constant current of 10 µA. Solid lines are the theoretically predicted titration curves.

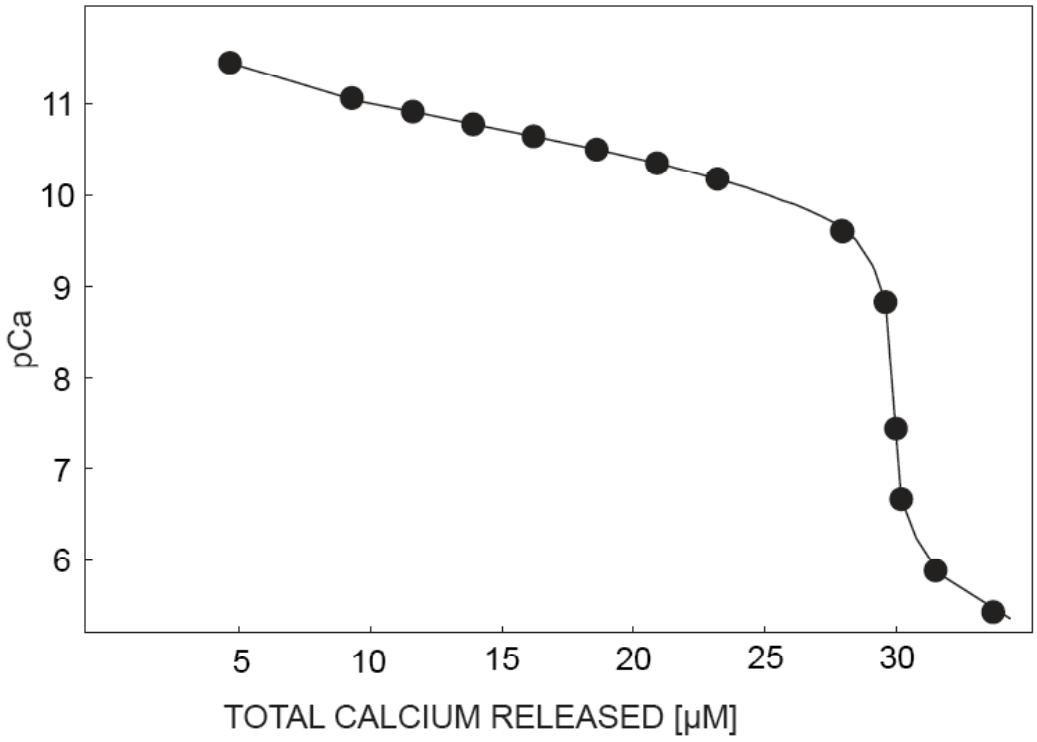

Figure. 2.6. Estimation of nickel by coulometric back titration of EDTA with calcium at a sample pH of 11.6. The titration was carried out at a constant current of 20 μA. Solid line: theoretical titration curve.

The coulometric titrations were carried out by the electro-generation of calcium ions through a 0.65 cm^2 area calcium ion selective membrane into a 1-mL sample volume containing 1, 5, 10 mM of Na_2EDTA buffered at pH 10, respectively. The titration rates were sufficiently high for these sample concentrations to be practically useful. Titration times totaled 24, 120, and 240 s, respectively, with release step durations of 3, 10, and 30 s. Here, however, step durations were shortened near the equivalence point to obtain a sharper end-point.

The observed endpoints showed good reproducibility, with a relative standard deviation on the order of less than 3 %. All the titrations were carried out at 10 μA constant current pulses. The theoretical titration curves shown in Figure 2. 5 were constructed from calculating pCa values from effective complex formation constants, as established.[23] The solid lines in Figure 2. 3 are theoretical predictions calculated using Faraday's first law of electrolysis. Overall, the experimental titration curves were in excellent agreement with theoretical predictions. The endpoints shift to the right with increasing concentrations of EDTA. The sample was buffered to pH 10 and titrations were carried out at a constant current of 10 μA. Solid lines are the theoretically predicted titration curves.

2.3.3 EDTA back titrations

As a second application for coulometric calcium release, an EDTA back titration was explored. A remaining excess of EDTA was titrated with coulometrically generated calcium to determine nickel(II) in a solution containing a large excess of iron(III) in a weakly acidic solution in the presence of triethanolamine,

following established volumetric protocols.[26] The titration was carried out at a constant current of 20 mA in a 1-mL sample volume with the sample solution buffered to pH 11.6. The resulting titration curve is shown in Figure 6. In this present setup, total titration times were on the order of 5 min. The excess EDTA was titrated with coulometrically released calcium and the amount of nickel was estimated as 6.84 ± 0.16 x 10^{-5} M. It corresponds well with initial added concentration 7.0 x 10^{-5} M nickel in the solution Again, the experimental data were in good correspondence with the theory. This is a strong indication that many other classical direct and back-titrations may be performed coulometrically by the use of ion-releasing ion-selective membranes.

2.4 Conclusions

An attractive semi-automatic coulometric method employing an ionophore-based ion-selective membrane as a coulometric electrode is reported here for the first time. Various types of titrations such as precipitation and complex formation were carried out using ion-selective membrane based coulometric electrodes, demonstrating its wide applicability. The use of ion-selective polymeric membranes for coulometric release broadens the spectrum of reagents that can be electro-released. Attractive applications include the generation and detection of various metals ions, including magnesium, and important non-electroactive reagents such as protamine for the detection of heparin via heparin–protamine titration. Further work in this direction is in progress.

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CHAPTER 3

OPTIMIZATION OF THE SELECTIVE POLYMERIC MEMBRANE ELECTRODES FOR UPPER AND LOWER LIMITS BY CONSTANT CURRENT COULOMETRY

3.1 Introduction

Constant current coulometry is an accurate and much desired method in terms of determining concentration of the analyte in the sample. Earlier, Nagy et al., demonstrated[1-6] that triangle-programmed coulometric titrations have several advantages. Flow titration combined with electro-generated concentration gradient brings about sample automation, low noise and easy coupling of on-line sampling devices to monitor the chemical and biotechnical processes. The microflow titration procedure with an electrogenerated titrant is valuable for determining low concentrations of nitrite, a common and toxic pollutant found in many natural and artificial water reservoirs,[7] and highly toxic hydrazine in the sample.[8] Miniaturization of these coulometric titrators further reduces the analysis time to seconds and increased the precision and sensitivity. [9-10]

However, non-electroactive titrants cannot be generated using metal-based coulometric electrodes. Attempts have been made to electrogenerate these titrants using an ion exchange membrane,[11,12] Coulometry based on metal and ion exchange

membranes are seldom used as routine quantitative analysis techniques like titrimetry, except for water determination (Karl-Fischer titration). The reason that this method is not broadly applied as a commercial technique is perhaps because of serious interferences arising from the greater complexity of the electrochemical reactions and also due to limited selectivity. Imparting selectivity to coulometric electrodes by using ionophore based ion-selective polymeric membranes to electro generate titrant makes coulometry more efficient in real time analysis in the presence of interferents. This method is also calibration free.[13]

In an ideal case, when a constant current of +i is applied across the membrane, a well defined flux of primary cations are expected to flow from an ion-selective membrane into the sample. However, there are two types of major limitations that plays key role in the efficiency of electro generation. They are mainly due to selectivity limitation and also due to perm-selective limitation.

Selectivity is mainly dictated by the neutral carrier, which favors the extraction of primary ion by selective complexation during conditioning. This selectivity is indeed the most important property of an ion selective coulometric membrane electrode as the above aids in selective expulsion of primary ions complexed to ionophore with 100% coulometric efficiency. The selectivity breakdown occurs by the use of a less selective membrane and also due to the membrane electrode's exposure to sample containing another equally or more preferred cation. In this case as the scheme shows, on application of constant +i the membrane releases both primary and interfering ion.

Ion-selective polymeric membranes have a definite concentration of ion exchanger, which is useful in maintaining the concentration of co-ions of opposite charges in the membrane in a controlled fashion. Although, such sites are known to inherently exist within the membrane at low concentrations additional sites are often needed to have improved membrane properties. If there is not sufficient ion exchanger sites or more lipophilic anions exist at higher concentration in the sample, the membrane loses its permselective properties which results in co-extraction of these anions. As the scheme 3.1 shows in these cases where perm-selective break down occurs instead of delivering an expected concentration of primary ion, the membrane actually extracts the co-ions of opposite charge to maintain the constant current.

This coextraction results in a break down in perm-selectivity. In coulometry, it is desirable to operate at the highest practicable current density in order to get the maximum ion flux per unit membrane area. Operating current levels are, however, restricted by concentration polarization of the components of the membrane that carries the charge such as the neutral ionophore complexed to cations and cations with anionic sites. It is important that the membrane remains perm-selective under those conditions.

The complexation and transport characteristics, which dictate the response behavior of ISEs were studied by electrodialysis experiments.[14] The electrodialysis experiments were carried out with stacks of membrane slices (40-50 μm thick each). Following the experiment, the membrane slices are separated and the concentrations of the free and complexed ionophore determined.

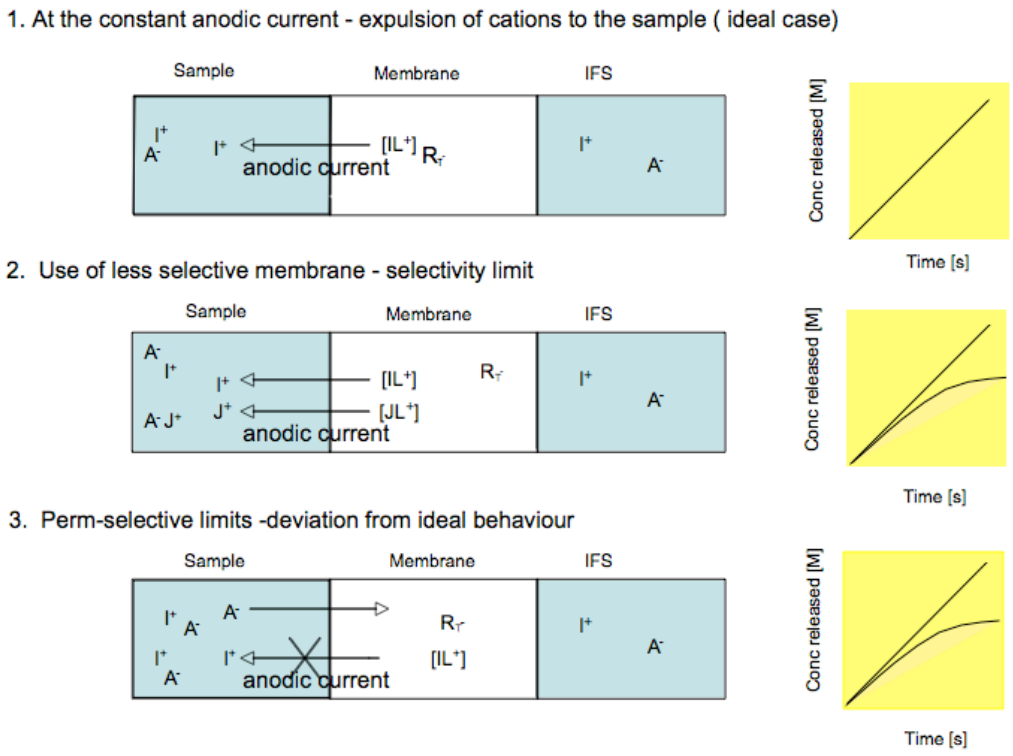

Figure 3.1 Scheme explaining the upper and lower limits of ion selective coulometric membrane electrodes

Ion-selective membranes have been characterized for electrical transport properties using impedance measurements.[15] Apart from that, Buck[16] used potentiometry along with impedance methods to characterize valinomycin with low anionic site density containing membranes for Donnan exclusion failure. Studies describing the transport mechanism and calculation of diffusion coefficients of neutral carriers[17] in ion-selective membranes using chronoamperometric transient responses were obtained as a result of an externally applied potential.[18-21]

The success of these types of matrices being used as coulometric electrodes is strongly dependent on the extent of control and understanding exerted on the limits of solvent polymeric ion-selective electrodes under current. In this chapter, therefore, the principle of trans membrane electrolyte diffusion is evaluated under constant current. Studies were also carried out to infer the effect of more lipophilic anion in the sample. Thereby, the permselectivity of the membrane subjected to constant current is also characterized. The effect of interfering ions on less selective membrane or a membrane similar to ion exchange membrane was also studied using potassium selective membrane.

3.2 Experimental

Reagents

High molecular weight poly(vinyl chloride) (PVC), the plasticizers 2-nitrophenyl-octyl ether (o-NPOE) and bis(2-ethylhexyl) sebacate (DOS), the ionophore

Calcium (IV) N, N-dicyclohexyl-N',N'-dioctadecyl-3-oxapentanediamide (ETH 5234), the lipophilic cation-exchanger salt sodium tetrakis [3,5,bis(trifluoromethyl)phenyl] borate (NaTFPB), the inert lipophilic salt tetradodecylammonium-tetrakis(4-chlorophenyl)borate (ETH 500), tetrahydrofuran (THF)and all salts were purchased from Fluka Chemical Corp. (Milwaukee, WI) in Selectophore quality or puriss quality. Aqueous solutions were prepared by dissolving the appropriate salts in nanopure-deionized water (18.2 MΩ cm).

Ion selective membrane preparation

The calcium selective membrane components consisted of 13 mmol kg^{-1} Ca(IV) ionophore, 4.5 mmol kg^{-1} NaTFPB and 10 mmol kg^{-1} of inert lipophilic salt ETH 500 together with o-NPOE and PVC (in a 2:1 mass ratio) with a total mass of 140 mg, dissolved in 1.5 mL of THF and pouring into glass ring (22 mm i.d.) affixed to a microscope glass slide. The solvent THF was allowed to evaporate overnight to give a membrane of 200 mm approximate thickness.

Electrodes

Ion-selective electrodes for the detection of the released calcium ions were prepared by punching a series of 3 mm discs from the parent membrane and gluing to plasticized PVC tube with PVC glue, which was mechanically affixed to a 1000 mL pipette tip. The detecting calcium electrodes were conditioned for 1 day in 10^{-3} M $CaCl_2$ followed by conditioning in 10^{-5} M of NaCl and HNO_3 each for one additional day. The inner filling solution contained $CaCl_2$ 10^{-3} M with 0.05 M Na_2EDTA at a pH of 5.8 adjusted by 0.01 M NaOH to give a calculated calcium activity of 3 x 10^{-8} M.

The calcium selective coulometric electrodes were prepared by punching a series of 9 mm discs from the parent membrane and gluing them to wide plasticized PVC tube with PVC glue, which was mechanically attached to a rigid Teflon tube. These electrodes were conditioned in 10^{-2} M $CaCl_2$ overnight. The inner filling solution was identical to that of the conditioning solution. The calcium selective electrodes characterized for the selectivity were conditioned in a 0.01 M NaCl solution identical to the inner filling solution over the night. Selectivity characterization required no prior exposure to the primary ion calcium before the measurement. Standard deviations were obtained based on the measurements of sets of at least three replicate membrane disks from the same parent membrane. The potentiometric calibrations with different lipophilic ions in the sample were carried out with 10^{-3} M $CaCl_2$ as inner filling solution.

Experimental setup for coulometry

Due to the simplicity of instrumentation for constant current coulometry, we built the apparatus from a galvanostat as the constant current source and the electrolysis cell, including end-point detection system. Traditional constant current coulometry consists of a 2-electrode set-up with the coulometric electrode as the anode in the case of cations.

A high surface area coiled Pt wire was used as a counter electrode. The experiments were performed with an AFCBP1 Bipotentiostat (Pine Inst., Grove City, PA). The detection circuit consisted of an ISE and a reference electrode isolated from the current generating galvanostat ground loop.

In our setup ISE potentials were recorded with a battery-powered high impedance amplifier (AD820, Analog Devices) connected to the data acquisition system (AduC824, Analog Devices) controlled by handheld computer (Palm III) at room temperature. The external reference electrode consisted of a double-junction Ag/AgCl electrode with a 1 M LiOAc bridge electrolyte and 3M KCl as reference electrolyte. All EMF values were corrected for the liquid junction potential using the Henderson equation. The activity coefficients were calculated according to Debye-Huckel approximations.[22]

3.3 Theoretical discussion

Prediction of the effect of interfering ion concentration on coulometric release:

When anodic current $+i_{app}$ is applied across the membrane, a constant flux of cations is expected from the membrane to the sample. If no interference is observed, ion selective coulometric electrodes will release a known concentration, c of the so-called primary ion I^+ according to the Nernst equation.

$$E_{PB} = \frac{RT}{z_I F} \ln \frac{K_I a_I(aq,PB)}{a_I(org,PB)}$$

Where E_{PB} is the phase boundary potential of the coulometric electrode at the aqueous and organic interface governing the constant current controlled release of I^+ without any interferent, R, T, F have their usual meaning. And $a_{I(aq)}$ and $a_{I(org).}$ The activity of the uncomplexed primary ion with charge z_I in the aqueous phase in

equilibrium with organic phase boundary. K_I is a function of the relative free energies of solvation in both the sample and membrane phase. Traditionally, the deviation from the Nernstian slope is due to the presence of other ions. Interference from the other ions, translates as a cation transference number of less than 1 in the coulometric release.

Use of less selective membrane for coulometric release:

When a less selective membrane is used as a coulometric electrode in the sample that contains a mixture of ions, the coulometric efficiency is compromised. When anodic current $+i_{app}$ is applied across the membrane, a constant flux of cations both i and j are expected from the membrane to the sample.

$$\text{Total flux, } J_{tot} = \sum J_i = \frac{i_{app}}{zFA} \qquad (1)$$

The flux of any transportable ion is given by Fick's law assuming a linear concentration gradient of ions complexed with ionophore in the membrane. This transport of ions is assumed to be only by diffusion, since electrical migration is underplayed by the presence of excess of background electrolyte in membrane. This concentration gradient is represented as follows

$$J_i = \left(\frac{D_{mem}}{\delta_{(t)}}\right)\left\{C_{i,Ln,T(mem,Bulk)} - C_{i,Ln,T(mem,PB)}\right\} \qquad (2)$$

Where, D_{mem} – diffusion coefficient in the membrane phase, $\delta_{(t)}$ – diffusion layer thickness with time. $C_{iLn,\,T}$ $(_{mem,\,Bulk})$ – are concentration of ions at the bulk and $C_{iLn,\,T}$ $(_{mem,\,PB})$ - Concentration of ions at phase boundary.

Ions exist in the membrane as either complexed or uncomplexed to ionophore in a

fixed stoichiometry; so, equation (1) can be rewritten as

$$J_i = \left(\frac{D_{mem}}{\delta_{(t)}}\right)\left\{C_{i(mem,Bulk)} + C_{i,L_n(mem,Bulk)}\right\} - \left\{C_{i(mem,PB)} + C_{i,L_n(mem,PB)}\right\} \quad (3)$$

Since concentration of ions complexed to ionophore in the bulk and phase boundary is very large, hence those terms with free uncomplexed ions can be neglected as there is an excess of ionophore, and equation 2 can be rewritten as:

$$J_i = \left(\frac{D_{mem}}{\delta(t)}\right)\left\{C_{i,L_n(mem,Bulk)} - C_{i,L_n(mem,PB)}\right\} \quad (4)$$

And the diffusion layer thickness as a function of time is given as:

$$\delta(t) \approx 2\sqrt{Dt} \quad \text{(From 2}^{\text{nd}}\text{ Fick's law)} \quad (5)$$

On application of constant current $+i_{app}$ for a less selective membrane in contact with a mixture of ions of same charge, z complexed with the ionophore, the equation (4) can be expanded

$$J_i + J_j = \frac{i_{app}}{zFA} = \left(\frac{D_{mem}}{\delta_{(t)}}\right)\left(\left\{C_{i,L_n(mem,Bulk)} - C_{i,L_n(mem,PB)}\right\} + \left\{C_{j,L_n(mem,Bulk)} - C_{j,L_n(mem,PB)}\right\}\right) \quad (6)$$

Suppose a mixture of two ions of same charge exists in the sample preferred equally by the membrane, then the ions complexed to the ionophore is given after substituting equation (4) into (5) and eliminating δ_{mem}, we get

$$\frac{i_{app}}{FA}\sqrt{\left(\frac{D_{mem}}{t}\right)} = \left\{C_{i,L_n(mem,Bulk)} + C_{j,L_n(mem,Bulk)}\right\} - \left\{C_{i,L_n(mem,PB)} + C_{j,L_n(mem,PB)}\right\} \quad (7)$$

If the sample phase boundary concentration is assumed to be equal to sample bulk as they are in equilibrium with each other then above equation can be written as

$$\frac{i_{app}}{FA}\sqrt{\left(\frac{D_{mem}}{t}\right)} = \left\{\left[IL_n^{zI+}\right]_{(mem,Bulk)} + \left[JL_n^{zI+}\right]_{(mem,Bulk)}\right\} - \left\{\left[IL_n^{zI+}\right]_{(mem,PB)} + \left[JL_n^{zI+}\right]_{(mem,PB)}\right\}$$

As for a membrane with interference, the lipophilic ion exchanger concentration dictates the complexes of primary and interfering ions

For a K (III) ionophore with calcium ion as primary and magnesium as interfering ion

n = m = 1

And z = 2, Simplifying,

$$L_T = [L] + [IL_n^{zI+}] + [JL_n^{zJ+}] \quad (8)$$

L_T = Total ionophore concentration. Always an excess of ionophore is present.

Charge balance for the membrane phase is written as

$$[R^-_T] = [IL_n^{ZI+}] + [IJ_n^{ZI+}] \quad (9)$$

$$E_{PB} = \frac{RT}{z_I F}\ln\frac{R_T}{Z_I} + \frac{RT}{z_I F}\ln\left[\frac{a_I}{IL^{ZI+}{}_n}\right] \quad (10)$$

This relationship is inserted into eq 1, together with the mass balance and charge balance in eq 8 and 9 to describe the phase boundary potential as a function rewriting above,

$$E_{PB} = \frac{RT}{z_I F}\ln\left[K_{IJ}^{pot} a_J^{Z_I/Z_J}\right] \quad (11)$$

The pulsed coulometric mixed-solution membrane response is expected to obey the Nicolsky equation, where the selectivity coefficient is given by K_{IJ}^{POT} the relationship between the phase boundary potential and selectivity is given as follows

$$\frac{c_I}{\left[IL_{n(mem,Bulk)}^{zI+}\right]_{(t)}} = \frac{z_I}{R_{T(t)}}\left[c_I + K_{IJ}^{pot} c_J\right]$$

$$Z_I\left[IL_{n(mem,Bulk)}^{zI+}\right]_{(t)} = \frac{c_I R_{T(t)}}{\left[c_I + K_{IJ}^{pot} c_J\right]}$$ and from equation (9)

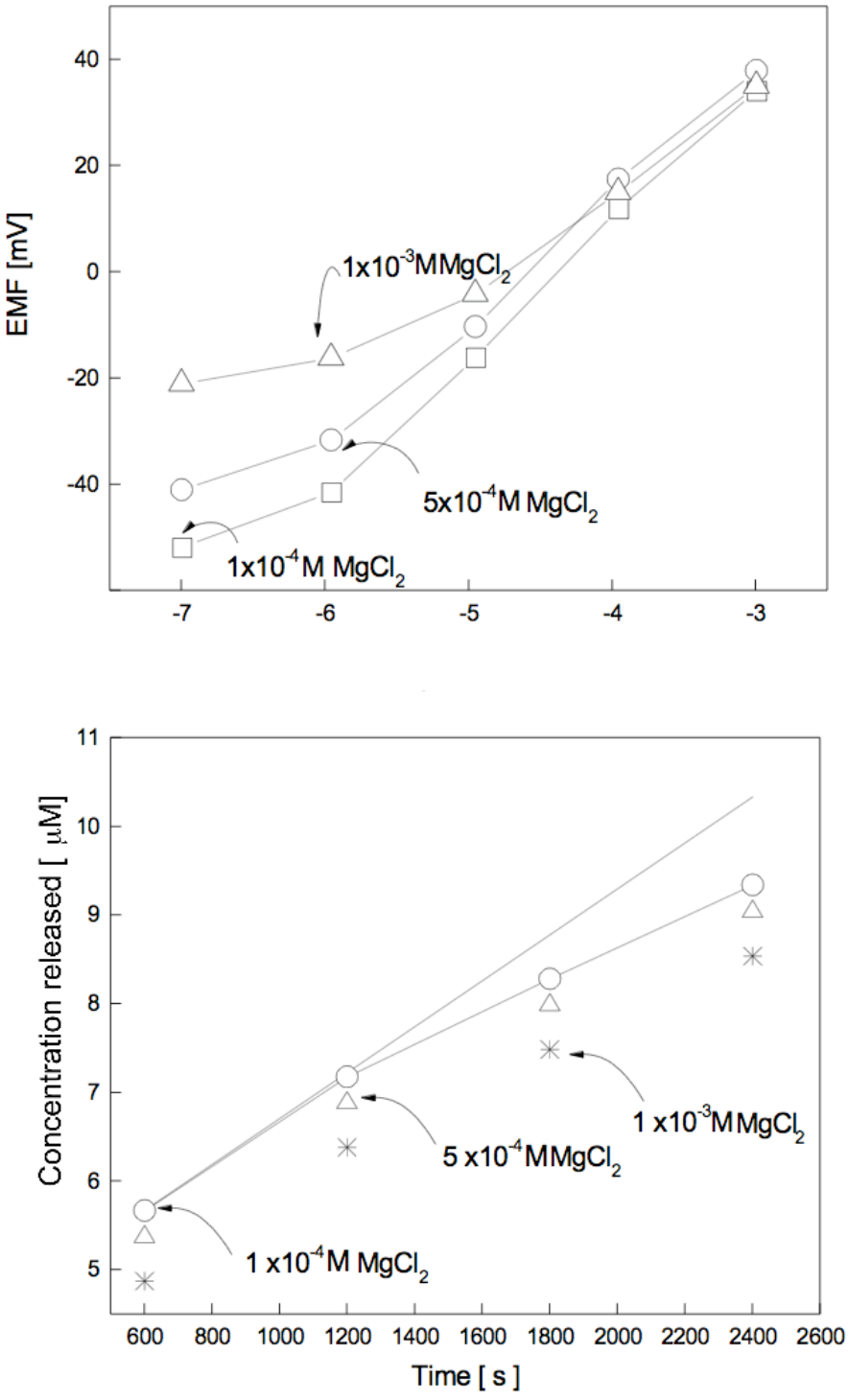

Figure 3.2 a,b. Potentiometric calibration curves for K(III) membrane in the presence of magnesium in varying concentrations and coulometric release of calcium from the K(III) membrane in the presence of varying magnesium concentrations

$$\left[IJ_{n(mem,Bulk)}{}^{zI+}\right]_{(t)} = R_{T(t)} - \left[IL_{n(mem,Bulk)}{}^{zI+}\right]_{(t)} \quad (13)$$

As we apply current, the ion release process is eventually no longer a selective transfer by the ionophore. And found to deviate from the slope of the electrode response observed for the same membrane. For coulometric ion selective membrane electrodes, the higher the concentration of interfering ion in the background, the higher will be the suppression of coulometric release of primary cations. This relationship is again analogous to the Nicolsky equation for ion-selective electrodes, now with K_{IJ} as the selectivity coefficient.

3.4 Results and Discussion

Effect of background electrolyte concentration on the less selective membrane

The Fig.3.2a illustrates effect of increasing magnesium concentration in the background on the potassium selective membrane with calcium as primary ion determined potentiometrically. As the figure shows magnesium interferes strongly bringing the detection limit down with increasing concentrations. Fig.3.2b shows the coulometric release of calcium into the samples containing different concentrations of $MgCl_2$ as the background electrolyte. Coulometric release was from a potassium selective membrane conditioned with calcium. The electro release is carried out 5mA of constant current.

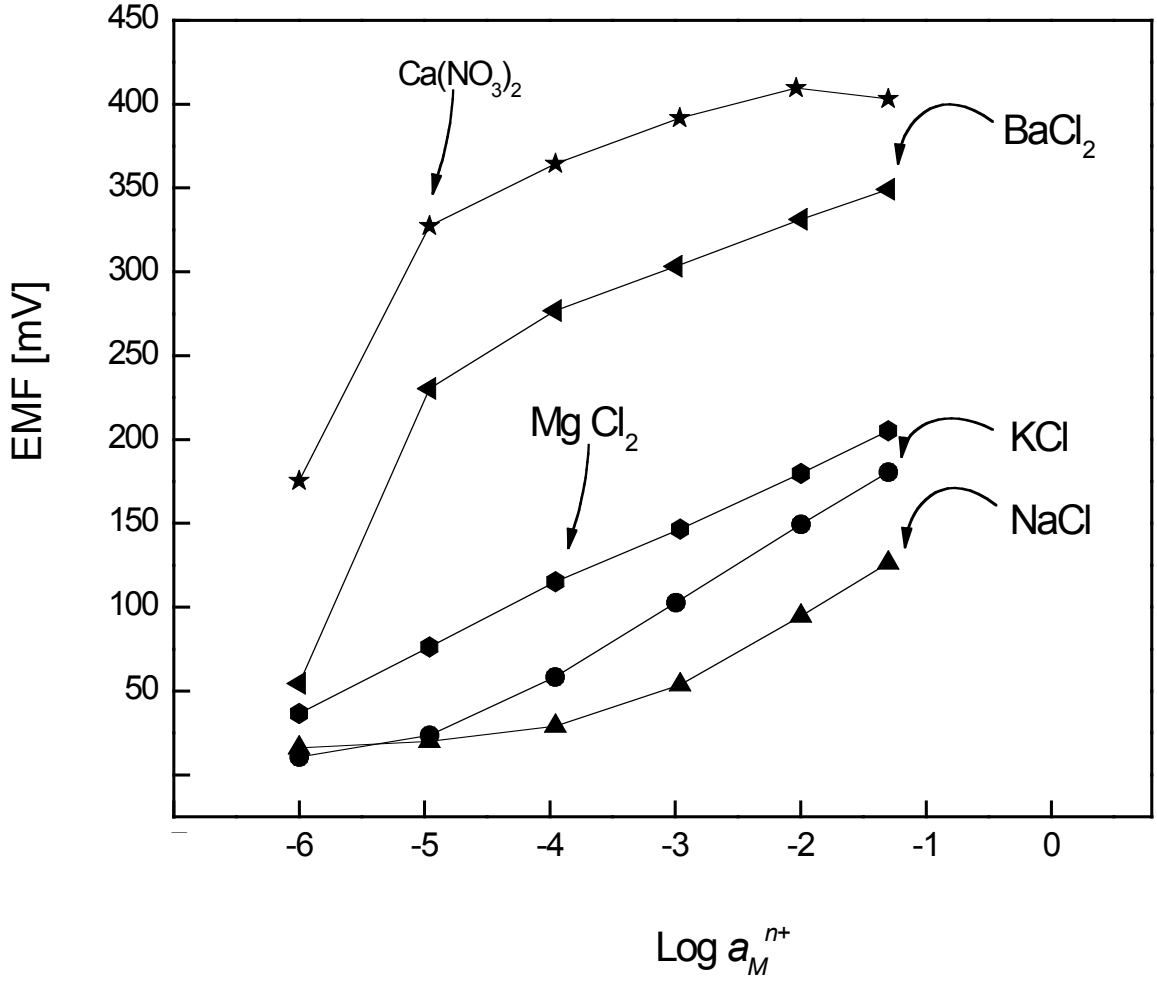

Figure 3.3. Potentiometric calibration curves of various interfering ions for ca selective membrane

Measured selectivity coefficients for potassium selective membrane shows that it is not very selective towards calcium. $MgCl_2$ is highly interfering, and it actually has the same selectivity coefficient as that of calcium for K (III) ionophore.

Lipophilic anion interference

Unbiased selectivities of the Calcium selective release membrane containing NaTFPB and calcium ionophore ETH 5234 were obtained without prior exposure to the primary ion calcium. The Fig. 4.2 shows the calibration curves obtained for different cations Mg, K, Na, Ba in the listed order with chloride as their counterion. A calibration curve for calcium with lipophilic NO^{3-}counter anion was also obtained after barium. It showed Nernstian response from 10^{-4} to 10^{-3} M. With increasing calcium ion activity, typically around 10^{-2} M interfering NO_3^- anions may penetrate the membrane through coextraction owing to a saturation of neutral carrier.

After which the membrane will ideally become anion selective in a Nernst fashion. Coextraction determines the true ion exchange capacity of the ion selective membranes. Coextraction could occur either due to the binding characteristic of the ionophore, the lipophilicity of the interfering anions or due to high sample electrolyte concentrations. The anion interference always follows the Hofmeister selectivity sequence. Therefore, we can see that in the calibration curve of calcium with Cl^- counter anion, the active range where coulometric electrodes can be used is about an order of magnitude more than that of calcium with lipophilic NO_3^- counter anion.

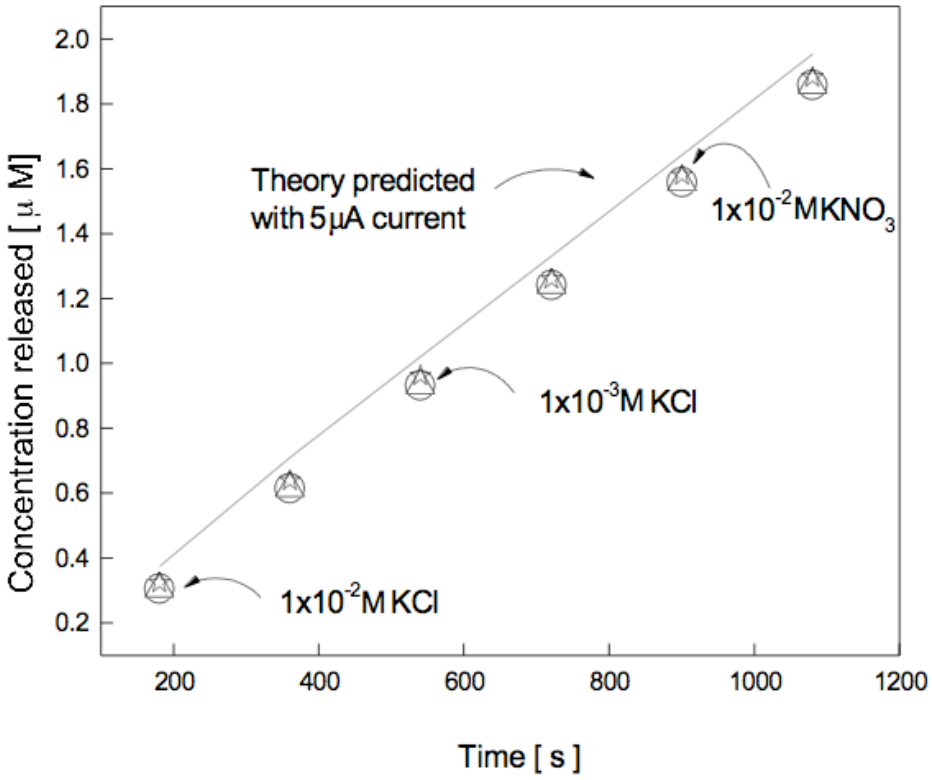

Figure 3.4. Coulometric release of calcium in the presence least interfering ion potassium in varying concentrations and with varying lipophilic anions

Effect of interfering lipophilic anion in the sample on the highly selective membrane

The Fig. 4.3 shows coulometric release of calcium from the calcium selective membrane into the samples containing different concentrations upto 10^{-2} M KCl and a higher concentration of 10^{-2} M KNO_3 as the background electrolyte. From the unbiased selectivity measurements above, we know that potassium is one of the least interfering cations for the calcium selective membrane based on Ca (IV) ionophore. Ligands exhibit carrier properties for the ions in question and therefore induce permeability selectivity in the corresponding membranes. As a consequence, there should be a correlation between the ion selectivity of membranes observed coulometrically and the ion transport selectivity of the same membranes.

The membrane behaves perm-selective under ideal conditions, so higher a concentration of least interfering background electrolyte does not lead to breakdown in perm selectivity. Throughout, a cation transference number of nearly 1 is found which is in perfect agreement with the slope of the electrode response observed for the same membranes potentiometrically. No extraction of anions from the sample results, if higher concentration of lipophilic nitrate is present in the sample for a highly selective membrane for calcium.

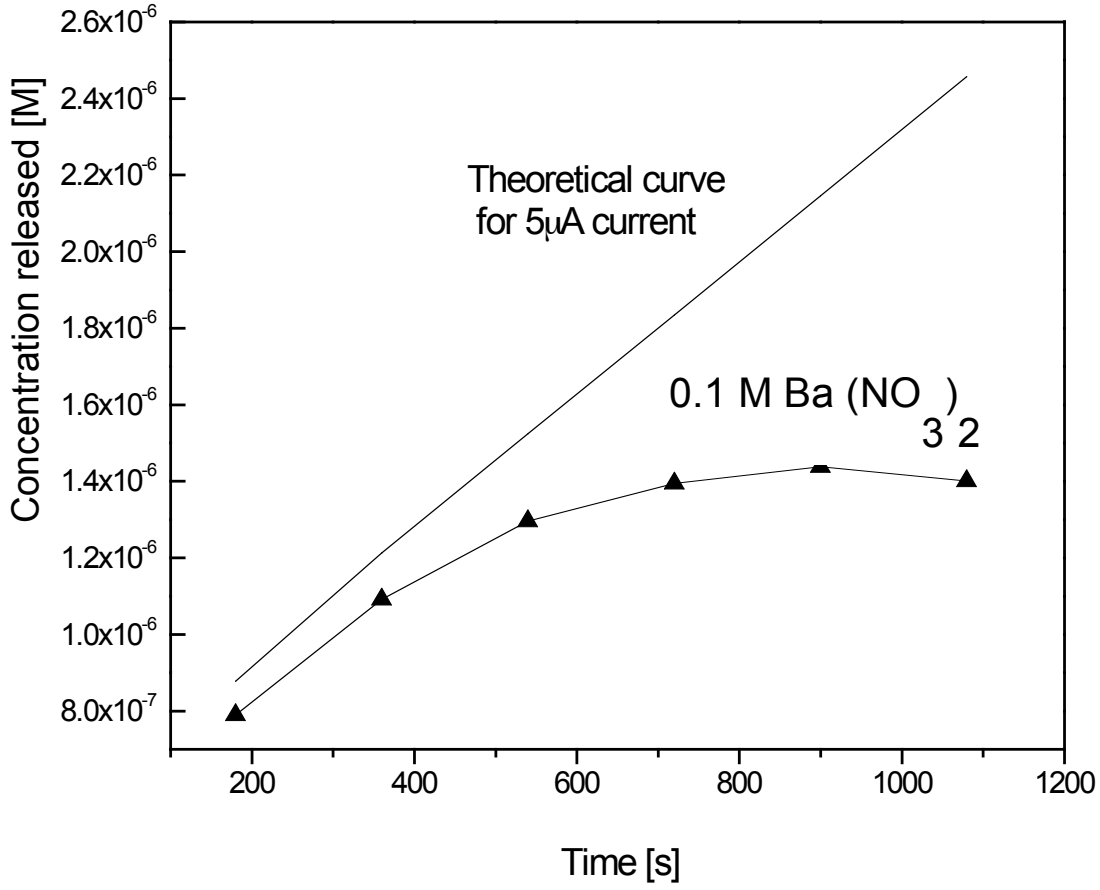

Figure 3.5. Coulometric release of calcium in the presence highly interfering ion barium with liphophilic anion nitrate as counterion

Effect of interfering lipophilic anion on the concentration polarization of ion selective membrane based coulometric electrodes

A constant current of 5 μA is applied across the ion selective membrane coulometric electrode for a short duration of seconds and after which released calcium ions are measured using calcium lower detection limit potentiometric electrode palm based hand held computer. As we know, these ion selective membrane based electrodes have polarizing interfaces and depletion of the ionophore complexed with primary calcium ions occurs at the inner filling membrane phase boundary.

As membrane relaxes between the current pulses, the release of the cations continues with 100% coulometric efficiency before it really undergoes concentration polarization. The solid line theoretical prediction calculated using Faraday's 1st law. And the points are experimentally obtained and as you can see from the figure 3. 5 this process are not efficient for the samples having higher concentration of interfering ions in the background. The break down in permselectivity occurs at a shorter time for this calcium selective membrane as we see.

3.4. Conclusions

Coulometry could be made a routine analytical technique by the use of solvent polymeric membrane with the selective ionophore as even, nonelectroactive analytes could be delivered. This article summarizes the main limitations involved in the membrane based coulometric technique.

Two main factors defining the selectivity limits of the polymeric membrane based coulometric electrodes will be; 1) Limits due to the discrimination of the interferent 2) The upper limit which is dictated by the analyte to ionophore binding stability and coextraction due liphophilic counter anion in the sample. Hence, high selectivity is required for the practical realization of ion selective polymeric membrane based coulometry. However, the practical limit depends on the sample concentrations range targeted. Complete evaluation of ionophore based polymeric ion selective coulometric electrodes to the effects of use of less selective ionophore, a higher concentration of interfering ion as a background electrolyte and to the presence of more lipophilic anion in the sample was carried out. The next main limitation is concentration the polarization of the ion selective membrane, which brings the process efficiency down to less than 100%.

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CHAPTER 4

CHRONOPOTENTIOMETRIC CHARACTERIZATION OF THE PERMSELECTIVE PROPERTIES OF ION SELECTIVE POLYMERIC MEMBRANES DURING CONSTANT CURRENT COULOMETRY

4.1. Introduction

Constant current coulometry is an appealing replacement in many aspects to regular titrimetry. In a very recent application of constant-current coulometry in clinical diagnostics, a method of determination of human serum albumin using electrochemically generated Br_2 or I_2 in human blood was introduced. This constant current coulometric method proved to be more accurate, precise, and sensitive compared to the spectrophotometric procedure. Coulometry is useful especially, wherever handling of highly toxic chemicals are required. Very recently, arsenic oxide was estimated as the standard reagent by the use of constant current coulometry. A constant current was used to electrogenerate the titrant I_2 at the platinum-working electrode by passing through the weak basic water solution with potassium iodide and standard reagent arsenic oxide, then the I_2 reacted with arsenous acid. The electrolytic current and time were ascertained to estimate the recovery of the method to be 100%.

Coulometric nanotitrators helped in miniaturization of these coulometric titrators effectively by reducing the analysis time to seconds, and increasing the precision and sensitivity.[3,4] However, non-electroactive titrants cannot be generated using metal-based coulometric electrodes. Attempts have been made to electrogenerate these titrants using an ion exchange membrane.[5,6] However, it could not be used as a commercial technique, perhaps because of serious interferences arising from the greater complexity of the electrochemical reactions and also due to limited selectivity. Imparting selectivity to coulometric electrodes, by using ionophore based ion-selective polymeric membranes to electro generate titrant, makes coulometry more efficient in real time analysis in the presence of interferents. This method is also calibration free.[7]

Ion -selective electrodes are currently one of the most extensively used field in areas of applications, including industrial process control, biomedical screening, pollution monitoring and clinical laboratories. Ionophore based ISEs are made out of plasticized polymeric PVC matrix along with ion exchanger sites and a lipophilic ionophore. The passing of current or voltage across the PVC membrane is not entirely new. The electrical and transport characteristics dictating the response behavior of ISEs were studied by electro dialysis experiments.[8,9]

Following the experiment, the membrane slices have been separated and the concentrations of the free and complexed ionophore were determined in the membrane slices. Impedance measurements[10-13] and conductivity measurements[14] were used to understand and describe the transport mechanism of the neutral carrier based ion selective membrane. Horvai and Buck extensively characterized the fixed site ion selective membranes using a.c. impedance by varying the bathing concentrations, the

time of soaking of membranes in the bathing electrolytes and the use of different plasticizers and so on. In a pioneering work, Armstrong was able to show how impedance measurements could be used to map both the bulk and interfacial charge-transfer of valinomycin-based PVC matrix potassium selective membranes in contact with aqueous solutions.

Calculation of diffusion coefficients of the neutral carrier in ion-selective membranes were based on chronoamperometric transient responses obtained as a result of externally applied potentials.[15-18] An ionophore concentration profile under a voltage step was completely researched. Current-voltage curves obtained gave an idea about field induced extraction that was confirmed with the evidence from the impedance data. Cyclic voltammetry, which was widely studied in liquid/ liquid interfaces, was applied as a technique to explore ion transfer at the solvent polymeric membranes used as ion selective electrodes.[19] Jadhav et al., also explored important response features of voltammetric and amperometric ion selective membranes with two polarizing interfaces.

Ion transfer mechanisms for voltammetric sensor membranes were studied with and without ion exchanging capabilities. Dummy membranes containing a lipophilic salt additive were examined with symmetric and asymmetric bathing.[20] All these electrochemical techniques, which studied the ion fluxes as a result of electro-assisted transport, improved the chances of reaching lower detection limits in ion selective electrodes. Evidently, researchers showed that lower detection limits are achieved not only due to the selective ionophore, but it also depend upon the elimination of greater ion flux resulting from the use of a traditional inner filling solution to the sample.

Pretsch and coworkers[21,22] used buffered inner filling solution to prevent leaching of primary ions from the membrane sample interface, there by improving the detection limits to the sub nano-molar range. Erno Lindner and colleagues[23] showed another way to modulate transmembrane ion fluxes and eliminate primary ion flux across the membrane by applying nano-amperes of direct current. The current is passed to remove the leaching ions that increase the ionic concentrations in the interface hence; the micro molar and sub-micro molar sample activity gradient remains flat. With this phenomenal invention, Nernstian response up to $3x10^{-12}$ M was achieved

In another method of application of an external current, Shvarev et al. described a pulsed galvanostatic technique to investigate ion-selective electrodes (ISEs) having no essential ion-exchanger properties. [24,25] Morf attempted to provide a theoretical explanation for the effects of externally applied current on the transmembrane ion fluxes and on the potential response of ISE membranes.[26-28] The main transport mechanism under current was identified as a current-induced migration of ions, which is overlaid on the zero current diffusional fluxes that usually influence the detection limits of ISEs.

Recently, the chronoamperometric transients were used to estimate the ionophore loss from fixed-site, solvent polymeric membranes.[29, 30] The effect of applied voltage on the current time transients of a valinomycin-based potassium electrode loaded with varying concentrations KTpClPB was interrogated by Lindner group.

They attempted to calculate the diffusion coefficient of the cation exchanger TPB^-, and suggested that the flux of $Kval^+$ and TPB^- is dependent on the slower moving TPB^-. Coulometry could be made a routine analytical technique by the use of solvent polymeric membrane with the selective ionophore as even, non-electroactive analytes could be delivered. Two main factors defining the selectivity limits of the polymeric membrane based coulometric electrodes will be; 1) limits due to the discrimination of the interferent based on the selectivity of the membrane. Hence, high selectivity is required for the practical realization of ion selective polymeric membrane based coulometry. However, the practical limit depends on the sample concentration ranges targeted. 2) The perm-selective limit is dictated by the analyte to ionophore binding stability and interference due to lipophilic counter anion in the sample.

In coulometry, it is desirable to operate at the highest practicable current density in order to get the maximum ion flux per unit membrane area. Operating current levels are, however, restricted by concentration polarization of the components of the membrane that carries the charge such as the neutral ionophore complexed to cations and cations with anionic sites. It is important that the membrane remains perm-selective under those conditions. In this paper, therefore, the principle of trans membrane electrolyte diffusion is evaluated under constant current. A simple model was developed to predict the steady-state delivery of cations as a function of current and observed limiting concentration polarization of the membrane using chronopotentiometry.

The transition time obtained experimentally gives the ionophore concentration. Studies were also carried out to infer the effect of more lipophilic anion in the sample. Thereby, perm-selective break down of the membrane subjected to current is also characterized. This paper introduces a chronopotentiometric technique as a diagnostic tool for the interrogation of ionophore-based polymeric membranes based on a simple model system containing a silver-selective ionophore.

4.2 Theory

Upon applying an external constant current pulse across the membrane, a flux of positive charge in direction of the sample solution results. It is assumed that the initial membrane composition will favor the release of primary ions from the membrane, rather than the uptake of anions from the sample in direction of the membrane. The loss of primary ions from the membrane will lead to gradual accumulation of uncomplexed ionophore at the sample side of the membrane, coupled to a decrease in primary ion complex concentration.

Elecroneutrality is maintained by a concentration of lipophilic ion-exchanger that matches that of the primary ion complex. At the inner membrane side, the reverse process must take place, with a loss of free ionophore coupled to a gradual increase of ion-exchanger and complex concentrations. The loss of either one of these components may lead to breakdown of coulometric efficiency. Mathematically, a simple diffusion model based on Fick's first law of diffusion can approximate these processes.

If no interferences take place before one of the critical membranes decrease to zero, the applied current *i* relates to the primary ion flux *J* as follows:

$$i = zFAJ \tag{1}$$

Assuming mass transport by diffusion only, and linear concentration gradients across the diffusion layer thickness d of the membrane, the flux may be approximated by the concentration gradient of ionophore or ion-exchanger within the diffusion layer of the membrane:

$$J = \frac{D_m}{\delta_m}\left(C_{m,bulk} - C_{m,PB}\right) \tag{2}$$

Which is rewritten after considering $\delta_m \approx 2\sqrt{Dt}$:

$$\frac{i}{zFA} = \frac{1}{2}\sqrt{\frac{D_m}{t}}\left(C_{m,bulk} - C_{m,PB}\right) \tag{3}$$

Once the concentration at the phase boundary approaches zero, t reaches the critical time, t_{crit}, and one obtains:

$$t_{crit} = \frac{D_m}{4}\left(\frac{zFA}{i}C_{m,bulk}\right)^2 \tag{4}$$

A similar type of equation has been earlier introduced in similar form by Buck[23] and Lindner[25], who used it to determine the residual ionophore concentration in the membrane by chronopotentiometry. Diffusion coefficients in the membrane are typically on the order of 1 x 10^{-8} cm^2 s^{-1}.

With typical values of i/A = 10 μA cm^{-2}, z = 2, $C_{m,bulk} = 10^{-2}$ mol dm^{-3}, a critical time t_{crit} = 93 s is estimated from equation 4. Note that equation 4 should be adequate to assess the breakdown on the basis of loss of ionophore or ion-exchanger from the phase boundary.

4.3 Experimental

Reagents

High molecular weight poly(vinyl chloride) (PVC), the plasticizers 2-nitrophenyl-octyl ether (o-NPOE) and bis(2-ethylhexyl) sebacate (DOS), the ionophore Calcium (IV) N, N-dicyclohexyl-N',N'-dioctadecyl-3-oxapentanediamide (ETH 5234), the lipophilic cation-exchanger salt sodium-tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), the inert lipophilic salt tetradodecylammonium-tetrakis(4-chlorophenyl)borate (ETH 500), tetrahydrofuran (THF) and all the salts were purchased from Fluka Chemical Corp. (Milwaukee, WI) in Selectophore quality or puriss quality. Aqueous solutions were prepared by dissolving the appropriate salts in nanopure-deionized water (18.2 MΩ cm). The Silver ionophore IV (O, O"-bis [2-(methylthio) ethyl]-*tert*-butylcalix [4] arene was synthesized in our lab similar to the procedure mentioned[31.]

Ion selective membrane preparation

The silver selective membranes contained 15 mmol kg^{-1} Cu (II) ionophore I, 5.54 mmol kg^{-1} of NaTFPB, DOS and PVC (in a 2:1 mass ratio) with a total mass of 240

mg. These were dissolved in 2 mL of THF for about 2 hours and poured into glass ring (24 mm i.d.) affixed to glass microscopic slide to give a membrane of 200 μm approximate thickness. The membranes for solid contact microelectrodes used for the detection were prepared by dissolving 50 mg of components in 0.8 ml of methylene chloride: copper (II) ionophore (I) (17 mmol/kg), lipophilic cation-exchanger (7 mmol/kg), ETH 500 (12 mmol/kg), MMA-DMA. The membrane cocktail was degassed by sonication for 1 min prior to coating the microelectrodes.

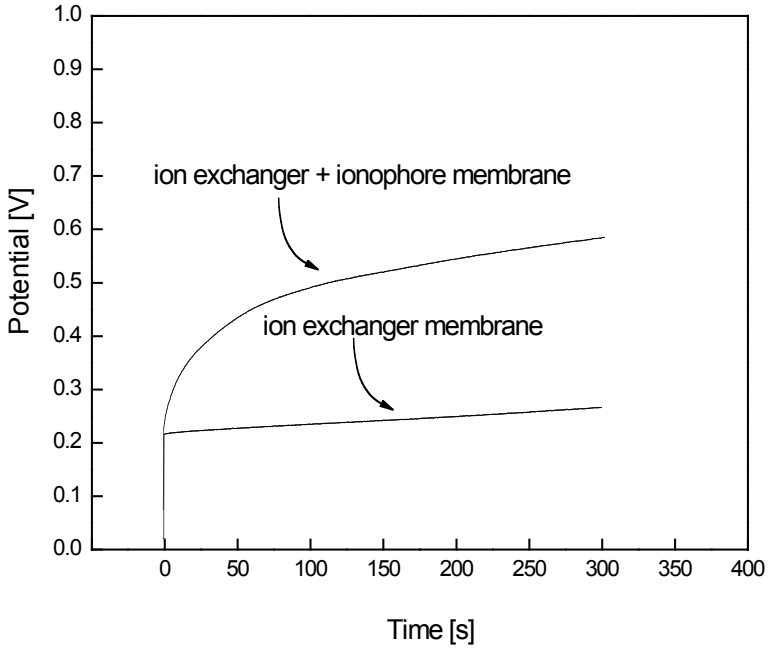
Electrodes

Regular silver electrodes were prepared by punching a series of 3 mm discs from the parent membrane and gluing to plasticized PVC tube with PVC glue, which was mechanically affixed to a 1000 mL pipette tip. All types of silver membranes used for chronopotentiometric studies were conditioned with 10^{-3} M $AgNO_3$ in the background of 0.1 M $NaNO_3$ for 48 hours. For the experiments with different concentrations of lipophilic ions and different lipophilic ions at least a day with above conditioning solution followed by $5x10^{-4}$ M $AgNO_3$ in 0.1 M Na salt with respective lipophilic anion. The inner filling solution was identical to that of the sample solution in all experiments execept for the experiments with the different concentrations of lipophilic ions and different lipophilic ions. Standard deviations were obtained based on the measurements of sets of at least three replicate membrane disks from the same parent membrane. For solid contact microelectrodes followed by 1 day in 10^{-9} M $AgNO_3$ in the background of 10^{-5} M $NaNO_3$. Standard deviations were obtained based on the measurements of sets of at least three replicate membranes.

Membrane cell

The experimental cell used to obtain chronopotentiograms was made out of Plexiglas. The cell contained a pair of solution ports for inlet and outlet. The cell was composed of two equal volume ($6cm^3$) compartments. The effective area of the membrane was 1.2 cm^2. The experiment was carried out by applying a constant current between the two large surface area Pt electrodes across the membrane. Chronopotentiometric studies were carried out with 0.001 M $AgNO_3$ in the background of 0.01 M $NaNO_3$ as the electrolyte in the case of silver selective membranes and 0.001 M silver with respective counter anion like acetate, perchlorate and nitrate and their respective sodium salt of 0.01 M concentrations as their background. Two Ag/AgCl electrodes were used as the references.

Chronopotentiogram of a coulometric membrane electrode was obtained in a transport cell with both compartments filled with in 1mM of silver nitrate in the background of 0.1 M $NaNO_3$. Fig. 4.1.a shows a typical chronopotentiograms of a silver selective membrane and an ion exchanger membrane containing NaTFPB conditioned in 1 mM of silver nitrate in the background of 0.1 M $NaNO_3$ at 20 μA current. These are the typical experimental conditions for all the experiments in this article unless otherwise mentioned. On application of constant electric current across an ion selective membrane placed in contact with an aqueous electrolyte solution, the potential (E) immediately rises because of the membrane and solution resistances.



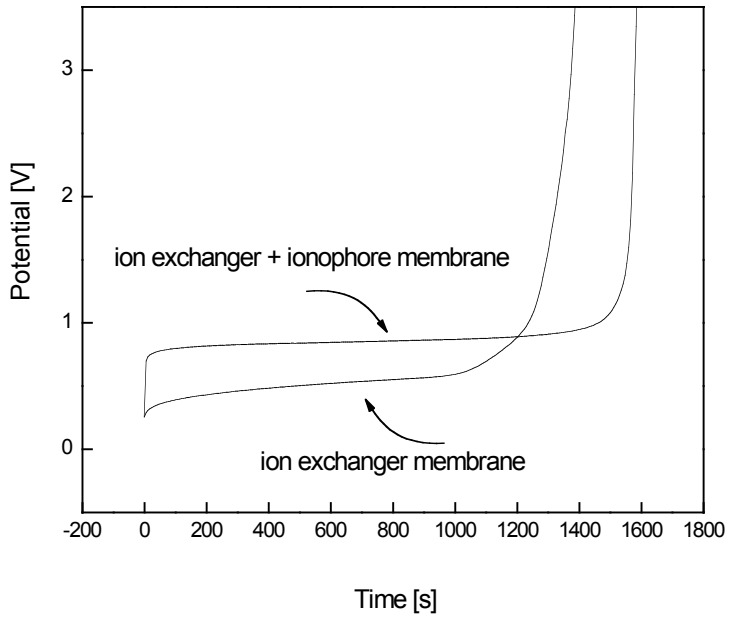


Fig. 4.1a, b. Chronpotentiograms obtained at 20 μA current for the ion exchanger only and the normal membrane containing both ion exchanger and Ag/Cu(II) ionophore in a stochiometric proportion in the electrodialysis cell with symmetric $AgNO_3$ 0.001 M concentration with 0.1 M $NaNO_3$ in the background. A) is the initial portion of the chronopotentiogram obtained.

After the initial ohmic region, the chronopotentiogram obtained for a regular membrane formulated with stochiometric proportions of ionophore and cation exchanger had 3 distinct regions. Out of the three, there were two transitions As illustrated in Fig.4.1.inset, an initial transition was observed around 100 to 200 seconds for that particular electrochemical cell configurations and a final drastic transition region around 30mins for the Ag^{+} selective membrane (see Fig 4. 1.).

In order to characterize the transitions, it was proposed to record chronopotentiogram for the membrane containing only ion exchanger and compare it with the regular ion selective membrane. When compared to the chronopotentiogram of regular membrane, notice that the first transition is absent in the chronopotentiogram obtained for the membrane containing only the ion-exchanger (see Fig 4. 1. inset). It seems only fair to conclude that the first transition could be from ionophore, which may be due to concentration polarization of ionophore. The final drastic transition seems to be common to both the ion exchanger and as well as regular membrane.

Characterization of initial transition

In order to verify our hypothesis, chronopotentiograms of membranes containing different Ag/Cu (II) ionophore (15mmol/Kg and 30 mmol/Kg) loading were obtained (see Fig.4.2). The membrane with twice the ionophore loading shows an increase in initial transition time (t_{crit1}) when compared to membranes with regular ionophore loading. This behavior in transition time may be attributed to the fact that an increase in ionophore concentration forces it to take more time to

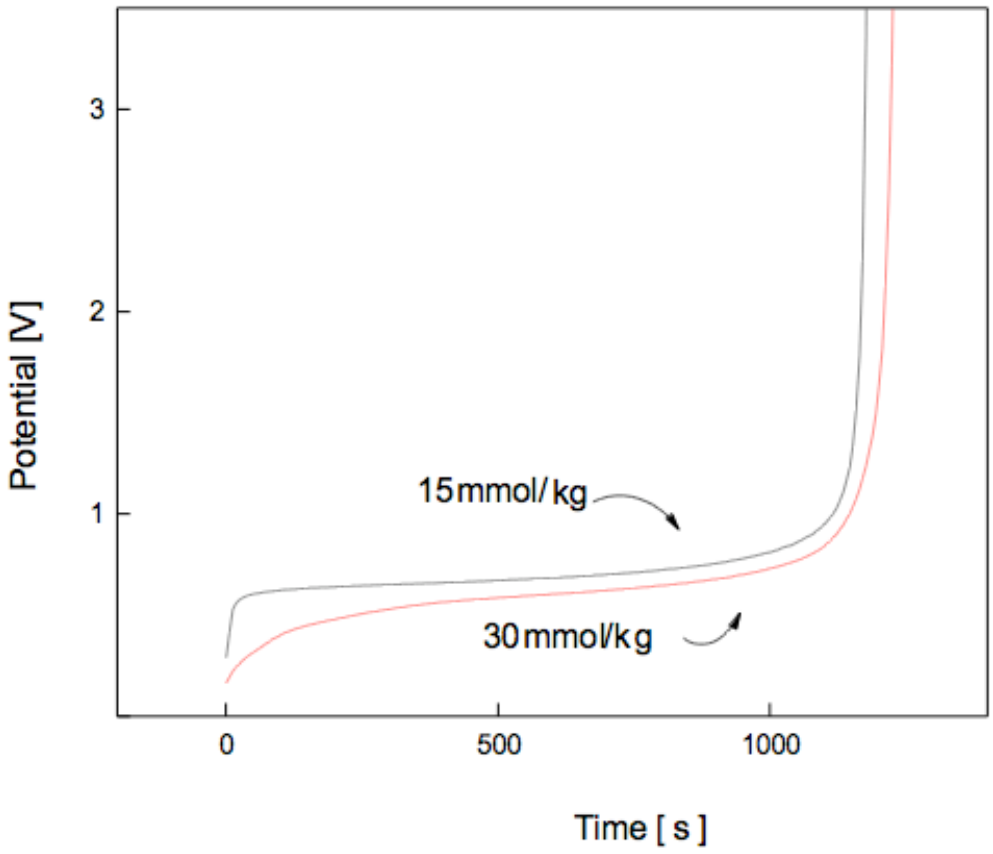


Fig.4.2. Chronpotentiograms obtained at 20 μA current for various Ag/Cu(II) ionophore concentrations in Ag^{+} selective membranes in electrodialysis cell with symmetric $AgNO_3$ 0.001 M concentration with 0.1 M $NaNO_3$ in the background.

establish steep concentration gradients, which brings about the concentration polarization. And as evident from the figure, the second drastic transition remained almost the same for both the membranes. This evidence reinforces strongly that the initial transition in the regular membrane is due to ionophore. The chronopotentiogram for 0.1 M t-butyl ammonium nitrate was recorded following the above with the same 200 μm thick silver selective membrane conditioned as above.

The chronpotentiograms were recorded in the transport cell where the sample compartment contained solution same as the conditioning solution where as the inner filling compartment was filled 0.001 M t-butyl ammonium nitrate solution for the experiment. The reason to change solutions on the inner filling side was so that ionophore depletion occurred for the constant current coulometry in the inner filling phase boundary.

Fig.4.3 illustrates the chronopotentiogram obtained for 0.001 M tetra butyl ammonium nitrate salt, a highly liphophilic cation as inner filling solution. This was done in order to test our hypothesis that after the initial polarization of the ionophore, the membrane behaved as an ion exchanger membrane losing selectivity in the process. Chronopotentiogram obtained shows significant changes in both the initial ohmic and plateau regions confirming the fact that the membrane had no selectivity and this lipophilic cation extracted itself into the membrane as a result.

To confirm the fact this transition is indeed due to ionophore, the chronopotentiometric studies of silver ion-selective membranes were carried out in 0.001 M silver nitrate solution in the background of 0.1 M $NaNO_3$ at current densities

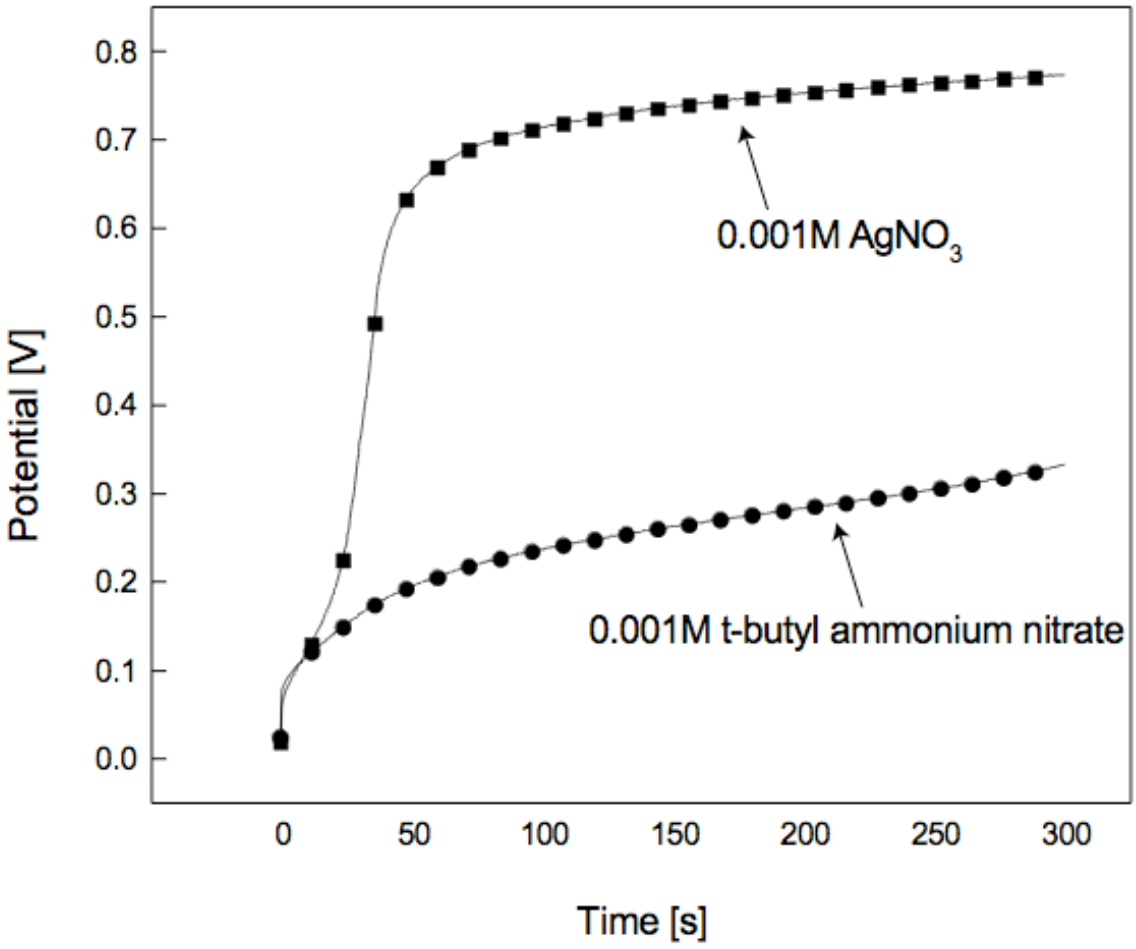


Fig. 4. 3 Chronopotentiograms of silver selective membrane obtained for different inner filling solution such as silver nitrate and lipophilic tetrabutyl ammonium nitrate.

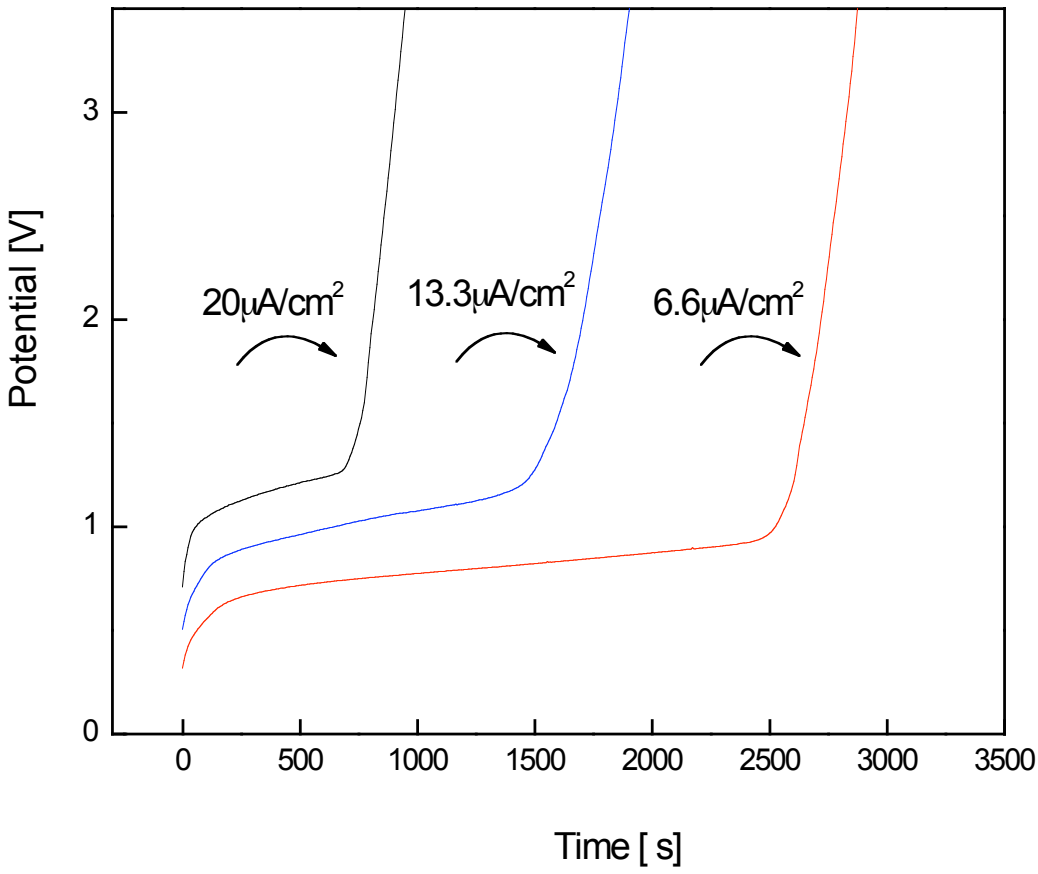


Fig. 4. 4. Chronopotentiograms of silver selective membrane obtained for different current densities.

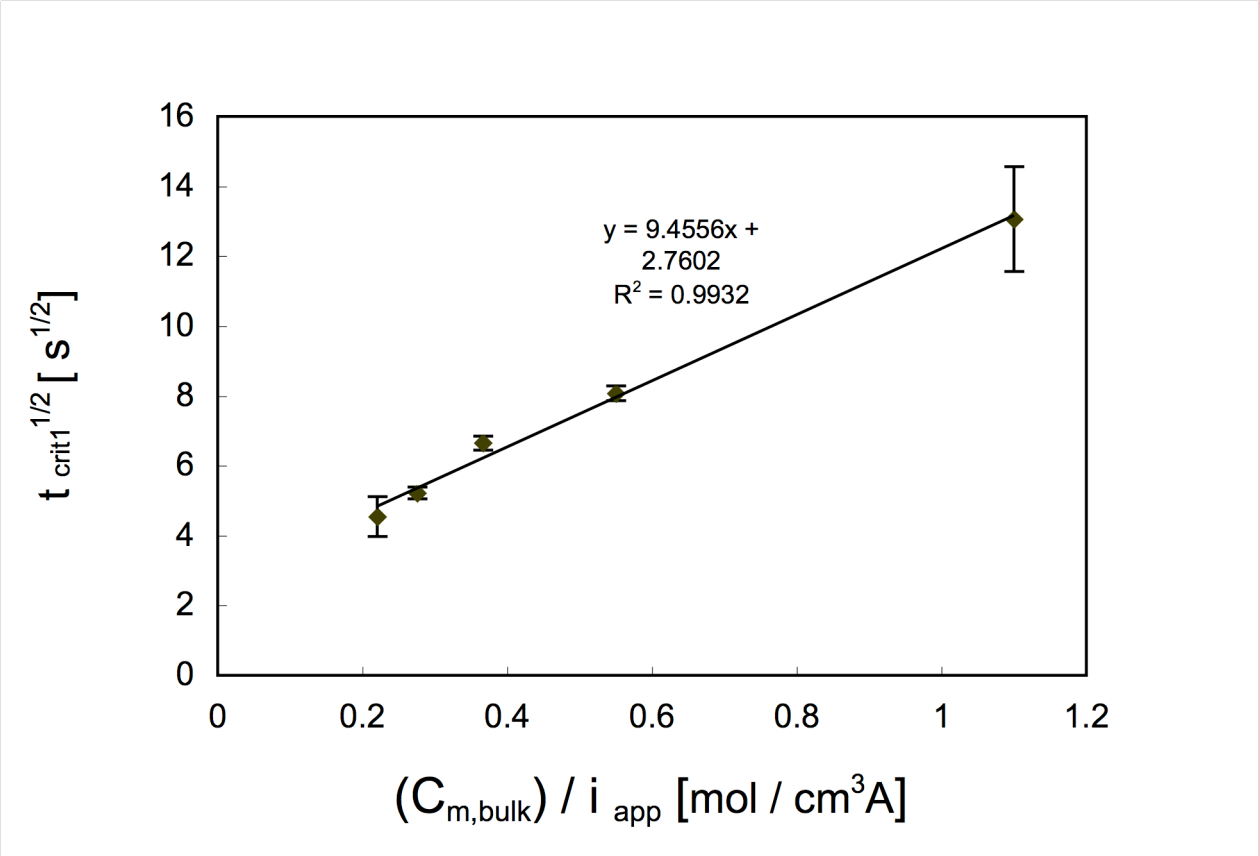


Fig.4.5. The calibration curve from a plot constructed between the experimentally obtained $t^{1/2}_{crit}$ computed from the slope of chronopotentiometric curves against the $(L_T\text{-}R_T) / I_{app}$.

of 6.6, 13.3, 20, 26.6, 33.3 μA/cm^2. Typical chronopotentiograms thus obtained for 200 μm thick membranes at three different current densities are shown in Fig. 4.4. This break is indicative of the total depletion of ionophore at one interface of the membrane and can be related to the ionophore concentration. The transition time values for these membranes at low current densities are larger in comparison to those of higher current densities due to delay in formation of diffusional layers of ionophore at the interface. As evident in the figure, the increase in current densities altered the potential at the initial stage across the membrane and shifted the inflection point close to the Y-axis. This is due to the quick polarization of the ionophore in the membrane with increasing current densities.

In the theoretical section, a mathematical model developed by Buck and coworkers relates the experimentally obtained $t^{1/2}_{crit1}$ of a cation selective membrane to the free ionophore concentration and applied current. According to the equation (8) obtained, slope of the chronopotentiometric transients is a function of the free ionophore concentration in the membrane. So, t_{crit} should be inversely proportional to $I_{applied}$ and it should also be a linear function of $(L_T\text{-}R_T) / I_{app.}$ Experimental data was obtained for different currents. In order to check the theory, a plot was constructed between the experimentally obtained $t^{1/2}_{crit1}$ computed from the slope of chronopotentiometric curves against the $(L_T\text{-}R_T) / I_{app}$. We can also obtain the diffusion coefficient of ionophore from the theory.

Fig. 4.5. shows the calibration curve showing the strong suggestion that it followed our hypothesis that this transition is due to the ionophore concentration polarization as the diffusion coefficient 2.65×10^{-8} cm^2/s obtained from the above proved to be close to the earlier literature.[18] Linear regression analysis of these data points was found to be 0.97. As evident from earlier figures, the inflections in all cases were diffusive in nature and measurement of t_{crit1} value in these cases will not be absolute. And as a result, error bars obtained for the diffusion coefficients calculated were wider. However, this initial transition time does not appear to be a function of both the ionophore and the cation exchanger concentration.

This was proved by sandwich membrane technique where an ion exchanger membrane without ionophore is sandwiched to membrane containing ionophore in different concentrations such as 10, 20 mmol/kg. Upon contact of the Ag/Cu(II) ionophore I with membrane having 10 mmol/kg of NaTFPB, a 498 mV potential increase was observed, which corresponds to estimated complex formation constant obtained in earlier. Figure 4.5.shows the sandwich membrane potential-time response after initial contact of the two segments, which is known to reflect the diffusion kinetics of the ionophore toward the undoped segment side.

For membranes containing ionophore, the potential was essentially stable for more than 2 h, confirming that the free ionophore diffused at a slightly faster rate than the ion exchanger. In a second experiment, membrane with 10mmol/kg ion exchanger only was sandwiched to the reference segment containing only PVC-DOS.

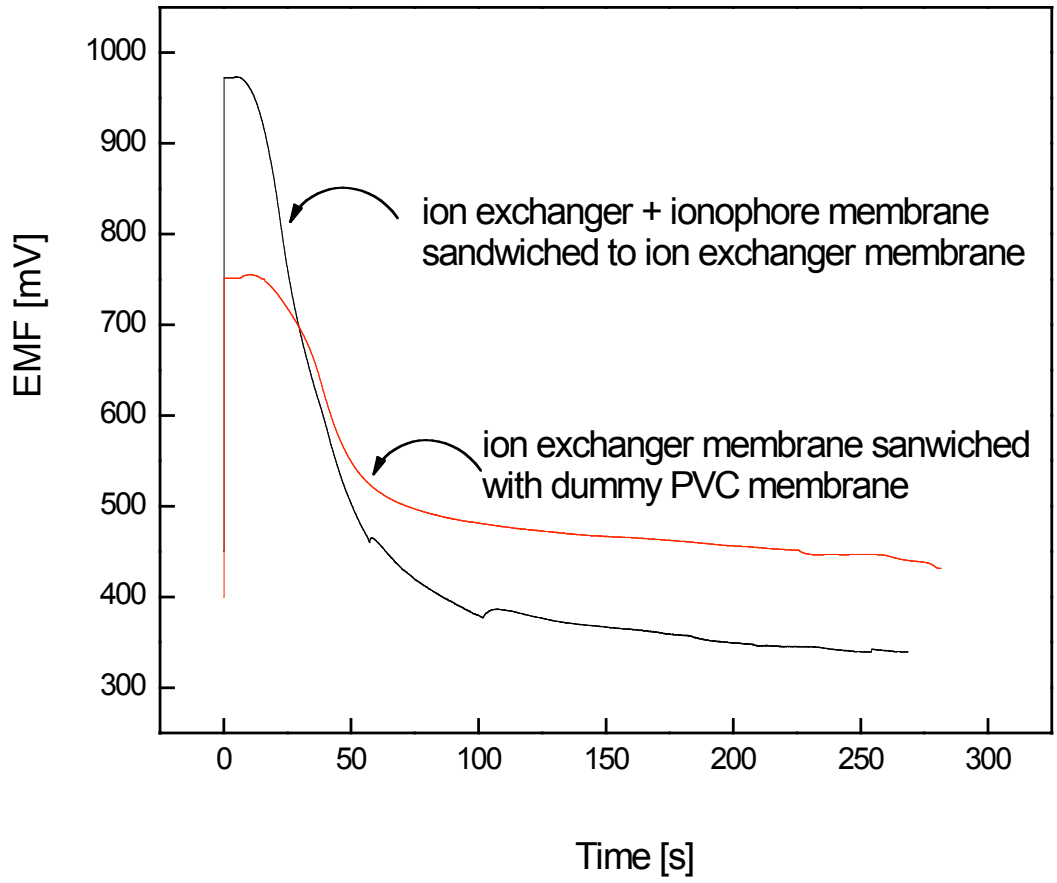


Fig. 4.6. Sandwich membrane assay obtained the ion exchanger only with dummy membrane and the normal membrane containing both ion exchanger and Ag/Cu(II) ionophore in a stochiometric proportion with ion exchanger with symmetric $AgNO_3$ 0.001M concentration.

The 310 mV increase found upon the fusion of the two segments, corresponds to a logarithmic stability constant less than the ionophore containing membrane. The potential was stable for more than 2 h (see Figure 4.6), indicating that the ion-exchanger diffuses at a slightly slower rate than freely dissolved ionophore. The sandwich membrane results agreed very well with the diffusion coefficients obtained by Armstrong[32] which showed a very slight difference in diffusion coefficient obtained for the ion exchanger as well as ionophore complexed to silver ions.

Characterization of second transition

Absence of 1st transition in ionophore free membrane and existence of 2nd drastic transition in the regular, ionophore free and also in the dummy membrane without ionophore or ion-exchanger proves that this transition could be due to the fixed site impurities in the membrane (see Fig.4.1.a and see Fig. 4.1.b). So the concentration polarization of entire fixed site ion exchanger takes longer to occur. Since, the ion exchanger dictates the perm-selectivity of the membrane, it was hypothesized that the membrane remains perm selective until the drastic transition indicating polarization of the ion exchanger.

In order to verify that coulometrically, an experiment with silver releasing membrane was carried out, with the potentiometric detection of the concentration of silver released. The experiment was carried out at 5μA of current, and measurements of concentration released were typically carried out at about 925 s and 3942 s. The experimental value obtained matched very well with the expected concentration computed from the Faraday's 1st law for 925 s.

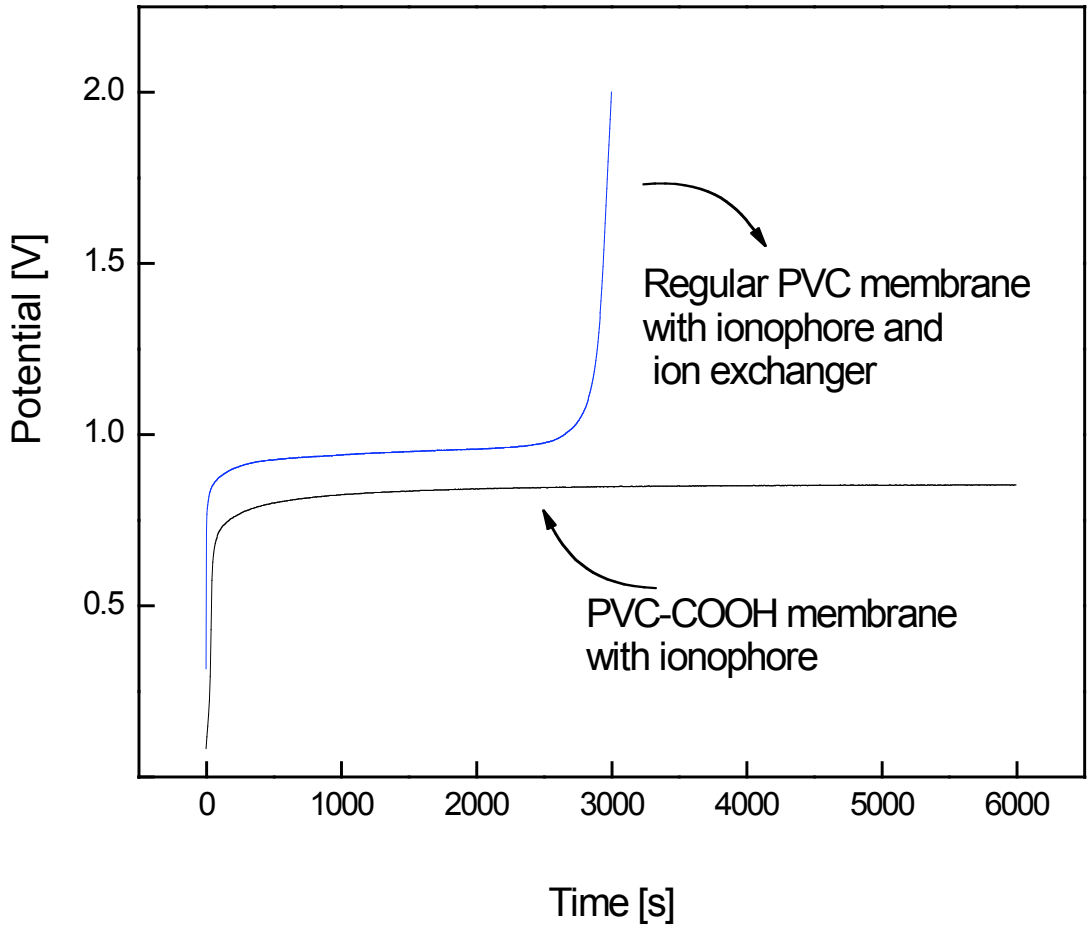


Fig. 4.7. Chronpotentiograms obtained at 20 μA current for the fixed site ion exchanger only PVC-COOH membrane and the normal membrane containing both ion exchanger and Ag/Cu(II) ionophore in a stochiometric proportion in the electrodialysis cell with symmetric $AgNO_3$ 0.001 M concentration with 0.1 M NaNO3 in the background.

But for measurements done at 3942 s, it was about 30% less than the expected concentration to be released. The experimental durations were typically chosen as 925 s, as this was close to the time obtained chropotentiometrically for the final drastic transition to occur (see fig 4.1b) and the duration 3942 s is almost 4 times of that 2^{nd} drastic transition where fixed site ion exchanger polarization has already occurred. Above fact was also confirmed with an experiment where chronopotentiograms were obtained in the transport cell with the sample and inner filling solution same as that of conditioning solution of 1mM $AgNO_3$ in 0.1M $NaNO_3$ for the membrane containing only PVC-COOH and only high molecular weight PVC as the polymer component of the coulometric membrane electrode (see fig.4.7).

The chronpotentiometric curves show that with the PVC-COOH in matrix composition the second transition shifted to longer times, emphasizing that this transition is very much the perm-selective break. In order to verify that coulometrically, an experiment with silver releasing membrane with PVC-COOH as the matrix was carried out with the potentiometric detection of the concentration released. The perm-selective breakdown doesn't occur before the second transition is also proved by recording chronopotentiograms for a silver selective membrane having a silver inner filling solution with various liphophilic anions at a concentration of $5*10^{-4}$ M (Fig.4.8.). This concentration was chosen, since the potentiometric response curves obtained for all the lipophilic anions with silver remained Nernstian potentiometrically and also because it allowed us to characterize the Donnan failure occurring under current.

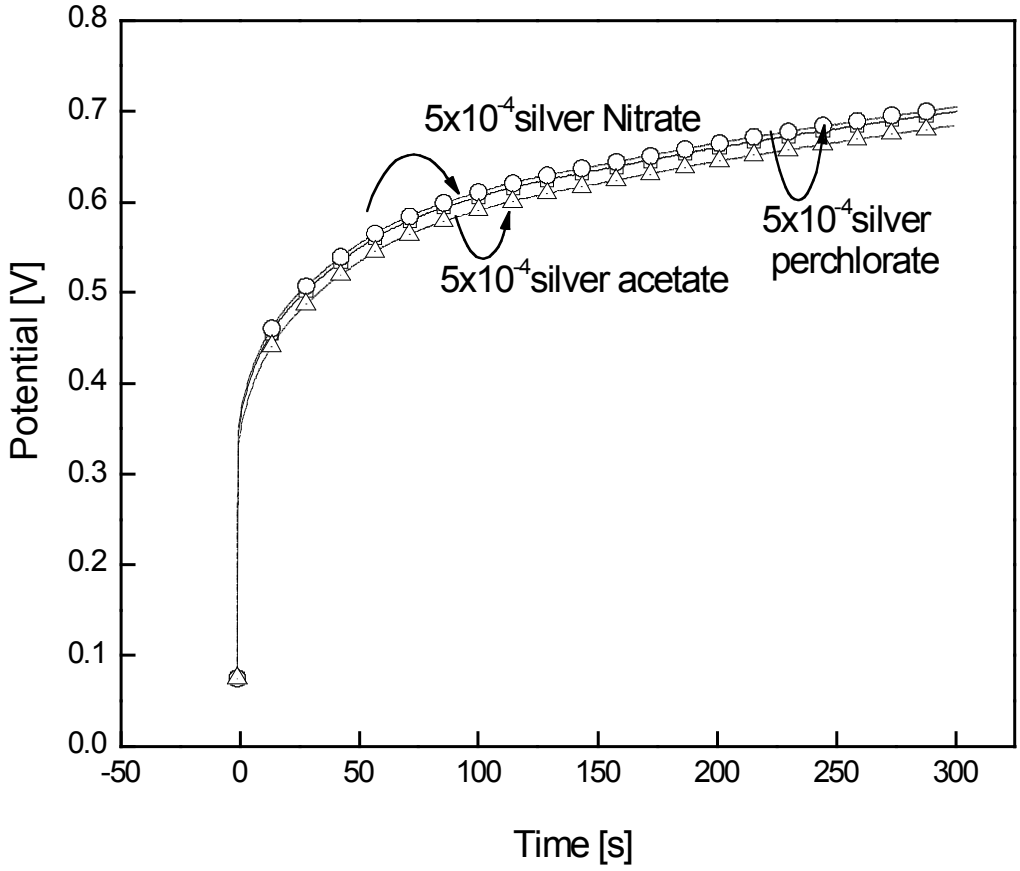


Fig.4.8. Chronopotentiograms of silver selective membrane containing Ag/Cu(II) obtained at 20 μA for samples containing 0.005 M of increasing lipophilic salts of silver such as acetate, nitrate and acetate in the background of their sodium salt of 0.1 M concentration.

Subsequent potentiometric measurements of the calibration curves for silver with increasing liphophilic counter anions like acetate, chloride and iodide showed that the breakdown in perm selectivity followed a Hoffmeister series. The region after the initial transition observed in the chronopotentiogram in Fig.4.8. indicates the presence of a current induced extraction region. This region should give us idea about the Donnan failure as a result of extraction under application of current. So, if the coions extraction is taking place under current, when exposed to samples containing liphophilic anions, then, it should show a Hoffmeister sequence just as it did potentiometrically.

The potentiometric calibration curve of silver with NO_3^- as counter anion is not anion selective at least up to 0.01 M, which allows us to use it as coulometric electrodes 1.5 order of magnitude more than that of silver with liphophilic ClO_4^- counter anion in the sample. The region after initial transition shows slight changes, but if the membrane extracts NO_3^- under current it should show a response to nitrate by at least an order of two or at least 180 mV more than the acetate as it showed in the potentiometric response curve.

The Fig.4.8. shows when membrane under current on exposure to more liphophilic anions, in fact did not even follow Hofmeister series. And according to Buck [17] if the membrane had co-extracted the hydrophilic anions, it would simply have increased the local primary ion concentration relative to the bulk and it would have resulted in decrease of the bulk resistance. The result would have been, the breakdown in perm-selectivity.

The recurrence of the initial ohmic and region of extraction of the same magnitude in these chronopotentiogram obtained for successive measurements of silver salts with lipophilic nitrate anions proves that ionophore is still in the membrane at the same initial concentration and the membrane still retained its perm selectivity. Further proof was obtained when there was no extraction under current even after the exposure of samples containing higher concentrations of liphophilic anions. The region after initial break remained the same showing no marked increase in the extraction of silver concentration up to 0.001 M $AgNO_3$. Consumption of free ionophores and the membrane resistance depended on the silver concentration.

Observations for this membrane show in the plateau region a constant number of charges existing until up to the concentration of 0.001 M $AgNO_3$. The above was once again confirmed by successive runs of 0.001 M $AgNO_3$ in the sample concentration of 0.001 M $AgNO._3$ Reproducibility of this is a proof that the membrane still contained an initial approximate concentration of calcium and Donnan failure had not occurred. However, Donnan failure under current occurred, when the membrane was exposed to a very high concentration 0.01 M in the region of exclusion failure as determined already by potentiometry. There was significant change in both the initial ohmic and plateau regions owing to the fact that the violation of Donnan exclusion occurred.

4.4. Conclusions

This article summarizes the main limitations due to the concentration polarization of components of the solvent polymeric membrane based coulometric electrode. This chronopotentiometric technique was able to discern the ionophore concentration polarization which occurring by a transition at a time t_{crit1} under constant current and from slope of the initial transition, the diffusion coefficient of ionophore was estimated to be $2x10^{-8}$ cm^2/s close to the reported value. The second drastic transition was characterized to be concentration polarization of the fixed site ion exchanger of an ion selective membrane from the transport studies. The perm-selectivity loss was characterized in detail by varying the concentration of a fixed site ion exchanger such as PVC-COOH and the currents applied. This once again can be eliminated by the use of large area coulometric electrodes, small current, smaller sample size and also by blending a quantity of PVC-COOH with the regular high molecular weight PVC so as not to compromise the selectivity of the matrix. The above studies mark a new level of approach toward coulometry being elevated to the routine quantitative analysis status.

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CHAPTER 5

PULSE GALVANOSTATIC PVC/DOS BASED PROTAMINE SENSORS

5.1. Introduction

Heparin is a highly sulfated glycosaminoglycan with average molecular weight of 15,000 Daltons. Heparin has different biological properties that are useful for the treatment of several human diseases. It is very well known for its anti-coagulant and anti-thrombosis properties.[1, 2] The anti-coagulant activity is believed to be due its complex formation with ATIII that causes inhibition of ATIII on enzymes involved in coagulation cascade. So monitoring heparin continuously with accuracy and precision is very important as it could cause bleeding complications resulting in death, especially in children.

Potentiometer ion sensors based on solvent polymeric membranes are versatile analytical tools that are well established for a number of pharmaceutical applications either for quality control or for clinical monitoring. In fact, in the recent years, potentiometer measurements for various important polygons such as heparin, low molecular weight heparin, dermatan sulfate and protamine and synthetic poly cationic drugs in blood.[3-16] The heparin is usually measured quantitatively by titration with its antidote protamine, a poly cationic polypeptide with average charge of +20. An illustration of heparin and protamine is shown in Fig 5.1.

Potentiometric polyion sensors functions based on the non-equilibrium steady state principle. Here, it undergoes a selective extraction polyion into the membrane as a result of formation of cooperative ion pair with lipophilic exchangers. As a result, the EMF of this potentiometric sensor changes and polyion response obtained obeyed equation put forward by Meyerhoff.[13] This equation is written for a polycation protamine and hence gives positive change in potential with the increase in protamine concentration in the sample C_{prot}.

$$\Delta EMF = + \frac{RT}{F} \ln\left(1 - \frac{zD_{org}\delta_{org}}{R_T D_{aq}\delta_{aq}} C_{prot}\right) \quad (1)$$

z is charge on the polyion. R_T is the total concentration of lipophilic ion exchanger. D_{org} and D_{aq} are diffusion coefficients of polyion in the aqueous and organic phases respectively and δ_{org} and δ_{aq} are the diffusion layer thickness of polyion in the respective phases. Meyerhoff group worked in improving the detection limits of the planar protamine sensors and they achieved a detection limit of an order lesser than what usually is obtained by employing rotating electrode potentiometry.[14] This also improved the precision in determination of endpoint in heparin vs protamine titration. They also utilized the above method for low levels of low molecular weight heparins (LMWH) approved by FDA. However, potentiometric polyion sensors are irreversible owing to their high charge, which renders the equilibrium extraction process to be free of the influence of sample polyion concentration.

This forces the sensor to lose its response for the polyion in a relatively short time and cannot be used further unless otherwise it can be renewed.

Mathison et al., tried to alleviate this problem by modifying membrane composition, so as to renew it by adjusting the pH in the sample.[15] Shvarev et al., reported first ever completely reversible protamine sensor using galvanostatic current control.[16] This chronopotentiometric sensor membrane had no ion exchanger, which helped to restrain the spontaneous polyion extraction. Here, the diffusion layer thickness is dictated by galvanostatic current. A particular current is chosen to give maximum EMF for that specific concentration. In between the extraction pulses, it is maintained in potentiostatic mode to remove the extracted ions from the membrane. They predicted protamine response was dependent on the background sodium ions, when protamine concentration is low in the sample. The protamine response at low polyion concentrations is given by: [17]

$$E_{PB} = E^{\circ} + \frac{RT}{F}\ln\frac{a_{Na}}{\frac{\delta_m}{D_{m,Na}}\left(\frac{-i}{FA} - Z\frac{D_{aq,PA}}{\delta_{aq}}C_{PA,bulk}\right)} \qquad (2)$$

Shvarev et al., used PVC/NPOE based membrane for their pulse galvanostatic sensors.[18] Here, the pulse galvanostatic polyion sensors based on the PVC / DOS based membrane is explored. Choice of plasticizers is important in polymeric membrane electrodes as they solvate the charged components within the polymeric phase and they also influence the diffusion coefficients of the polyions in the membrane.

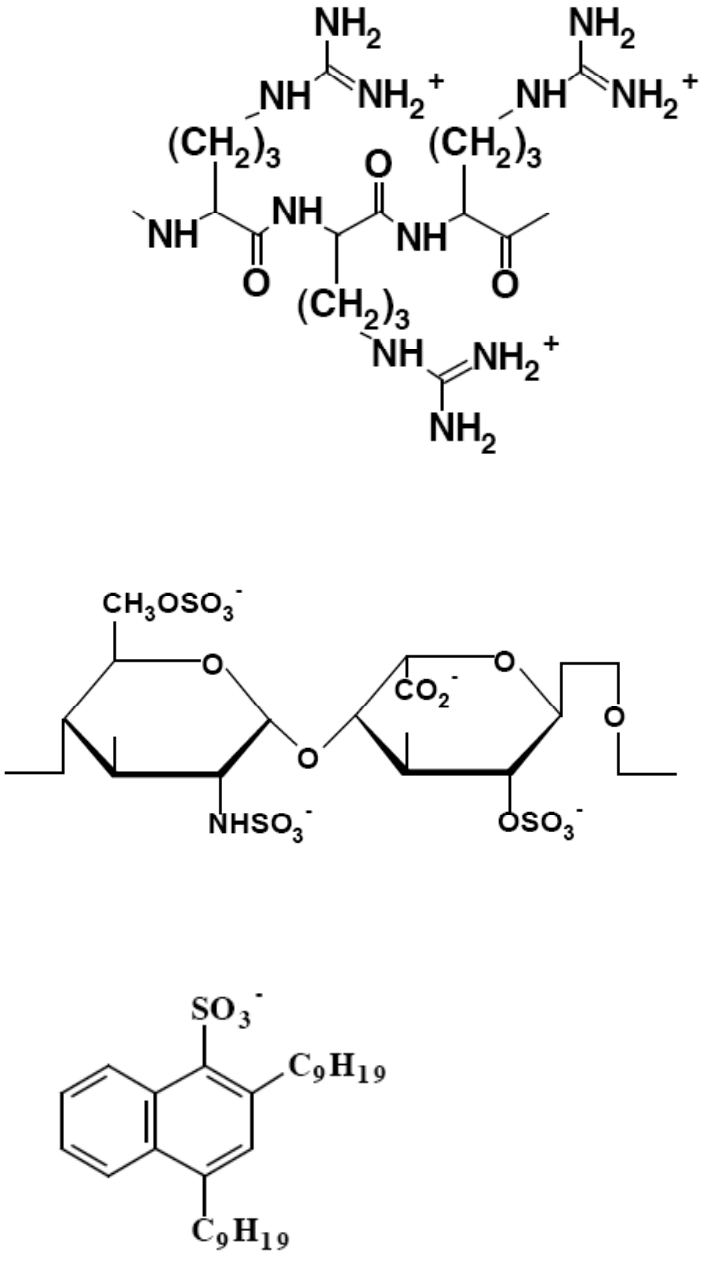

Fig. 5.1. Chemical structures of protamine, heparin and DNNS (in the order of top to bottom) respectively.

The motivation behind this work was basically as a result of PVC/DOS membranes being a better choice when used in clinical analysis as it causes less tissue inflammation due to leaching in comparison to O-NPOE. Although, membrane with 1:1 PVC/DOS ratio was explored as potentiometric polyion sensor,[7] PVC/DOS 1:2 ratio was not explored as a matrix for either for pulse galvanostatic polyion sensors or for potentiometric sensor. This chapter talks about potentiometric and pulse galvanostatic selectivities of PVC/DOS matrix for protamine. Further, it examines the use of the above electrodes in heparin determination of clinically relevant concentrations in sheep blood via titration.

5.2 Experimental

Reagents

High molecular weight PVC, DOS (Di-n-octyl sebacate), tetradodecylammonium chloride (TDDACl), dionylnaphthalene sulfonic acid (DNNS) as 50% solution in heptane, tetradodecylammonium tetrakis (4-chlorophenyl) Borate (ETH 500), Tetrahydrofuran (THF). Heparin, sodium salt (from bovine intestinal mucosa, 151 units/mg), protamine sulfate (from herring) and organic solvents were purchased from Sigma (St. Louis, MO). Aqueous salt solutions were prepared by dissolving the appropriate salts in nanopure de ionized water (18.2M Ω/cm).

Membrane preparation

Ion selective membranes (ca. 200 μm thick) for potentiometric selectivity measurements, containing PVC and DOS 1:2 by weight, 1 wt% of DNNS and 10 wt % of lipophilic inert electrolyte ETH 500 were obtained by dissolving all components in THF, followed by its evaporation. Membranes for pulse galvanostatic protamine sensing contains 10 wt% of lipophilic salt DNNS-TDDA in additional to other components mentioned above. The lipophilic salt DNNS-TDDA was prepared by earlier described method. [16]

Electrodes

The ion selective membranes were cut with a cork-borer (6mm in diameter) from the parent membrane and pasted to PVC tubes using THF. The inner filling solution was 0.1 M NaCl for galvanostatic sensor NaCl buffered to pH 7.4 using Tris 50 mmol. The inner solution was in contact with an internal Ag/AgCl reference electrode. The electrodes were conditioned before experiments in a solution identical to the inner filling solution overnight. The external reference electrode consisted of a double junction Ag/AgCl electrode with a 1 M LiOAc bridge electrolyte.

Experimental setup

A conventional three-electrode setup was used for measurements. A stranded platinum wire was used as a counter electrode; an external reference Ag/AgCl electrode (Mettler-Toledo, InLab 302) contained saturated KCl and a salt bridge filled with 1 M LiOAc.

Chlorinated silver wires were used as inner reference/working electrodes. The experiments were performed with an AFCBP1 Bipotentiostat (Pine Inst., Grove city, PA). Controlled by a PCI-MIO-16E4 interface board and LabVIEW 5.0 Software (National Instruments, Austin, TX) on a Mac computer. Here in triple pulse, first in mode I, a pulse of dc current of fixed magnitude and duration was applied galvanostatically and the potentials were recorded as a function of time during the pulse. Followed by a zero current measurement pulse II.

During this pulse, the previously extracted background ions are removed from the membrane according to zero current counter diffusion transport principles. Second mechanism is analogous to that observed in zero current potentiometry. Details on the low noise potentiostatic/galvanostatic control switching device may be found elsewhere. [18] The last, third, pulse was applied potentiostatically with the especially adjusted baseline potential to ensure effective stripping of ions extracted during the current pulse. The magnitude of this potential pulse III, which should be close to the open circuit potential, was chosen as 0 V vs Ag/AgCl. Duration of this pulse was usually 30-50 times longer than the corresponding current pulse.[19]

In the following experiments, the pulse galvanostatic sensing uses triple pulse as follows, which includes an applied constant cathodic current pulse (of 1s duration) followed by a 0.5 sec of zero current measurement pulse followed by constant potential pulse (of 15s duration). Sampled potentials, which represent the sensor response, were obtained as the average value during the last 100 ms of each current pulse.

All experiments were conducted in a laboratory ambient temperature. For calibration and titration curves of protamine and protamine with heparin, -2 μA constant cathodic current pulses were applied. For titration of heparin with protamine, 200 μL of Heparin stock solution (7.5g/L, 5 x 10^{-4} M) was added to 1 mL of 0.1 M NaCl background before titration. Protamine stock solution of 5g/L was used for calibration of the sensor. Potentials were obtained as the average of 15 uptake pulses after every addition. Heparin and protamine concentrations were calculated by assuming molecular weights of the above to be 12,500 and 5000 Daltons respectively. All the solutions were buffered to pH 7.4 with 50 mM of TRIS-HCl.

5.3 Results and Discussion

Selectivity is the most important characteristic of an ion selective membrane. So, the first step would be to determine the unbiased selectivity coefficients for the PVC/DOS based sensing membranes. From the above step, membrane of high selectivity towards protamine would be chosen to carry out the heparin vs protamine experiments. The potentiometric selectivity of the membrane to be used as electrode material for the sensor was determined by carrying out individual calibration curves for the chloride salts in the order of Hoffmeister series like sodium, potassium, magnesium, calcium etc., (Fig 5.2).

The sodium and potassium calibration curves show sub-Nernstian slopes. The subsequent calibrations were carried out with magnesium and calcium, also showed poor sub-Nernstian slopes.

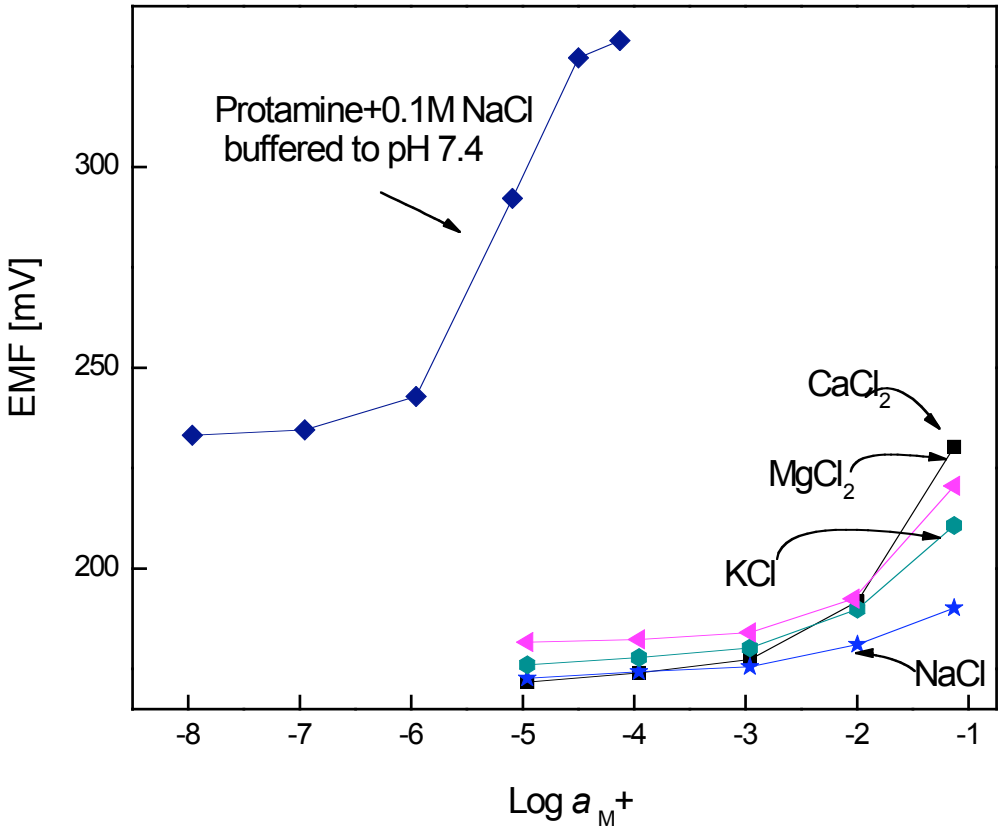

Fig. 5.2. Unbiased selectivites of PVC/DOS based membrane containing 1 wt% DNNS along with 10 wt% lipophilic salt ETH 500.

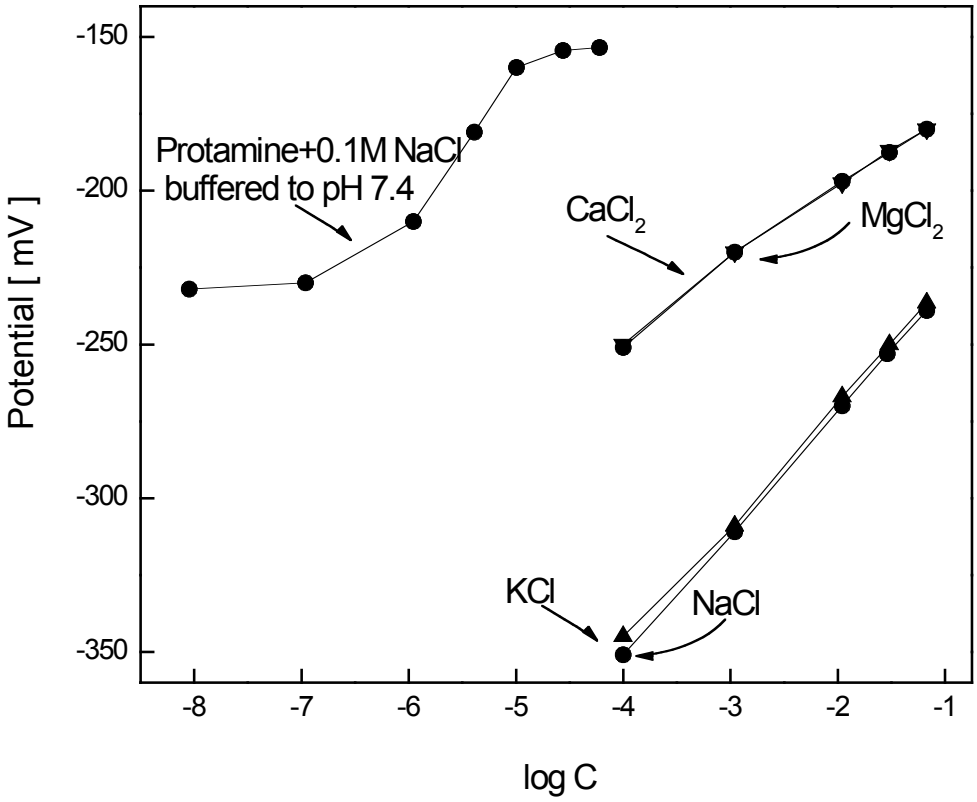

Fig. 5.3. Pulse galvanostatic unbiased selectivites of PVC/DOS based membrane containing 1wt% DNNS along with 10wt% lipophilic salt ETH 500.

All the ions showed some response at a very high concentration of 0.1 M of the respective electrolyte. A protamine calibration curve in 0.1 M NaCl buffered to a pH of 7.4 was also carried out. The ΔEMF response for protamine was good. The higher potential readings obtained for protamine calibration than for any other cations tested demonstrate a preference of this membrane electrode for protamine over all the tested cations. The over all EMF difference for entire calibration is around 120 mV. This shows that, this is one of the best membrane compositions that can be used as a potentiometric electrode material in the presence of all other common ions in higher concentrations.

Since, we know DNNS-TDDA is a lipophilic salt used in membrane composition of the pulse galvanostatic sensor under current control, it could have affinity for other cations as well. So, it is very important to know the selectivity coefficients for those common ions such as sodium, calcium and so on, which are found in high concentrations in the blood.

The use of polymeric membrane electrode to sense polyion would be questionable if a very high concentration of sodium ions in the background ion-exchanges with the membrane electrode if it were not so selective for the polyion to be sensed. It is also very important to know selectivity of this electrode to sodium as some suppliers of blood use sodium citrate as an anti coagulant in the blood. If membrane is not selective to protamine, then it may undergo partial ionexchange with sodium. The determination of unbiased selectivity coeffients[20] for sensing membranes containing 10 wt% DNNS-TDDA with PVC / DOS in 1:2 ratio was carried out.

The pulse galvanostatic unbiased selectivities were determined by recording individual calibration curves for the chloride salts in the order of Hofmeister series such as sodium, potassium, magnesium, calcium as shown in Fig 5. 3. The calibration curves of sodium and potassium showed near- Nernstian slopes for the DNNS based membrane. The electrode showed near-Nernstian slopes of 26 mV for both calcium and magnesium. A protamine calibration curve in 0.1 M NaCl buffered to pH 7.4 was also carried out. This higher potential reading obtained demonstrates a strong preference of this electrode for protamine over the sodium and potassium in tested concentrations.

However, this electrode prefers calcium and magnesium ions as well. They showed about 110 mV of total EMF change as response toward protamine from the baseline potential obtained from the background of 0.1 M NaCl. This shows that this membrane can be used as electrode material in constant current coulometry. Membrane with DNNS-TDDA was used as a sensor to carry out the following experiments in buffer and blood.

5.3.1 Response of PVC/DOS based sensor

Figure 5.4. shows time traces for calibration of protamine in the entire concentration range as measured by PVC/DOS based electrode at the cathodic current of -4 μA. Protamine was added into the sample containing 0.1 M NaCl at pH 7.4. After the addition of protamine, the pulse galvanostatic sensor was used to sample for the protamine concentration. The potential response of these sensors was quite stable in the measured concentration range with high reproducibility.

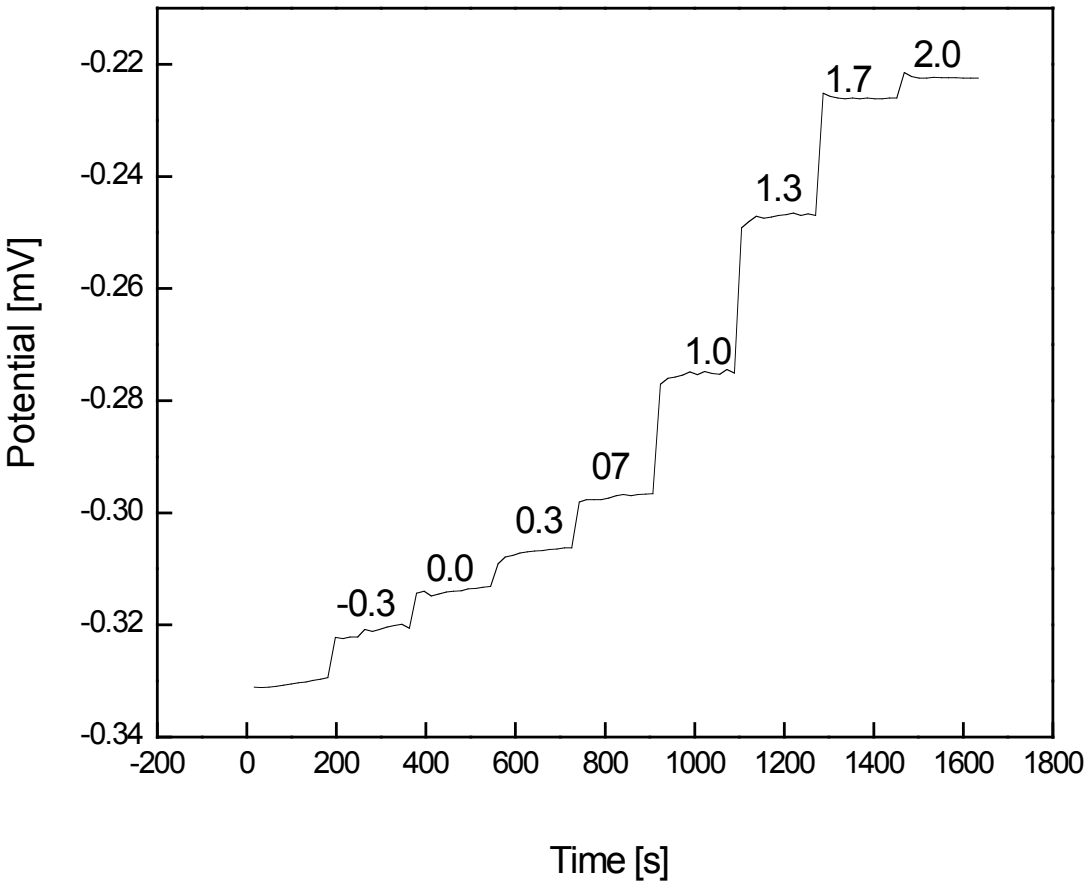

Fig. 5.4. Time traces obtained for the protamine calibration using pulse galvanostatic PVC/DOS based membrane sensor in 0.1 M NaCl in pH 7.4.

This is highly significant with regard to its use as a sensor in heparin-protamine titrations. The sensor potential was quite reproducible with a complete capability to estimate clinical heparin concentration range by titration. The total EMF change was ~ 100 mV with a good detection limit.

5.3.2 Heparin vs Protamine titrations in clinically relevant concentrations

The typical titration curves are obtained in 0.1 M NaCl buffered to physiological pH of 7.4 spiked with heparin. The sample titrations are carried out by the addition of known quantity of heparin to the background electrolyte to acquire different sample concentrations in the clinical range of 0.6-4.5 kU/L. Prior to the titration the sensor was characterized for its current response and calibrated with protamine using the stock solution. The protamine complexes with heparin when added in microliter quantities and the EMF response of free protamine in the sample are sensed using pulse galvanostatic protamine detection.

The titration curves has increased EMF response even before the end point and this is attributed to the extraction of low molecular weight components available in heparin forming a positively charged complexes with protamine. The titration endpoints shift stoichiometrically with respect to change in heparin concentration of the sample. The total EMF response of the sensor towards protamine using PVC/DOS in the buffer is comparable to what our group reported earlier with PVC/o-NPOE.

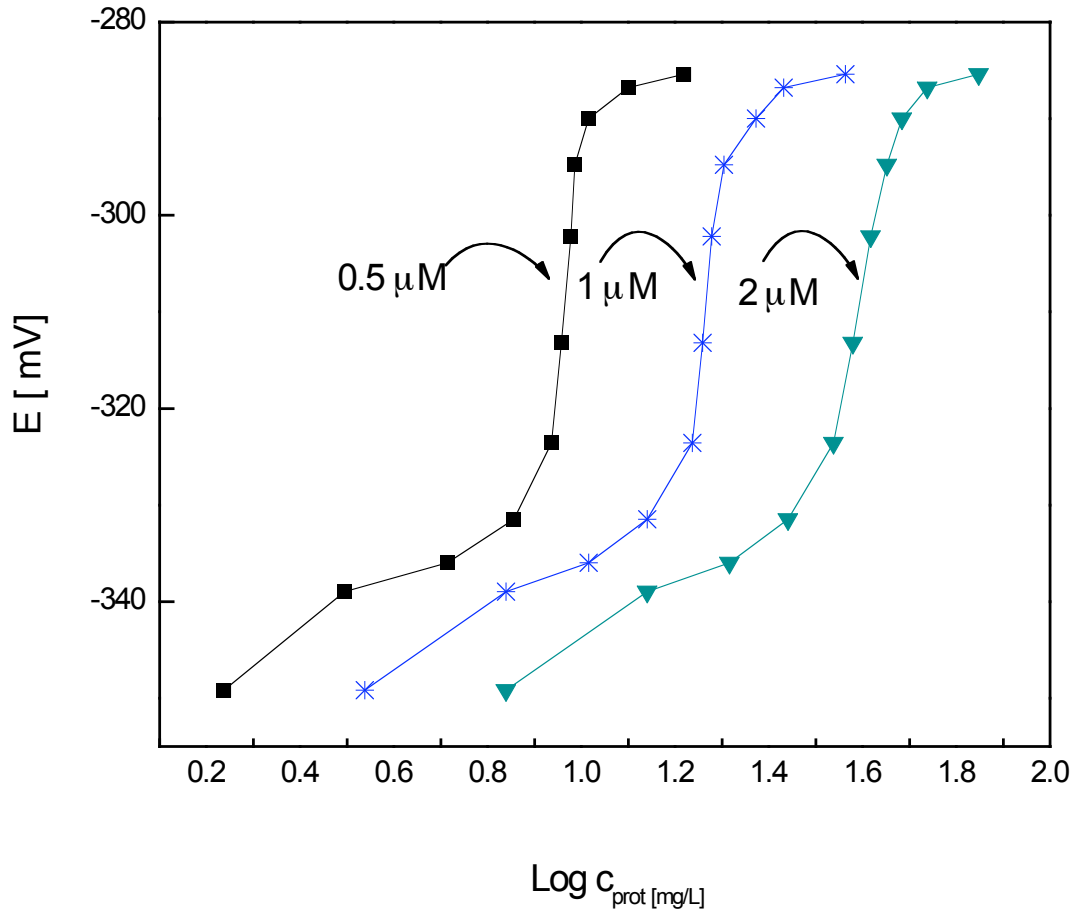

Fig. 5.5. Heparin vs protamine titration curves obtained for the 0.5, 1, 2 μM, concentrations of heparin spiked into the whole blood samples using pulse galvanostatic PVC/DOS based membrane sensor.

The titrations were carried out in the volume of 15ml and a constant cathodic current of -1 μA was used for sensing.

5.3.3 Heparin Determination in Blood

As shown in Figure 5.5, the protamine sensor proves to be a convenient tool to quantify heparin concentrations in whole blood and plasma. Figure 5.5. shows the typical titration curves obtained in 0.1 M NaCl buffered to physiological pH of 7.4 spiked with heparin. Figure 5.5 shows three titration curves carried out consecutively in the order of lower to higher concentrations of heparin. The stoichiometric ratio of heparin to protamine is found to be 1:4 for beef lung heparin.

The total EMF obtained for titration endpoint when compared with what was obtained in buffer was less. The ΔEMF was found to be comparable to the response obtained with PVC/o-NPOE based electrode. The reduction in EMF response towards protamine by this matrix could be attributed to proteins in blood adsorbing onto the surface of the electrode membrane. The sample volume is 2 mL of citrated sheep's blood.

5.4 Conclusions

As shown from above experiments, a significant change in EMF is obtained with pulse galvanostatic sensors, which is reversible and reproducible compared to its potentiometric counter parts in whole blood. So it can be used to monitor the protamine concentration continuously.

This sensor based on PVC/DOS is a better matrix in terms of its ability to cause less tissue inflammation, which makes it a likely candidate for an implant sensor. The sensor's pulse galvanostatic selectivity is found to be not so good. As the response of PVC/DOS sensor is comparable to PVC/o-NPOE, it could be of use, where plasticizer leaching is undesirable.

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CHAPTER 6

COMPLETELY AUTOMATED CALIBRATION FREE COULOMETRIC ESTIMATION OF HEPARIN WITH A VISION TOWARDS ONLINE MONITORING IN CLINICAL LABORATORIES

6.1 Introduction

Heparin is an anticoagulant used during several clinical procedures including open-heart surgery and kidney dialysis.[1,2] Protamine is a polycationic peptide rich in arginine residues with a specific affinity to heparin. It may be as such administered to cardiopulmonary bypass patients following surgery to reverse the blood thinning effects of heparin.[3,4] Each 1 mg of protamine will neutralize approximately 90–115 USP units of heparin stochiometrically.

A fast and accurate determination of heparin in blood is very critical in case of the patients who undergoes above procedures, as they are exposed high doses of heparin. Protamine titration with heparin compared to other heparin assays [5-7] reduces the dose of protamine administered thereby decreasing the chance of adverse reactions such as excessive bleeding and immune-mediated heparin-induced thrombocytopenia (HIT)[8], a potentially life-threatening complications associated with a fall in platelet count and a high incidence of thromboembolic condition dangerous to children.[1]

A heparin/protamine titration system for the determination of heparin levels (Hepcon) was found to be effective with whole blood heparin measurements correlating well with plasma heparin concentration.[9] However, discrepancies in ACT prolongation times and lack of speed in analysis, brings up the question whether ACT is a better indicator of heparin neutralization compared to other techniques. For the monitoring of heparin levels in blood, several electro analytical sensing principles such as the voltammetric,[10] Amperometric,[11] chronoamperometric,[12] potentiometric,[13] and pulse galvanostatic[14] have been employed in the recent years.

Some other methods investigated also includes flow injection analysis, [15] ion channel sensors,[16] resonance Rayleigh scattering spectra,[17,18] capillary chromatography,[19] high-performance liquid chromatography,[20] surface plasmon resonance sensor analysis,[21] rotating electrode potentiometry,[22] piezoelectric quartz crystal sensor[23] and extracorporeal membrane oxygenation,[24] And several optical methods such as spectrophotometric method,[25] spectrofluorimetry [26]and optodes[27] so on.

The polyanion-responsive ISEs exhibited significantly large and reproducible EMF responses for clinically applicable concentration ranges of 0.2-5.0 units mL^{-1} heparin. However, the sensor EMF response is mainly dependent on the background electrolyte activities in the real samples;[28] hence, quantitative detection of heparin is best reached by titration with the polycation protamine, with an analogous polycation-sensitive membrane electrode.

Since, protamine over-dose could cause a trigger into other toxic effects in-patients, the titration is best method where addition of excess protamine could be avoided once the endpoint is reached. Coulometric titration has several advantages compared with volumetric titrations. The automatic coulometric titrator eliminates the need for the bigger volumes and can be continuously run without systematic errors, making headway into clinical laboratories. In a significant recent advance, chemically selective coulometry used in regular complexometric and precipitation titrations showed very good precision and accuracy.

The need for coulometric protamine release system arises from the minimal protamine dose required to neutralize the anti-coagulant activity of heparin in the small volume of blood/plasma at the conclusion of cardio-pulmonary bypass surgery. This coulometric device could be envisioned as a step towards completely automated online monitoring device used in clinical analysis during CPB as to needs of saving lives showing adverse reactions.

The novelty for developing this type of actuator and sensor pair is to test the feasibility of automated titrator where protamine can be coulometrically generated to neutralize heparin concentration. The above aim is to be realized by the use of galvanostatic sensor of fast response and use of small sample volumes compared with Hepcon assay. Usually, Hepcon assay required at least 3cc of blood and if the tested cartridge does not lie within the range of channels capable of testing the heparin it may require more blood.

The coulometric titrators would be much more efficient than the conventional titrators as there will be no need to worry about accurate titrant additions and standardization of titrant in small volumes. The main idea behind this experiment is to coulometrically generate the protamine using protamine-generating electrode from solvent polymeric membrane doped with protamine selective ionophore. Coulometry is very elegant in its delivery of protamine, as this technique will control the concentration of released ions according to Faraday's law hence; fairly accurate estimation of endpoint of the titration is possible. As a result, reduction of the risk of administering over-dose of protamine to the patients could be accomplished. The coulometric device requires a mere 1 to 1.5 ml of the sample, which could further be reduced.

6.2 Experimental

Reagents

High molecular weight PVC, 2-nitrophenyl octyl ether (O-NPOE), tetradodecylammonium chloride (TDDACl), dionylnaphthalene sulfonic acid (DNNS) as 50% solution in heptane, tetradodecylammonium tetrakis (4-chlorophenyl) Borate (ETH 500), Tetrahydrofuran (THF). Heparin, sodium salt (from bovine intestinal mucosa, 151 units/mg), protamine sulfate (from herring) and organic solvents were purchased from Sigma (St. Louis, MO) Aqueous solutions were prepared by dissolving the appropriate salts in nanopure de ionized water (18.2M Ω/cm).

Membrane preparation

Ion selective membranes (ca.200 μm thick) contained PVC and o-NPOE 1:2 by weight. Membranes are prepared by solvent casting, with THF as the solvent. Pulse galvanostatic sensor membranes contains 10 wt% of lipophilic salt DNNS-TDDA. Coulometric electrode membranes also contained 1 wt% of KTClpB, DNNS with excess of background electrolyte ETH 500 about 10wt%. The liphophilic salt DNNS-TDDA was prepared by earlier described method. [14]

Electrodes

The ion selective membranes were cut using a cork-borer (6mm in diameter) from the parent membrane and pasted to PVC tubes using THF. The inner filling solution for the actuator was 0.001 M protamine in 0.1M NaCl buffered to pH 7.4 using Tris 50mmol and 0.1 M NaCl for galvanostatic sensor NaCl buffered to pH 7.4 using Tris 50mmol. The inner solution was in contact with an internal Ag/AgCl reference electrode. The electrodes were conditioned before experiments in a solution identical to the inner filling solution overnight.

The external reference electrode consisted of a double junction Ag/AgCl electrode with a 1 M LiOAc bridge electrolyte. The generating electrodes had a membrane area of 1.5 cm^2. The membrane was attached to end of Tygon tubing by THF and the membrane was conditioned in 10^{-3} M protamine for at least 24 hrs. This coulometric membrane electrode glued to tube is used the next day of conditioning as DNNS concentration is expected to decrease with more conditioning as described by Meyerhoff.[22]

Protamine conditioning solution is prepared by dissolving appropriate weight of protamine sulfate into 0.1 M NaCl solution prepared out of autoclaved water buffered to pH 7.4 to increase the stability of protamine solution.

Experimental setup

The coulometric measurements were conducted in a four electrode cell system, where the internal Ag/AgCl electrode worked as a working electrode, and the counter electrode, A Pt wire electrode of large surface area were immersed into the background electrolyte 0.1 M NaCl. The galvanostatic control of the ion-selective membrane utilized the pulse galvanostatic/potentiostatic technique, described in detail elsewhere.[14,29]

The experiments were performed with an AFCBP1 Bipotentiostat (Pine Inst., Grove city, PA). Controlled by a PCI-MIO-16E4 interface board and LabVIEW 5.0 Software (National Instruments, Austin, TX) on a Mac computer. Prior to the experiment, the operation of first electrode output of the bipotentiostat (K1) was switched to current control for coulometrically releasing electrode. The second working electrode (K2) output of the bipotentiostat was switched to current control for pulse galvanostatic sensor followed by switching to potentiostatic control. To apply anodic current, the coulometric working electrode was connected to the K1 output via analog switch controlled by external software.

To apply cathodic current, the sensor working electrode was connected to the K2 output via analog switch controlled by external software.

When the baseline potential between the current pulses was applied, the working electrode was still connected to K2. During coulometric release, different cycles contain several steps of equi duration for a constant anodic current is applied. The pulsed galvanostatic sensing follows every anodic current release step, which includes an applied constant cathodic current pulse (of 1s duration) followed by a constant potential pulse (of 15 s duration). Sampled potentials, which represent the sensor response, were obtained as the average value during the last 100 ms of each current pulse. The observed amplitude-time behavior of current and potential has been described.[29]

All experiments were conducted in a laboratory ambient temperature. For calibration and titration curves of protamine and protamine with heparin, -2 μA constant cathodic current pulses were applied. For titration of heparin with protamine, 200 μL of Heparin stock solution (7.5g/L, 5 x 10^{-4} M) was added to 1mL of 0.1 M NaCl background before titration. Protamine stock solution of 5g/L was used for calibration of the sensor. Potentials were obtained as the average of 15 uptake pulses after every addition. Heparin and protamine concentrations were calculated by assuming molecular weights of the above to be 12,500 and 5000 Daltons respectively. All the solutions were buffered to pH 7.4 with 50 mM of TRIS-HCl.

6.3 Results and Discussion

The preliminary studies involved observation of change in sensitivity of the polymeric poly ion selective coulometric electrode with time. Fig.6.1. Shows A.C.

impedance spectra obtained for coulometric polyion sensitive DNNS based PVC/O-NPOE membrane bathed in symmetrically in Tris Buffer pH 7.4 in the background of 0.1 M NaCl. The spectra were collected after conditioning in protamine solution buffered to physiological pH in the solution of 0.1 M NaCl for 1hr and after 24 hrs. The electro transport of poly ions across the interface of polymer ion selective membrane is dependent on the bulk resistance of the membrane. If the resistance of the membrane is very high, then higher currents cannot be passed through the membrane electrode.

The polyelectrolyte coat on the surface of the membrane blocking the interface of the membrane increasing the charge transport resistance,[30] that, was indicated by the presence of second semicircle in the impedance spectra obtained. Here, there is no significant change in the semicircle was observed and the bulk resistance remained in fact the same. This may be attributed to fact that protamine is small polypeptide having a molecular weight ~ 5000 Daltons which when compared with big proteins, it may not form strong coating. Selectivity is the most important characteristic of an ion selective membrane.

So 1st step would be determination of unbiased selectivity coeffients for both sensing and releasing membranes. From the above step, membrane of high selectivity toward protamine would be chosen to carry out the following experiments. The potentiometric selectivity of the membrane to be used as coulometric electrode material was determined by recording individual calibration curves for the chloride salts in the order of Hoffmeister series like sodium, potassium, magnesium, calcium

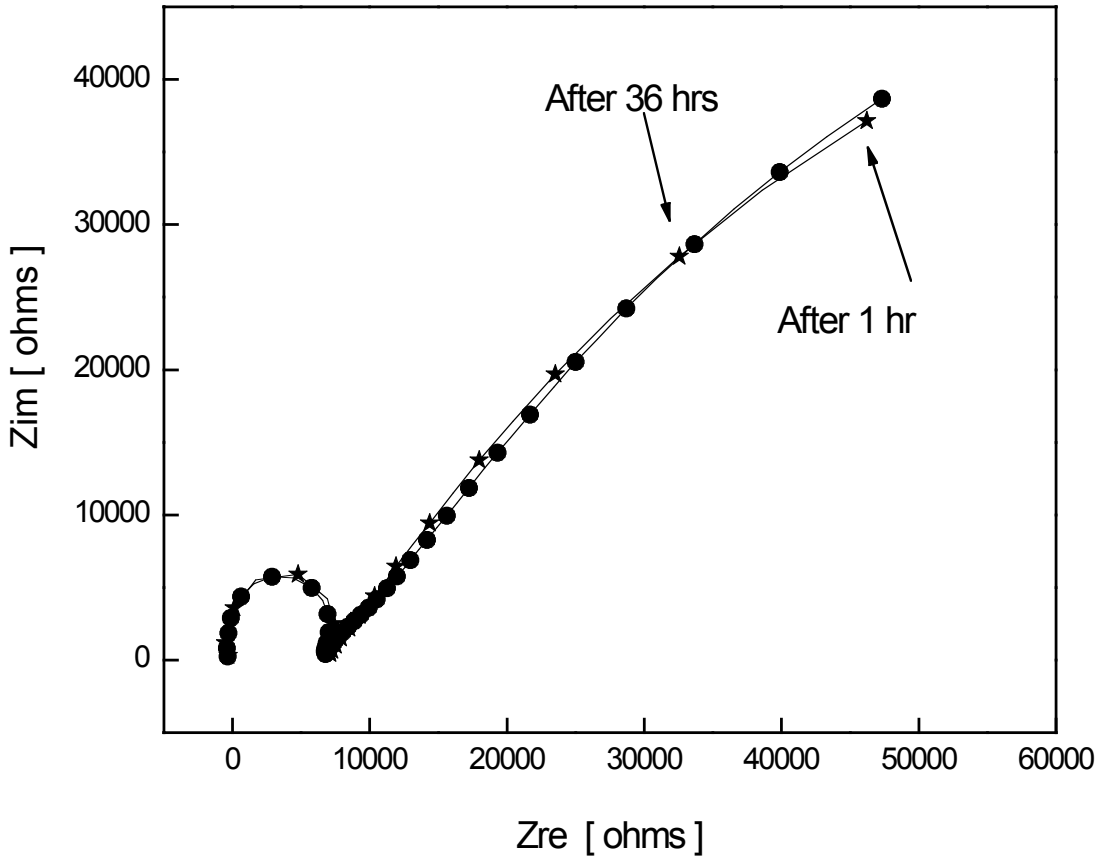

Fig. 6.1. Impedance spectra obtained for coulometric release electrode of PVC/o-NPOE membrane having 1 wt% of DNNS as the ionexchanger and 10wt% lipophilic salt ETH 500, conditioned for 1hr and 36 hrs respectively in 0.001 M protamine chloride in 0.1 M NaCl in pH 7.4.

see Fig.6.2. The sodium and potassium calibration curves show near Nernstian slopes. The subsequent calibrations carried out with magnesium and calcium shows super-Nernstian slopes from 10^{-6} to 10^{-5} M for magnesium and 10^{-6} M–10^{-4} M for calcium respectively. A protamine calibration curve in 0.1 M NaCl was also carried out. The higher potential readings demonstrate a preference of this membrane electrode for protamine over all the tested cations. However, the over all EMF difference for entire calibration is less than 30 mV. This shows that, this is barely enough to be used as an electrode material for coulometric release; it may not be an ideal candidate for a protamine release in the presence of several other ions in higher concentrations.

Since, we know DNNS is an anion, it could have affinity for other cations as well. So, it is very important to know the selectivity coeffcients for those common ions such as sodium, calcium so on, abundantly found in the blood. As the use of polymeric membrane electrode to coulometrically deliver polyion would be questionable, if very high concentration of sodium ions in Tris buffer, pH 7.4 was in the background and this membrane electrode was not so selective for the polyion to be released.

Especially, it is very significant to know selectivity of this electrode to sodium as some suppliers of blood use sodium citrate as an anti coagulant to the blood. If membrane is not selective to protamine, then, it may undergo partial ionexchange with sodium ions resulting in release of sodium ions instead of protamine.

Hence, selectivity for protamine is the main property for which a protamine selective membrane that is to be used as couolmetric electrode has to be characterized. The determination of unbiased selectivity coeffients[31] for releasing membranes containing 1 wt% DNNS and KTClpB with PVC, o-NPOE in 1:2 ratio was carried out.

The potentiometric unbiased selectivities were determined by recording individual calibration curves for the chloride salts in the order of Hoffmeister series like sodium, potassium, magnesium, calcium as shown in Fig 6.3. The calibration curves of sodium and potassium showed near Nernstian slopes for the DNNS based membrane. The electrode membrane showed good Nernstian slopes for both calcium and magnesium.

A protamine calibration curve in 0.1 M NaCl buffered to pH 7.4 was also carried out. The higher potential readings demonstrate a strong preference of this membrane for protamine over all the tested cations. It showed about 100 mV of total EMF change from the baseline potential obtained from the background of 0.1 M NaCl. This shows that this can be used as electrode material for coulometric release. Membrane with DNNS was used as a couometric electrode to carry out the following experiments in this article.

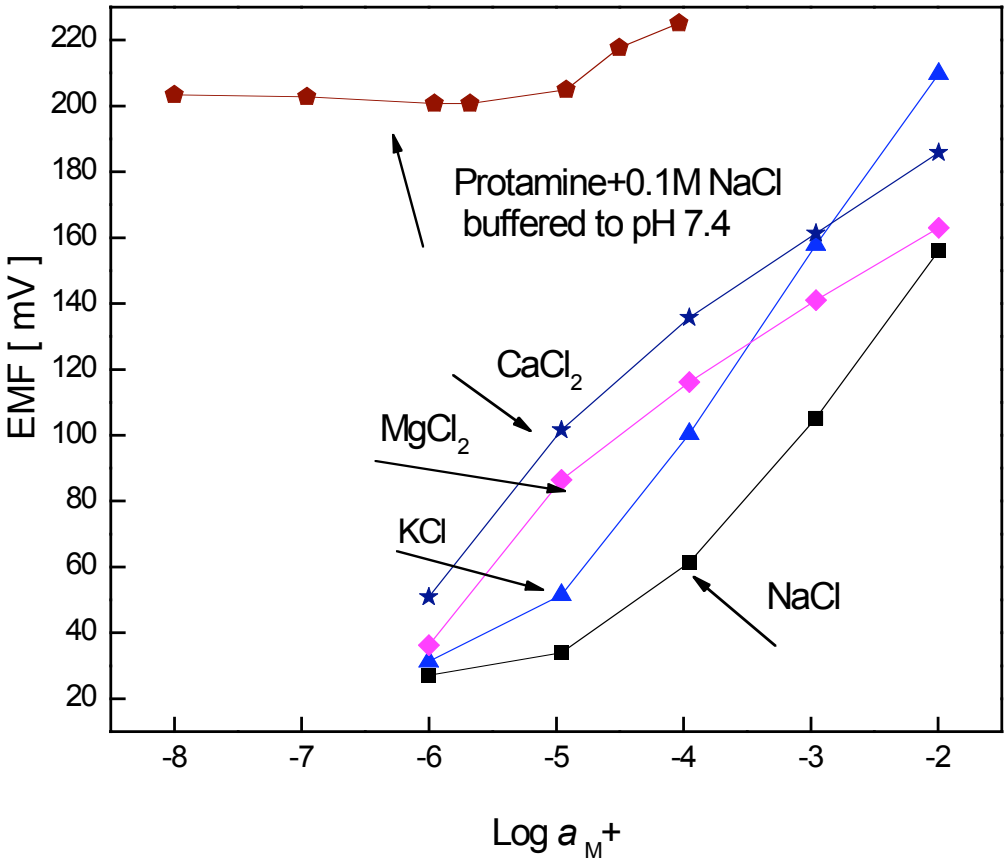

Fig. 6. 2. Unbiased selectivities of coulometric membrane electrode having PVC/o-NPOE in the ratio of 1:2, 1wt% of KTClpB and 10wt% lipophilic salt ETH 500.

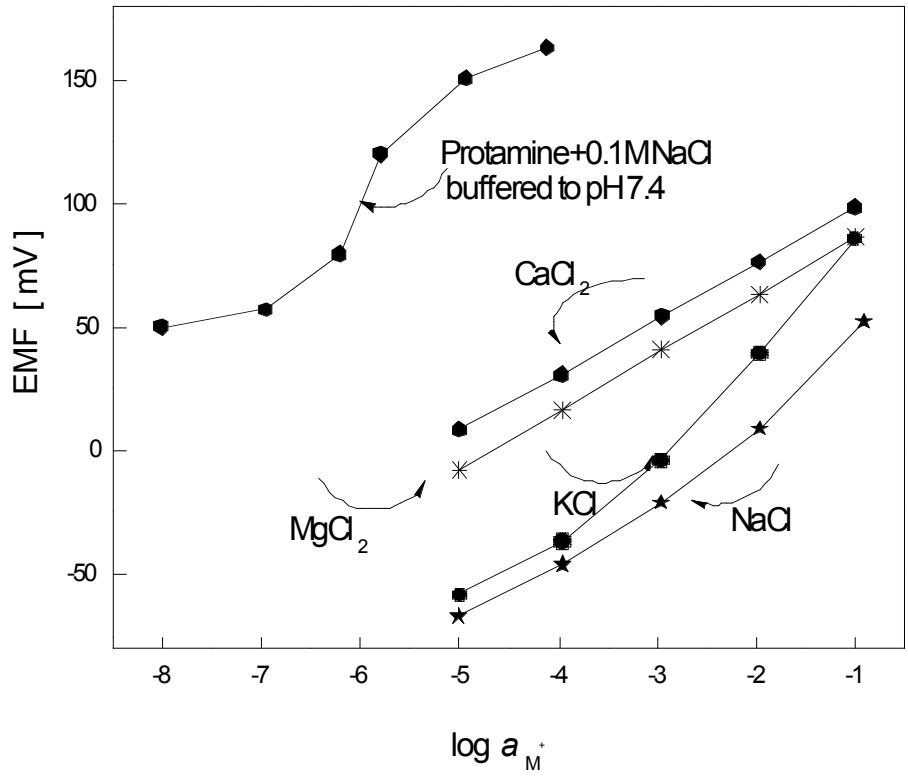

Fig. 6.3 Unbiased selectivities of coulometric membrane electrode having PVC/o-NPOE in the ratio of 1:2, 1wt% of DNNS and 10wt% lipophilic salt ETH 500.

Fig 6.4. Illustrates potential-time traces for the calibration of the sensor at a cathodic current of -1μA in the background of 0.1 M NaCl buffered to pH 7.4. This was to ensure the sensor was in working condition especially, in the relevant clinical measurable concentration. Calibration is carried out so as to measure from the sample if there was any undesirable spontaneous release of protamine from the large area protamine selective coulometric electrode. Following the entire calibration with protamine, the sensor was washed in the background solution and was promptly brought back to baseline potential. After this sensor was inserted into coulometric measuring set-up so as to check if there was a spontaneous release from coulometric polymeric membrane electrode during the time duration of 1752 s without the application of anodic release current.

The reason why this time duration chosen was, as the entire duration of calibration took the same duration and coulometric titrations shown in this work also were less than the time period for which check was performed. As the figure shows, for the entire duration of the experiment, the sensor stayed perfectly on the baseline potential. Sensor when under the influence of cathodic current, on exposure to polyions like protamine should extract the ions into membrane changing the baseline potential, if there was any spontaneous release from the coulometric membrane based electrode. As there was no visible drift from baseline potential itself was a proof that there was no spontaneous release whatsoever was there from coulometric membrane based electrode for the mentioned duration.

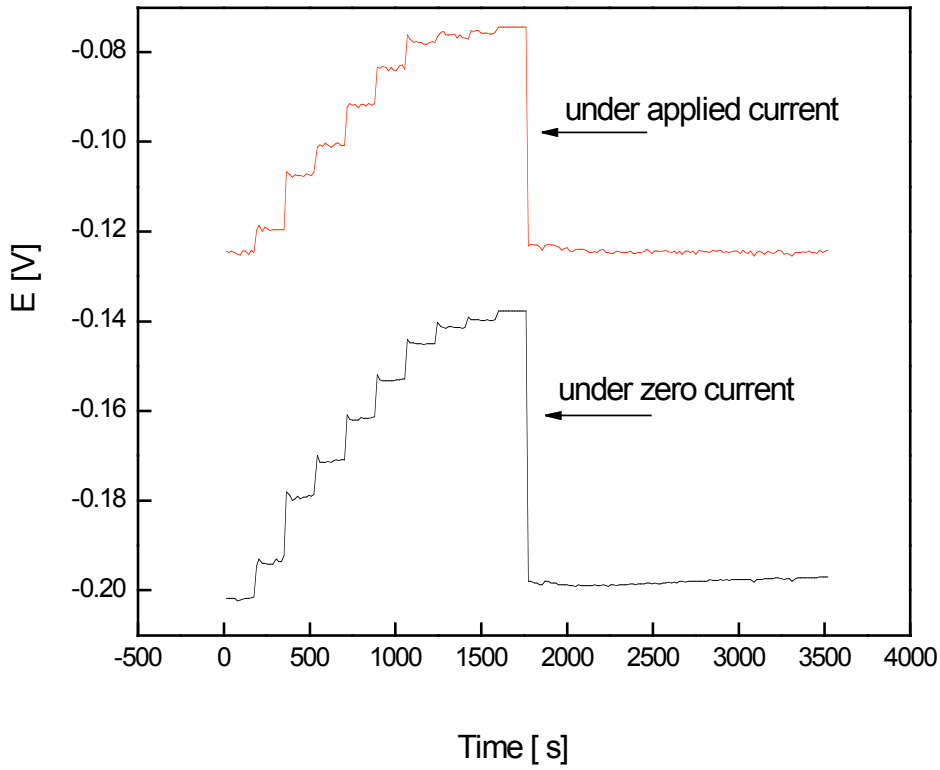

Fig. 6. 4. Check for any undesirable spontaneous release of protamine from the large area protamine selective coulometric electrode using pulse galvanostatic protamine sensor under -1μA after a complete calibration with protamine.

6.3.1 Coulometric release of protamine

Figure 6.5. Shows sensor response to the protamine released subsequently in the log C protamine mg/L. Constant current of 20 μA of equal duration pulses were applied to electro release protamine into the sample containing 0.1 M NaCl at pH 7.4. After the electrochemical generation of protamine, the pulse galvanostatic sensor used to sample for protamine signal. The potential response of these sensors was quite stable in the measured concentration range with high reproducibility. This is important with regard to its use as a sensor in heparin-protamine titrations. Coulometric release is performed using a protamine selective coulometric membrane electrode at the constant current of 20 μA in a 1.5 ml coulometric membrane cell.

According to Faraday's 1st law of electrolysis, the experiments were in good agreement with theoretical prediction; concentrations of protamine released were back calculated from the sensor calibration curve. The coulometric release was quite reproducible with a complete capability to estimate clinical heparin concentration range with titration. The reproducibility of the coulometric release was appraised by carrying out multiple release experiments at identical conditions. Error bars represent one standard deviation for 3 consecutive measurements with coulometric release performed with the same membrane electrode at 20 μA. The error bars obtained was very small compared with most of the techniques making it most desirable for quantitative estimation of heparin clinically.

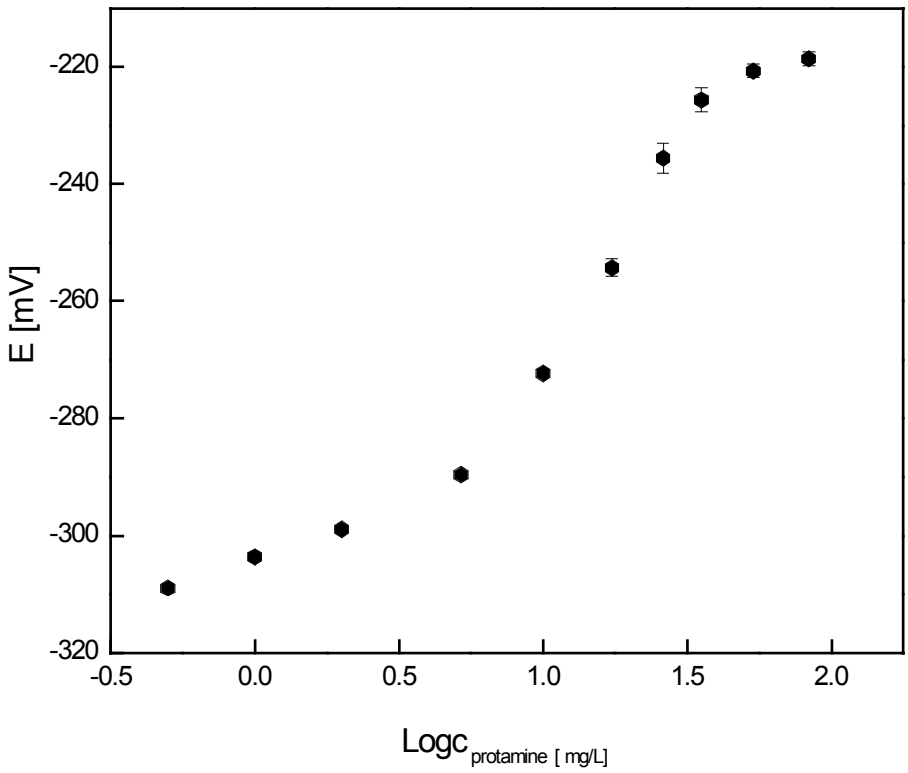

Fig 6.5. Coulometric calibration curve of protamine obtained in clinically relevant ranges of concentrations at a constant current of 20μA from a DNNS based membrane electrode having 1:2 of PVC/o-NPOE

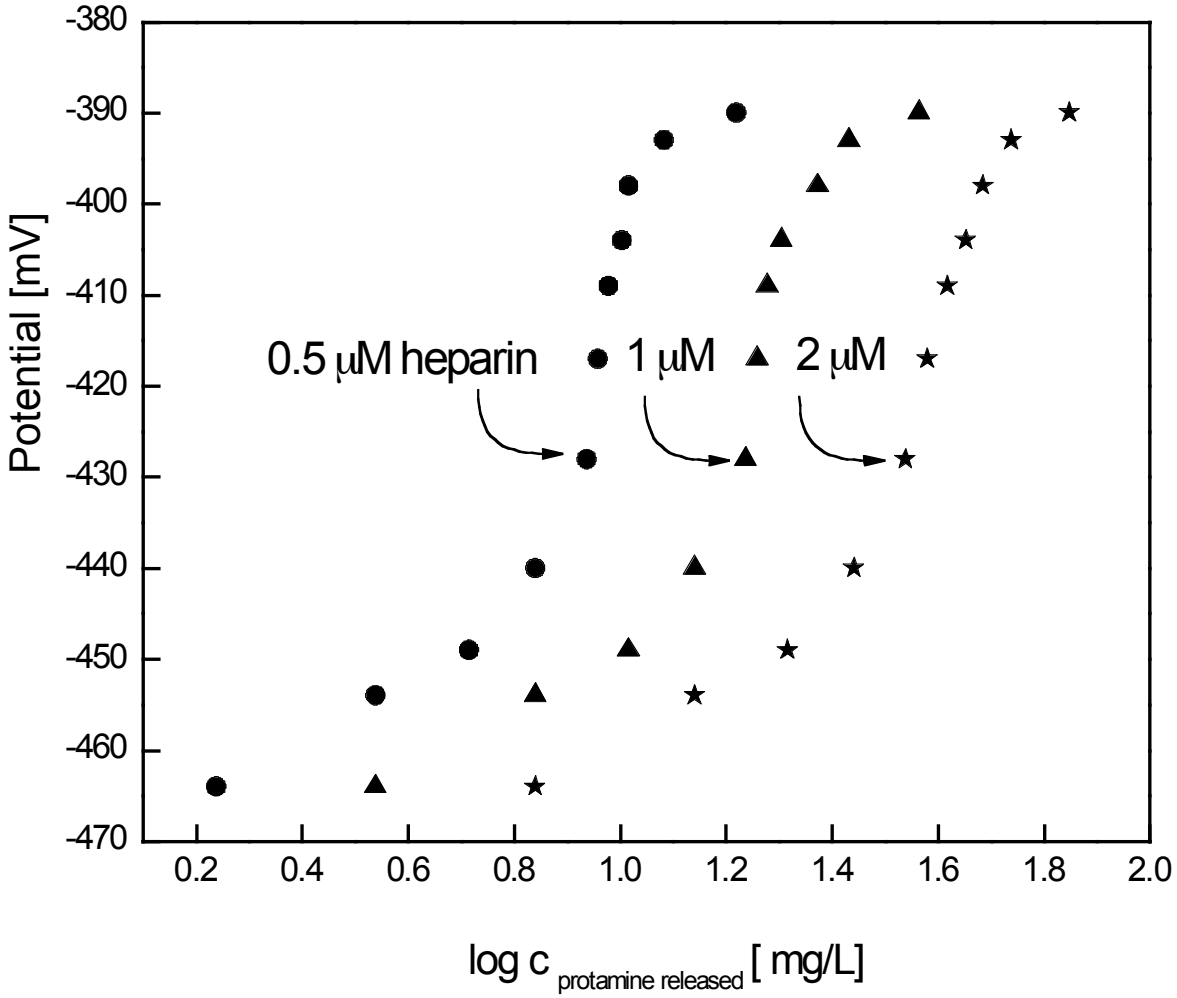

Fig. 6.6. Constant current coulometric titrations of protamine with different clinically useful concentrations such as 0.5, 1 and 2 μM of heparin

6.3.2 Release of protamine to titrate heparin coulometrically in clinically relevant concentrations.

Successfully automated coulometric titrations of beef lung heparin in 0.1 M NaCl buffered to physiological pH of 7.4 were carried out in the range of 0.6-4.5 KU/L. Figure.5.6 demonstrates the typical titration curves obtained with the coulometrically released protamine as log $C_{protamine}$ mg/L. The EMF response of free protamine in the sample is sensed using pulse galvanostatic protamine detection.

The coulometrically released protamine, complexes with heparin as it is released from the selective polymer membrane. The sample titrations are carried out by the addition of known quantity of heparin to the background electrolyte, so as to acquire different sample concentrations in the clinical range. Prior to the coulometric titration the sensor was characterized for the current response and calibrated with protamine using the stock solution. Figure illustrates three coulometric titration curves carried out consecutively in the order of lower to higher concentrations of heparin.

The titration endpoints shift stoichiometrically with respect to change in heparin concentration of the sample. The titrations were carried out in the volume of 1.5ml and at a constant anodic current of 20 μA. Titrations were carried out with several steps of equal duration of release followed by a sampling of every point by averaging ten consecutive potential readings. Repeating the same under identical conditions with the same coulometric electrode at least 4 times assessed reproducibility of these titrations.

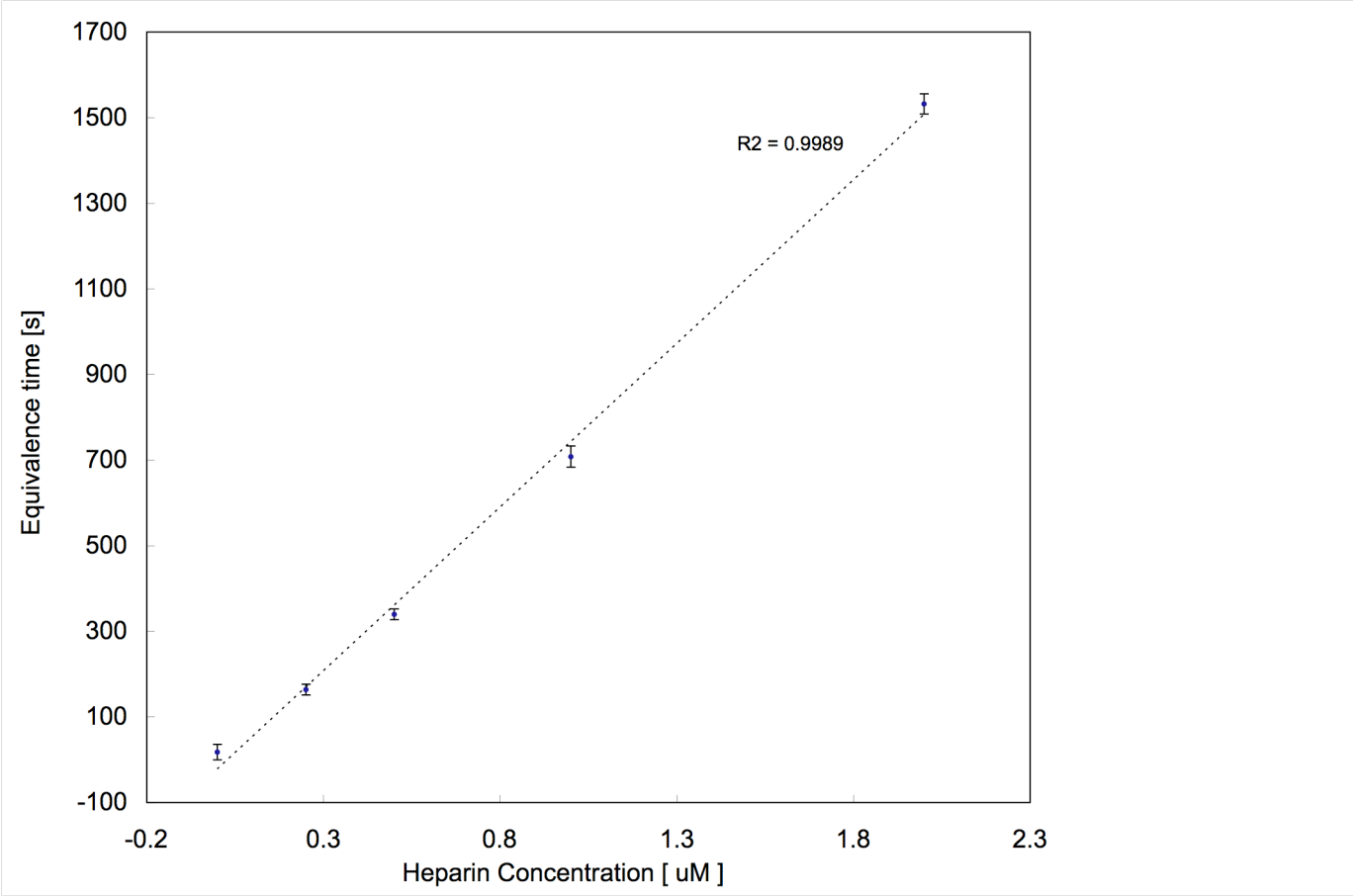

Fig. 6.7. Calibration curve for the titration of buffered samples containing 0, 0.25,0.5, 1, and 2 μM heparin with coulometrically released protamine solution. Dashed line represents the linear regression of the data

The protamine released coulometrically to titrate, explains the huge EMF response to the appearance of endpoint to the corresponding different sample concentrations of heparin. However, there is an increase in potential of detecting electrode initially, much before titration endpoint due to extraction of the positively charged low molecular weight complex of heparin into the polymer membrane as also observed by Meyerhoff.[32] Standard deviations of the endpoints in the titrations were calculated from several consecutively ran titrations. Depending on the Fig 6.6 Shows a plot of coulometrically observed titration endpoints as a function of heparin concentration in the sample. This is a linear curve with a correlation coefficient of 0.998. This technique is remarkable in its accuracy giving analytical chemist an edge in clinical studies.

6.4 Conclusions

Completely automated coulometric calibration free coulometric titrations have been successfully used to monitor the clinically relevant heparin levels in the buffer in physiological range. In addition, the selective and reversible pulsed galvanostatic sensors were utilized as suitable endpoint indicators. The statistics obtained showed remarkable accuracy and precision demonstrating it as a very attractive prospect in clinical laboratories and hospitals thus eliminating human error. The coulometric ion selective membrane had better selectivity over other common cations, which would otherwise cause interference during the release. Further studies on this quantitative determination of polyions and its application to whole blood and plasma measurements are underway in our laboratory.

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CHAPTER 7

COULOMETRIC PRECIPITATION TITRATION OF SULPHATE WITH BARIUM

7.1 Introduction

Antibiotics were the first ever neutral ionophores tried as ion carriers in ISE membranes.[1,2] Then the ionophore research followed the above with a large number of natural and synthetic uncharged carriers for cations and a series of charged electrically neutral, lipophilic ion-complexing agents which show characteristics of ionophores or ion carriers [3]. These neutral carrier ligands have been designed for selective extraction of specific ion from aqueous sample solutions into a hydrophobic membrane phase and transporting these ions by neutral carrier transport. The neutral carriers, when embedded into a organic membrane phase using an appropriate organic solvent or an inert matrix, functions as cation-selective sensors and found to show a Nernstian response to the primary ions. However, not all the ISEs are useful in terms of measurement of actual metal ion concentration.

A classical example of the above is a Barium ion-selective electrodes used for the determination of sulphate using either a precipitation titration with $BaCl_2$[4-8] or a method based on precipitation with barium (II) chloride followed by potentiometric

back-titration of the excess of barium (II) salt with sodium tetraphenylborate in the presence of polyethylene glycol.[9] The use of simple and convenient potentiometric sensors for the fast determination of sulfate in natural and drinking waters makes the procedure inexpensive and attractive. But, the natural water has plenty of calcium too. So, the barium ISEs would have to distinguish very well between calcium and barium. As selectivity of the carrier for an ion depends on how well it fits into the ring or cage formed by the atoms of the ionophore and a function of ionic radius and ring size.[10]

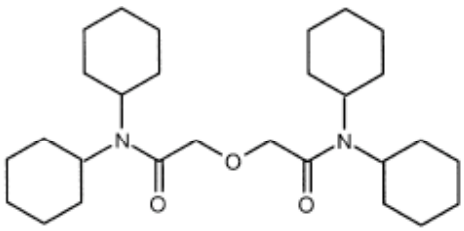
Several researchers tried the common poly alkyl ethers complexed with tetra phenyl borates dissolved in nitrobenzene as an ionophore with selectivity towards barium. However, it was found that the above complexes were water soluble resulting in potential drift. An ISE made with saturated solution of Igepal CO-880.Ba.2TPB in p-nitroethylbenzene as the ion exchanger was found to give linear, and near Nernstian response (26.6mV/decade) from 10^{-1} to 10^{-6} M $BaCl_2$.

Above ISE was found to have good selectivity to barium compared to other divalent ions. And the selectivity ratios obtained were greater than 10,000 to 1 for Ba^{2+} over Ca^{2+}, Mg^{2+}.[11] Some research groups also tried the natural monensin and their derivatives for Li^+ ISEs. The carboxylic polyethers such as natural monensin based electrode exhibits high selectivity for Na^+ and Ba^{2+}. Especially, the high Ba^{2+} selectivity could be attributed to the complex formation of negatively charged monensin deprotonated at the carboxylic group with Ba^2.[12]

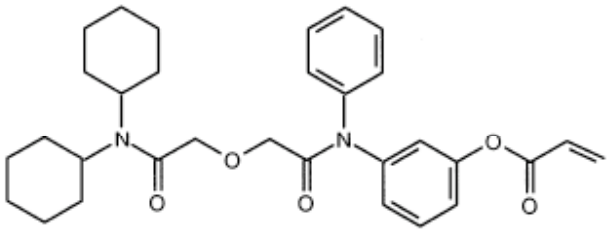
Simon group synthesized highly lipophilic electrically neutral ionophores for barium. They used the ionophore with the following chemical name 2{(2[Dicyclohexylcarbamoyl)methoxy]diphenoxy)ethoxyl}N,N,dicyclohexylacetami de for the sulphate estimation in the drinking water even though it had more interference from calcium and strontium. The reason to use the above ionophore was its ability to distinguish very well the other common ions available in water. The EMF jump of more than 60 mV was obtained for the above ionophore.[13]

Neutral organophosphorus compounds with phosphoryl complexing groups have successfully been used as ionophores for alkali and alkaline ion sensors.[14-23] Their complexing properties are mainly dictated by the structure of the linear backbone chain and the electronic nature of substituents at the phosphoryl groups. Many of the phosphoryl containing ionophores have useful selectivity towards barium ions.[21-23] Main limitations of barium sensors available today are insufficient selectivity, sub-Nernstian slopes and a very narrow response range.

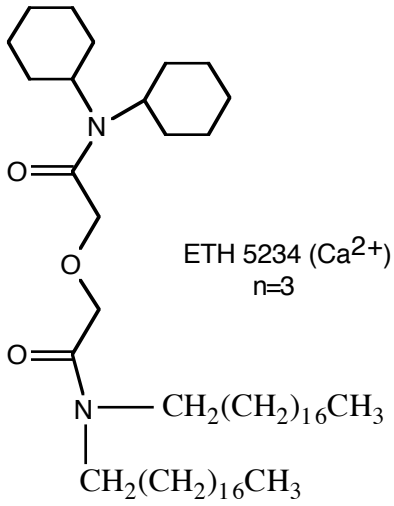
Barium ISEs still needed calibration and measurement of sulphate by analyte subtraction. Above problem of calibration in a monitoring device could be alleviated by employing a coulometric titration. The coulometric electrode releases a known quantity of barium into the sample sulfate solution. And the moles of barium released is given by no of coulombs passed through that membrane electrode divided by its charge times the Faraday's constant in a fixed volume. This elegant semi-automatic titration device if made portable could be used as a field device by environmentalists.

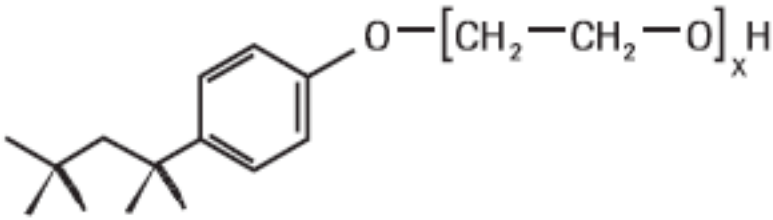


ETH 129



AU-1





Triton-X-100

Fig. 7.1. The chemical structures of the studied alkaline earth metal ion selective ionophores

The selective coulometric release of barium ions, were made possible by employing ion selective membrane coulometry.[24] The ionophores employed for selective coulometric release were selected from a group of calcium selective ionophore such as Ca (IV) and Ca (II) and AU-I , an ionophore synthesized by our own group. The structures of these ionophores were illustrated in Fig 7.1. This chapter talks about the process of screening these ionophores for their selectivities. And talks about a successful coulometric estimation of sulphate in concentration range of regular drinking water as a preliminary example of this type of titration carried out with a selective membrane based coulometry.

7.2 Experimental

Reagents

High molecular weight poly(vinyl chloride) (PVC), the plasticizers 2-nitrophenyl-octyl ether (o-NPOE) and bis(2-ethylhexyl) sebacate (DOS), the ionophore Calcium (IV) N, N-dicyclohexyl-N',N'-dioctadecyl-3-oxapentanediamide(ETH 5234), Ca(II) [N,N,N',N'- tetracyclohexyl-3-oxapentanediamide] ETH 129 and Au-I the lipophilic cation-exchanger salt sodium-tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), the inert lipophilic salt tetradodecylammonium-tetrakis(4-chlorophenyl)borate (ETH 500), tetrahydrofuran (THF) and all salts were purchased from Fluka Chemical Corp. (Milwaukee, WI) in Selectophore quality or puriss quality. Aqueous solutions were prepared by dissolving the appropriate salts in nanopure-deionized water (18.2 MΩ cm).

Ion selective membrane preparation

The barium selective membrane components consisted of 13 mmol kg^{-1} of Ca (IV) ionophore or Ca (II) ionophore or Au-I , 4.5 mmol kg^{-1} NaTFPB and 10 wt% of inert lipophilic salt ETH 500 together with o-NPOE and PVC (in a 2:1 mass ratio) with a total mass of 140 mg, dissolved in 1.5 mL of THF and pouring into glass ring (22 mm i.d.) affixed to microscope glass slide. The solvent THF was allowed to evaporate overnight to give a membrane of 200 □m approximate thickness.

Electrodes

Ion-selective electrodes for the detection of the released calcium ions were prepared by punching a series of 3 mm discs from the parent membrane and gluing to plasticized PVC tube with THF, which was mechanically affixed to a 1000 mL pipette tip. The barium selective coulometric electrodes were conditioned in 10^{-2} M $BaCl_2$ overnight with an identical inner filling solution. The barium selective sensors were conditioned in 10^{-3} M $BaCl_2$.

The calcium selective electrodes characterized for the selectivity were conditioned in a 0.01 M NaCl solution identical to the inner filling solution over the night. Selectivity characterization required no prior exposure to the primary ion calcium before the measurement. Standard deviations were obtained based on the measurements of sets of at least three replicate membrane disks from the same parent membrane of at least three replicate membrane disks from the same parent membrane.

Experimental setup for coulometry

Due to the simplicity of instrumentation for constant current coulometry, we built the apparatus from a galvanostat as the constant current source and the electrolysis cell, including end-point detection system. Traditional constant current coulometry consists of a 2-electrode set-up with the coulometric electrode as the anode in the case of cations. A high surface area coiled Pt wire was used as a counter electrode. The experiments were performed with an AFCBP1 Bipotentiostat (Pine Inst., Grove City, PA). The detection circuit consisted of an ISE and a reference electrode isolated from the current generating galvanostat ground loop.

In our setup ISE potentials were recorded with a battery-powered high impedance amplifier (AD820, Analog Devices) connected to the data acquisition system (AduC824, Analog Devices) controlled by handheld computer (Palm III) at room temperature. The external reference electrode consisted of a double-junction Ag/AgCl electrode with a 1 M LiOAc bridge electrolyte and 3M KCl as reference electrolyte. All EMF values were corrected for the liquid junction potential using the Henderson equation. The activity coefficients were calculated according to Debye-Huckel approximations.[25]

Calculations

The concentration of the electro-generated reagent was calculated according to Faraday's law:

$$C = \frac{it}{nFV}$$

where C is the concentration of reagent, i is the magnitude of the constant current pulse used to generate the reagent, t is the duration of current applied pulse, n is the valency of the reagent (here, n = 2), F is the Faraday constant and V is the volume of the sample into which the reagent is released. The precipitation reaction titration curve was calculated with the solubility product K_{sp} =1.1 x 10^{-10}.[26] End points were obtained according to established procedures from the second derivative plot of $[\Delta(\Delta pM^{2+}/\Delta t)] / \Delta t$ versus t. The end point is the t value when the curve crosses the abscissa.

7.3 Results and discussion

The coulometric release of barium ions was explored with an ionophore-based polymeric membrane calcium-selective electrode. In addition to a high concentration of inert lipophilic electrolyte, the membrane was formulated according to standard procedures with the ionophore ETH 5234 and an appropriate cation-exchanger, and backfilled with a relatively concentrated barium chloride solution. The ionophore ETH 5234 is known to be very selective to calcium[27]. So-called unbiased selectivity coefficients[28]of ETH 5234 based coulometric electrodes towards discriminated ions were measured and are reported.

Fig.7.2. shows calibration curves obtained induvidual chloride salts of common ions found in the water including barium. These were obtained from three identical Ca^{2+} selective electrodes conditioned overnight in 0.01 M NaCl.

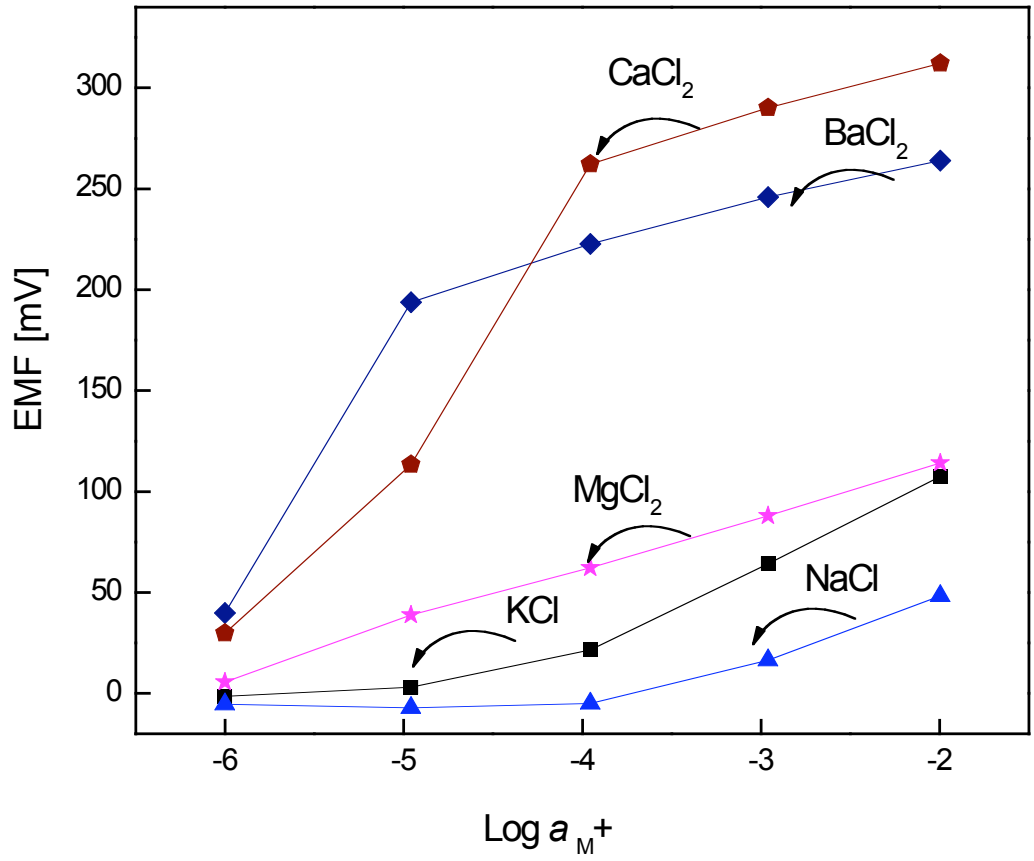


Fig. 7.2. Unbiased selectivities obtained for membrane containing Ca (IV) ionophore.

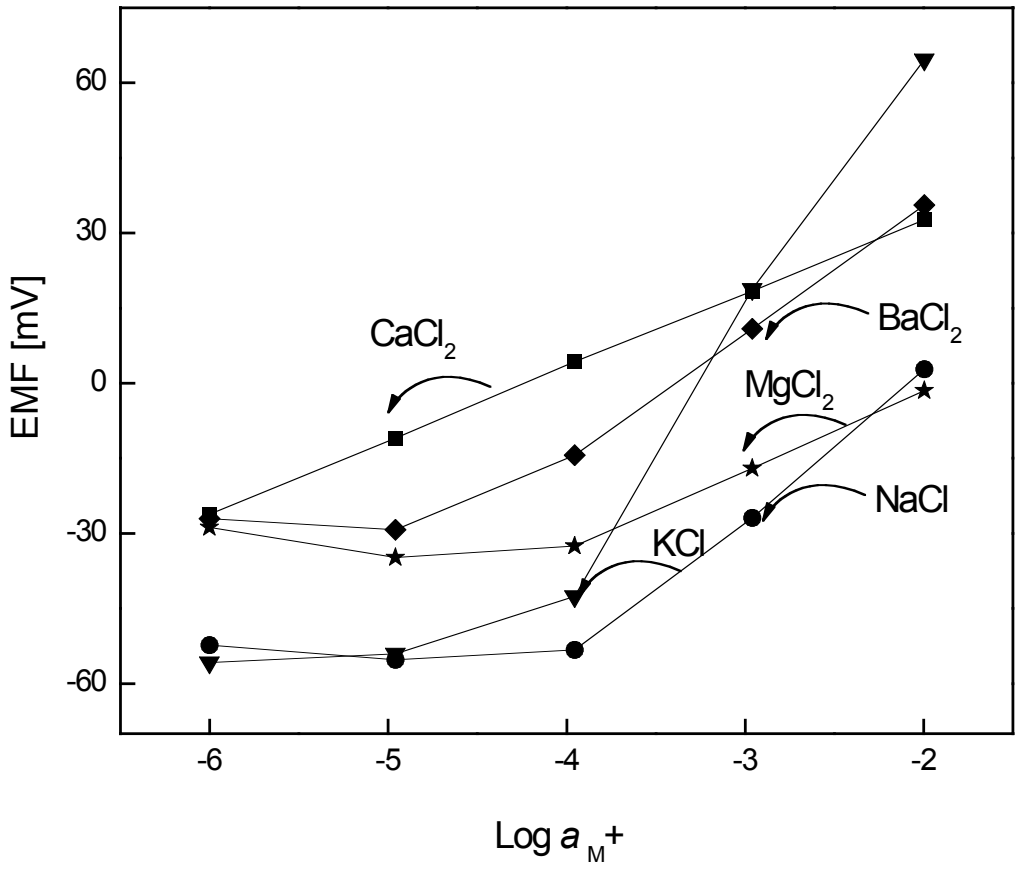


Fig. 7.3. Unbiased selectivities obtained for membrane containing Au-I ionophore.

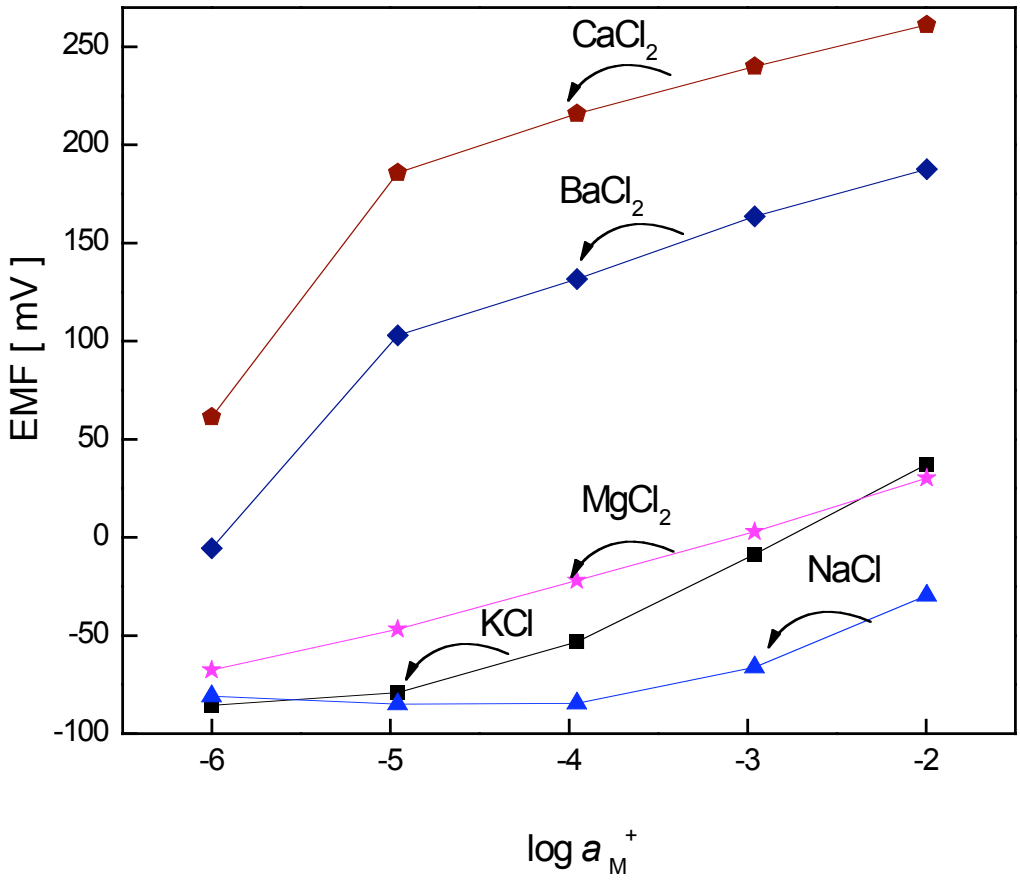


Fig. 7.4. Unbiased selectivities obtained for membrane containing Ca (II) ionophore.

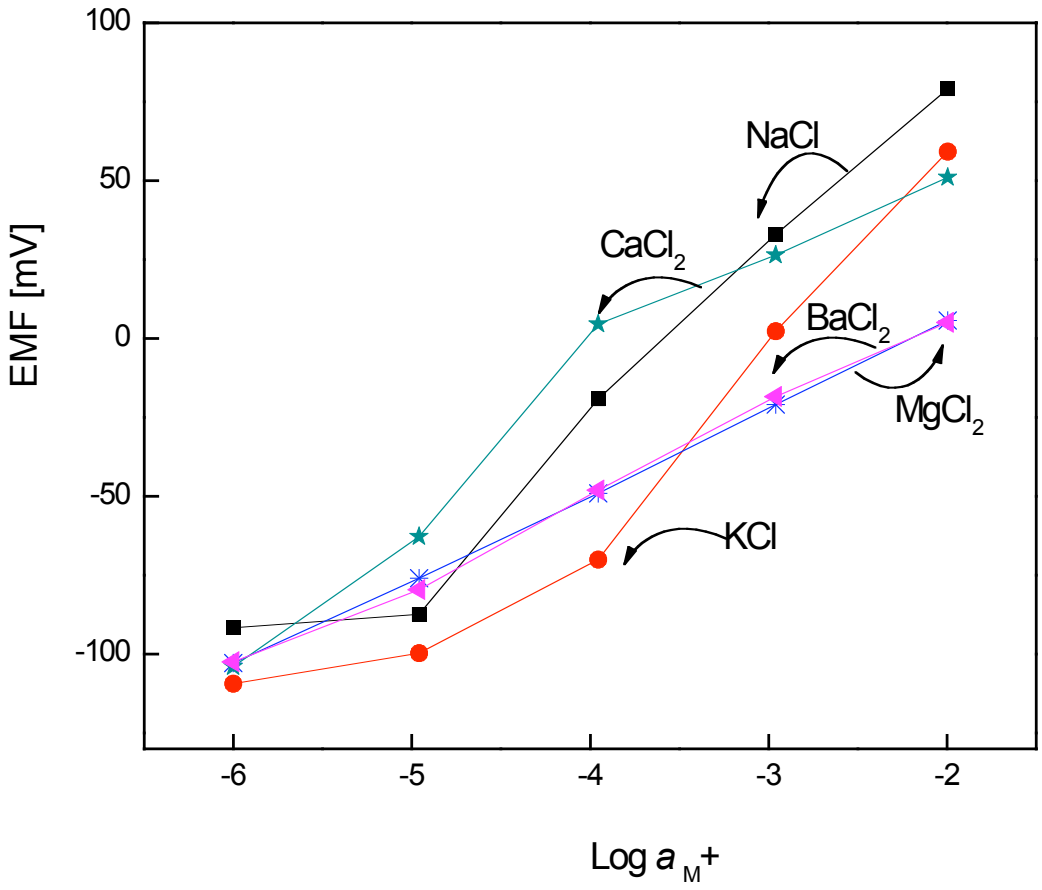


Fig. 7.5. Unbiased selectivities obtained for membrane containing Ca (IV) ionophore with the sample containing Triton-X-100.

The calbration curves obtained for calcium and barium show super- Nernstian slope around 10^{-6} to 10^{-4} M and 10^{-6} to 10^{-5} M respectively. The obtained response slopes were all near-Nernstian, suggesting that the selectivity coefficients shown are close to their unbiased values. From the small values of log $K_{Ba,J}^{POT}$ obtained for all the discriminated ions, it is evident that the membranes exhibited a high selectivity toward barium except for calcium. Since the nature of the study involved no calcium in the background, this was used to release barium.

Fig 7.3 shows the unbiased selectivties curves for chloride salts of sodium, potassium, magnesium, barium and calcium recorded for the ion selective membranes containing Au-I. The Au-I was not very selective to barium as we can see from individual calibrations recorded subsequently. The sodium and potassium give near-Nernstian slopes and K^+ shows a super-Nernstian slope from 10^{-4} to 10^{-3} M. For calcium it showed perferctly Nernstian slope and for barium and magnesium it showed a near Nernstian slopes. It did not really distinguish very well between barium and the commonly available ions in water. Hence it is not a good ionophore for sulphate estimation.

Fig 7.4 illustrates the unbiased selectivities obtained for calcium (II) ionophore chloride salts in Hoffmeister series. The calibration curves obtained for sodium and potassium were near-Nernstian. And Calcium (II) ionophore distinguished the sodium and potassium ions from barium very well. And the subsequent calibration curves with magnesium, barium and calcium were recorded.

The magensium is also well distinguished in a similar fashion as to Ca (IV). And calcium and barium calibration curves show super-Nernstian from 10^{-6} to 10^{-5}M. Calcium is preferred much more than barium. This could be potential candidate for barium release and detection other than Ca (IV) in the absence of calcium. Fig.7.5 shows unbiased selectivities of the membrane having Ca (IV) ionophore, obtained in the presence of a surfactant rich in polyoxy methylene units such as Trition-X-100. The poly ether groups are known for their selectivity towards barium ions from the literature[9]. Hence, it was tried with so far best available Ca (IV).

Separate calibration curves were recorded for a membrane shows that slopes obtained in the concentration range were found to be super-Nernstian which actually biases the selectivity coefficients obtained.[29,30] This super-Nernstian jump in potential is due to depletion at sample –membrane interface as the surfactants block the surface resulting in change of ion flux.[31] The slopes obtained for all common ions such as sodium,potassium and magnesium were near-Nernstian. Membrane did not show any increase in selectivity towards barium. In fact the membrane seems to prefer almost all ions to the same extent. So it was ruled out.

The indirect determination of sulfate as barium sulfate by precipitation titration was also explored. In the selectivity data shown in Table 2.1, the calcium-releasing membrane was found to be moderately selective for barium ions. They are less preferred than calcium ions by 3.5 orders of magnitude, but if calcium is absent, the other tested ions are still reasonably discriminated, even when compared to the commercially available barium ionophore I.[13]

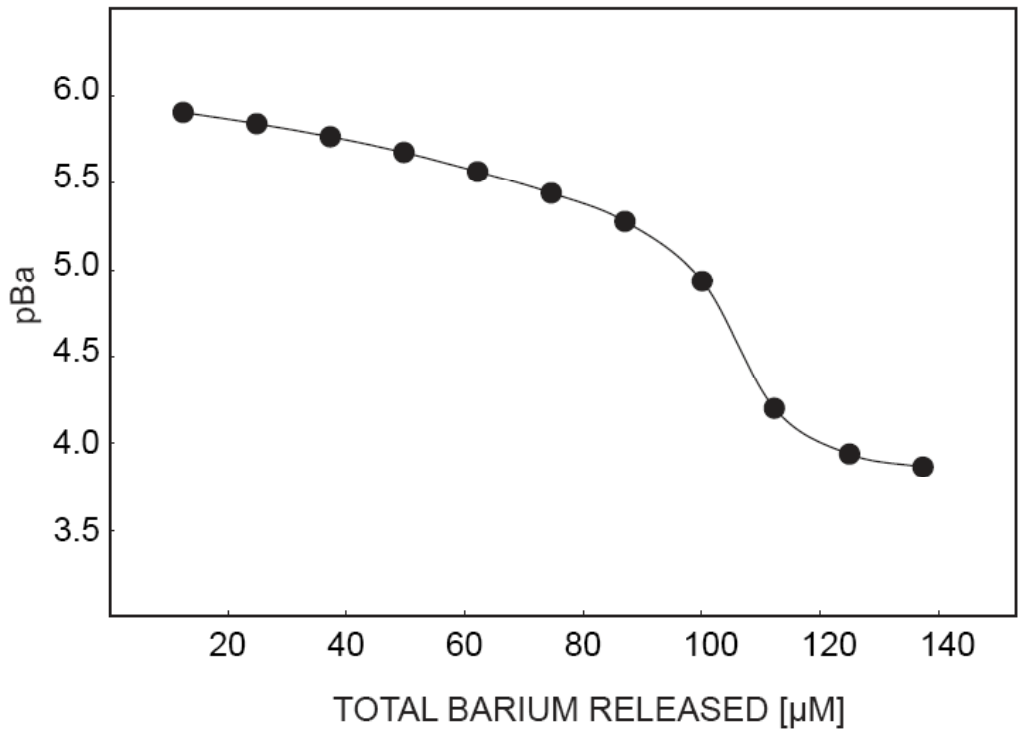


Fig. 7.5 Coulometric precipitation titration to estimate 1 x 10^{-4} M sodium sulfate with coulometrically released barium from an ion-selective membrane. The titration was carried out at a constant current of 20 μA. Solid line: theoretical titration curve.

A fresh releasing membrane was conditioned in and backfilled with barium chloride solutions for the purpose of releasing barium ions. The electro-release of barium into the sample containing sodium sulfate was achieved at 20 μA current pulses, as shown in Figure 7.5. For the current setup and with a 100 μM sulfate concentration, the end point was observed after 485 ± 6 s. The sensors used for barium detection also were calcium-selective membranes of the same type as used in the above experiments, but conditioned in barium chloride solutions. The experimental titration curves were in good correspondence with the theory, which was obtained from the solubility product after calculating the released barium concentration using Faraday's law.

7.4 Conclusions

Preliminary precipitation titration of sulphate with coulometrically released barium was carried out in concentration range it is available in natural waters. The time of entire titration was short with this semi-automated titration. The sample used is small. Constant current coulometry employing ion selective membrane has a good accuracy, making this technique more appealing. With a better selective barium selective membrane, this has a potential to be used as a regular monitoring device by environmental agencies.

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CHAPTER 8

CONCLUSIONS

Constant current coulometry using an ionophore-based ion selective polymeric membrane has been demonstrated to be a feasible technique with a potential to work in real world samples. Different types of semi- automatic titrations such as precipitation and complex formation were carried out as few examples using respective ion-selective membrane based coulometric electrodes, proving its wide applicability. Due to the available plethora of ionophores for various inorganic, organic and neutral analytes the use of ion-selective polymeric membranes for coulometric release broadens the spectrum of reagents that can be electro-generated.

The elegance of this technique lies in use of ion selective coulometric electrode for the applications where the generation and detection of various metals ions, including magnesium is carried out. As an application, coulometric complexometric titrations of dilute concentrations of calcium with EDTA were carried out to get sharp endpoints with a very calcium selective membrane electrode successfully. The polymeric ion selective coulometric electrode have polarizing interfaces and the passage of current across the membrane results in concentration polarization of charge carrying species such as metal ion complexed with ionophore and the ion pairs with ion exchanger sites having metal ions as their counter-ions.

An effective and simplified theoretical treatment is applied to model the concentration polarization of ionophore under current using chronopotentiometry. The coulometric membrane electrode has the same types of limitations as those of potentiometric electrode with respect to its loss in permselectivity on its exposure to inner filling solutions of higher concentrations of lipophilic counter anions. However, the coulometric electrodes were found to be robust under current application for longer durations, as they do not undergo extraction of anions in the concentrations range where the electrode obeys Nernst slopes. The selectivity change occurring at the membrane inner filling interface does not the coulometric membrane electrode in anyway. The coulometric technique employed is in fact the pulsed constant current coulometry, which ensures that these current carrying mobile species are not depleted by the use constant current pulses of shorter durations.

Sacrifice in current efficiency was therefore avoided cleverly. And also the endpoints obtained for the coulometric titrations had very good precision and accuracy for a 1mL small volume of samples making it an attractive and practically reliable technique. This technique is highly beneficial as a clinical method as it requires no calibration and the important non-electro active reagents such as polyion protamine could be electro-released from the protamine selective membrane for the detection of heparin via heparin–protamine titration in clinically relevant concentrations. Another successful application of ion selective membrane based coulometric electrodes is the estimation of sulphate in the concentration range available in natural waters using precipitation titration with barium.

Barium was electro generated with a Ca (IV) ionophore containing membrane found to be selective for barium in the absence of calcium. The membrane was found to discriminates rest of the interfering ions really well. Both pulse galvanostatic and potentiometric modes of detection have been utilized for the entire work to sense the respective concentrations ions that have been electro released. A pulse galvanostatic protamine sensor based on PVC/DOS matrix is described. The sensor contained a lipophilic electrolyte, which extracts the polyion into the membrane for short time and it is stripped out of the membrane by applying stripping pulse. Usually the stripping pulse is at least 30 times longer than the uptake pulse. The pulse galvanostatic PVC/DOS based protamine sensor was found to be comparable to that of PVC/o-NPOE in terms of its response to protamine in whole blood samples and it is useful matrix in clinically as there is reduced leaching of DOS out of the membrane.