REVIEW OF LITERATURE

The development and application of ion selective electrodes (ISEs) continue to be an exciting and expanding area in the field of analytical research. Clearly, the ability to make direct or indirect measurements in complex samples, without concern about sample colour or turbidity and the fact that such measurements require relatively inexpensive equipment make ISE based techniques attractive to scientists in many disciplines.

The first ion-selective electrode to be discovered was the glass electrode, first reported by **Cremer in (1906)** and later studied by **Haber and Klemensiewicz, (1909)**, which was sensitive to the concentration of H^+ ions in aqueous solutions. Quantum leap in the field of ion selective electrode was achieved with the development of new glasses sensitive to various ions namely Na^+, K^+, Ag^+, NH_4^+,Ti^+, Li^+, and Cs^+ by Eisenman and co-workers in the late 1950s and early 1960s **Eisenman, (1967); Eisenman *et al.* (1957); Eisenman (1962**).

These electrodes were followed by similar sensors for other cations and for anions, based both on dissolved salts and solid crystals **Shatkay, (1967); Coetzee and Freiser, (1968) and Eisenman (1968**), and absorbed onto conducting substrates **Hansen *et al.*, (1972**). The golden age of ISEs started in the 1960s with the discovery that some antibiotics are capable of selective binding of particular ions. Work with neutral carriers gained momentum with the discovery in 1955 of valinomycin **Brockman and Schmidt-Kastner, (1955**), a cyclic depsipeptide antibiotic that had the ability to selectively complex potassium ion. Other depsipeptides have also been investigated as electroactive materials, particularly the enniatins **Wipf *et al.*, (1968**). Several other compounds that have been tried as electro active materials are the peptides gramicidin **Meuller and Rudin, (1967**) and alamethicin (**Pressman,1968**), the macrotetralides of the homologous nactin series **Zust *et al.*, (1973), Stefanac and Simon, (1966) and Pioda and Simon, (1969**), and a series of carboxylic acid ionophores including nigericin **Lutz *et al.*, (1970) and Pointud *et al.*, (1983**), monensin **Lutz *et al.*, (1970**), grisorixin, X-206 (**Pressman, 1968**), X-537A and A23187 **Covington and Kumar, (1976**).

The difficulties experienced in extraction and purification of most of the substances have prompted the researchers to produce synthetic compounds that are capable of exhibiting similar ion-binding properties. First such compounds synthesised was a series of cyclic polyethers, the so-called crowns, produced by Pederson (**Pederson, 1967**), and afterwords all members of the homologous series, from 12-Crown-4 to 30-Crown-10 and their derivatives were synthesised, **Pederson and Frensdorf, (1972**), including several attempts to bind the polyether ring to a polymer chain backbone (**Sinta *et al.*, 1983**). Another series of ligands showing promise as electroactive materials are the bridged polyamine cryptands **Truter and Pederson (1971; Cote and Bauer, (1977); Cox *et al.*, (1984)**, and other ligands reported include a carboxylic acid polymer **Varoqui and Pefferkorn, (1976**), various cyclic peptides (**Karle,1978**), a large number of acyclic compounds produced by **Simon *et al.*, (1984)**, poly-(glycol)s **Jaber *et al.*, (1977); Levins, (1971**), and a novel series of compounds-based on THF subunits (**Nakazaki *et al.*, (1982**).

The first polymeric calcium - ISE appeared in 1967 but it was not until 1970, after the work of Moody and Thomas that it attracted interest and rapidly replaced the liquid membrane electrodes. An interesting configuration of the polymeric electrode was the coated-wire ISE presented **Cattrall and Freiser** in (**1971**). In this modification platinum or silver wire, coated with a polymeric matrix containing various electroactive materials was used. Recently electrodes have been invented for gases and enzymes. The carbon dioxide electrode **Stow *et al.*, (1957); Severinghaus and Bradley, (1958**) was the first designed for the measurement of the partial pressure of carbon dioxide. Later, other gas sensors became available for various gases such as NH_3, SO_2, NO_2, etc.

The year 1969 saw the development of the first ISE based on valinomycin (**Pioda *et al.*, 1969**), which proved to have excellent selectivity for potassium ions in the presence of sodium.

The development of solid-form ion-selective electrodes can be traced back to the work of **Tendeloo** (**1936**), and **Kolthoff and Sanders** (**1937**). Tendeloo's electrodes based on calcium fluoride and calcium oxalate in paraffin matrix were proved to be unsuccessful. The first non-glass ion-selective electrodes were prepared by **Pungor and co-workers** (**Pungor and Hallos-Rokosinyi**) in **1961**, whose membranes were made of embedded

silver-iodide precipitate in an inert matrix (silicone rubber). A series of heterogeneous solid membrane electrodes, such as the silver and halide ion-selective electrodes were later introduced by the same authors.

The first liquid-membrane electrode was introduced by (**Ross, 1967**). This was based on calcium didecyl phosphate with di-n-octylphenyl phosphonate. During the development of membrane electrodes, liquid membranes received greater attention due to the availability of numerous ion-exchange materials. Shortly after the calcium electrode, the emergence of other liquid electrodes based on organic materials such as, nitrate, perchlorate, chloride, cupric ions, etc were developed by many authors.

Recently electrodes have been invented for gases and enzymes. The carbon dioxide electrode **Stow *et al.,* (1957; Severinghaus and Bradley, (1957**) was the first designed for the measurement of the partial pressure of carbon dioxide. Later, other gas sensors became available for various gases such as NH_3, SO_2, NO_2, etc. The first enzyme electrode was introduced by **Clark and Lyons** (**1962**). The principles of these electrodes are similar to gas sensing electrodes, except that the reaction takes place between a biocatalytic layer and the substances to be measured. The product of this reaction was measured by a suitable electrode (**Vadgama, 1979**).

The group of Simon utilised valinomycin, monensin and nonactin to make potassium, sodium and ammonium selective electrodes, respectively (**Lutz *et al.,* (1970); Pioda *et al.*, (1967); Stefanac and Simon, (1966**). This was quickly followed by the realization that other compounds could be utilized and/or synthesized for the purpose of binding selected ions. The word “ionophores” was specially coined for such compounds. The 1960s and 70s were the time of exponential growth in the field of ISEs, so much so that Orion (a company that almost exclusively worked on development of new ISEs) featured “electrode of the month” **(Frant, 1994; Frant, 1997)**. Being responsive only to the bio available ion fraction, having excellent ability to discriminate ions of interest *versus* other ions, and having suitable sensitivity and quick response time, ISEs based on ionophores demonstrated sensing characteristics that were excellently suited for application in clinical analysis. The most valuable application was in the analysis of blood electrolytes, such as Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^-.

Brief history of calcium ion selective electrodes

The importance of calcium in the body was first demonstrated by **Ringer in 1883**. Serum calcium was found by dialysis experiments to be present in two forms, protein bound or non diffusible and diffusible **Rona and Takahashi, (1911**). **McLean and Hastings (1935)** showed that the diffusible fraction could be further divided into the ionised or free fraction which was biologically active fraction. They also noted a relationship between the protein, pH of the serum and ionised calcium and so developed the first nomogram or algorithm for the estimation of ionised calcium using total calcium determinations (**McLean and Hastings, 1935)**. Over the next two decades, further refinements to the nomogram took place but always a direct method of measurement was sought. Many different methods were tried such as bio-assay and photometric techniques but these were labour intensive and prone to interference and so never replaced the measurement of total calcium as the method of choice. The development of a direct method of analysis for ionised calcium came with the rapid development in potentiometry that took place in the 1960's and 1970's.

Ross developed the first calcium ion-selective electrode in 1967 for clinical purposes. Improvements in ionised calcium measurement have come hand in hand with developments in potentiometric techniques. The development of PVC membrane technology (**Shatkay, 1967**), i.e. immobilisation of an active agent in a poly (vinyl chloride) matrix allowed electrodes with longer lifetimes to be manufactured and made clinical analysers feasible. **Simon *et al.*,** developed the neutral carrier ionophore, ETH 1001, in 1972 (**Amman *et al.,* 1972**) and this has since become the most popular ionophore for calcium measurement (**George *et al.,* 1986**). Further improvements in electronics and microprocessor technology has resulted in analysers that can measure pH and ionised calcium simultaneously and so calculate the ionised calcium level corrected to a pH of 7.40. This value of pH is taken to represent the "normal" blood pH in human subjects.

The best development of calcium ISES was the incorporation of ion exchanger or neutral carrier in PVC matrix **Moody and Thomas (1978, 1979)**. Calcium ion-selective sensors based on a poly(vinyl chloride) (PVC) polymer membranes impregnated with different ionophores find wide applications in electroanalytical practice (**Schefer *et al.,* (1986**). They are widely used in medical and biological investigations as well as in the food industry. An important application is the determination of calcium ion concentration in milk

and milk products especially in cheese production during the processes of coagulation **Remeuf *et al.,* (1989) and Mary-Ann Augustin and Clarke, (1991).**

Application of ion selective electrodes

Ion-Selective Electrodes (ISEs) have been used for several decades to determine inorganic ions **Amman *et al.,* (1983), Bakker *et al.,* (1999**). Ever since **Baum (1970); (1971), Baum and Ward, (1971)** introduced the organic sensitive sensors in the seventies, A great amount of research has been done about the application of ISEs in various fields of science and Technology (**Coşofreț, 1991); Khalil and Borham, (1999); Aboul-Enein and Sun, (2000)**. For example **Coşofreț and Buck, (1986)** studied a polyvinyl chloride (PVC) membrane selective towards various drugs and also in particular for determination of phenytoin in pharmaceutical formulations **Coşofreț and Buck**, (**1985)**

Application of calcium selective membrane sensors

Calcium is an important mineral as far as the bone and tooth health is concerned. The main minerals (calcium, magnesium, phosphorus, sodium, chloride and potassium) are either demanded in the greatest quantities or they are present in large amounts in the body. The three basic functions of the minerals are as constituents of the skeleton, as soluble salts which help control the composition of the body fluids, and as essential adjuncts to the action of many enzymes and other proteins. There are more than 70 reports on Ca^{2+} selective electrodes. Most of them were used in biological and environmental analysis.

Table 2.1

Selective biological or environmental applications of calcium ion selective electrode

S.No	Application	References
1.	Rain Solutions	Lauver *et al.*, 1992

2.	Study of Protein Effect	Covington and Zhou, 1992
3.	In soil	Lemos *et al.*, 2007
4.	In natural and borehole water	Van Staden and Stefan, 1999
5.	In coconut water	Chumbimuni-Torres and Kubota, 2006
6.	In Serum	Cattrall and Fong, 1978
7.	In natural waters	Alvares-Ribeiro and Machado, 1998
8.	In erythrocytes	Malon and Maj-Zurawska, 2005
9.	In tap water and in different plants	Saleh,1991
10.	In natural waters	Chen and Adams, 1998
11.	Measuring Calcium in Plasma	Umemoto *et al.*,1994
12.	In wood pulp	Vazquez *et al.*, 2001
13.	In Apple Juice	Cooke, 1975
14.	To the detection of calcium release during bone resorption	Berger *et al.*, 1999
15.	In Pharmaceuticals and Serum	Veltsistas *et al.*, 1994
16.	In human milk	Allen and Naville, 1983
17.	Determination of ionic association constants for Ca^{2+} with various organic acids	Ernara *et al.*, 1982
18.	To study effects of various electrolytes on rates of $CaSO_4$ -$2H_2O$ precipitation	Mile *et al.*, 1982

At the start of review literature, an acquaintance with related literature of past studies is a must for any research for formulating sound methodology, which acts as a persevering force during the onset of research. From this literature new areas of research can be inferred.

Perusal of literature reflect the fact that till 2012 more than 20 000 publications related to ion-selective electrodes (ISEs) have been published, with approximately two new ones per day in recent years. Figure 3.1 shows, for the period between 1970 and 2009, the

continuing increase in the number of publications that could be readily recognized as reporting on the preparation or use of ionophore-based ISEs. Clearly, this is still a fast growing field. Until the mid 1990s, most of the research in this field focused on the development of new ionophores that provided improved selectivities.

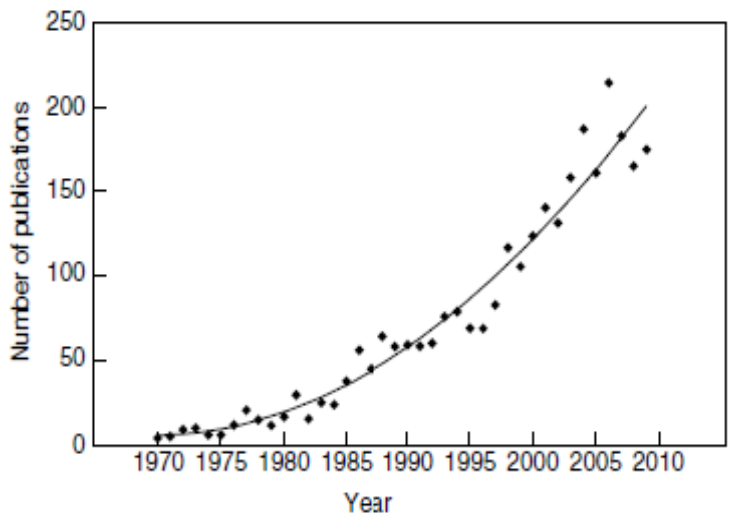

Figure 2.1
Publications related to ion selective electrodes

Ionized calcium was determined in serum from 84 normal individuals by using a flow-through calcium-selective electrode operated at 37^0C. The mean value was 1.08 ± 0.06 (2 SD) mmol/liter. The relationship of serum ionized calcium concentration to other serum constituents was examined. The effects of hyperventilation and food ingestion on ionized calcium were studied. Respiratory alkalosis, induced by hyperventilation, caused a decrease in ionized calcium of 0.05 ± 0.02 (2 SD) mmol/liter per 0.1 unit increase in pH. In contrast, the metabolic alkalosis induced by food ingestion caused a decrease in ionized calcium of 0.105 ±0.025 (2 SD) mmol/liter per 0.1 unit increase in pH (**Bette *et al*, 1972**).

Determination of calcium and potassium in human and sheep sera by differential Potentiometry with a flow-through cell was carried out by **Cattrall and Fong, 1978**. The electroactive reagent used was di(n-octylphenyl) phosphoric acid for calcium and valinomycin for potassium. The results reflect a generally good agreement and little difference in the precision for both the electrodes. The response of the electrode system to change in the ionized calcium level wheep as tested by infusing intravenously as with a

solution containing 4.4% EDTA (disodium salt) and 0.9% sodium chloride. The infusion rate was approximately 100 ml/hr, and blood samples were taken at 20-min intervals during the infusion and also before it. The results clearly inferred a decrease in the ionized calcium level. The results for potassium in a number of human plasma or serum samples and for the Versatol standards are in fairly good agreement with that of obtained by flame photometer.

Craggs (1979) proved that calcium ion-selective electrodes based on calcium bis [di (4-octylphenyl) phosphate] sensor can be used for determining free calcium-ion measurements utilizing various complexing anion ligands. The ion selective electrode was studied over a very wide concentration range from 10^{-7} to 10^{-3} free calcium-ion concentration. From the findings, it was inferred that log data fall in the range of those previously measured for the various equilibria by alternative methods, thus demonstrating the reliability of the calcium ion-selective electrodes. The investigation proved that these calcium ion-selective electrodes were not affected by added phosphate except insofar as free calcium-ion levels are lowered by complexation.

Sensors of calcium bis[di(4-alkylphenyl)phosphates] (alkyl = hexyl, octyl and 1,1,3, 3-tetramethylbutyl) in conjunction with dioctyl phenylphosphonate solvent mediator in PVC matrices give good calcium ion-selective electrodes with near-Nernstian slopes and detection limits of 1.9 x lo^{-6} to 2.7 x 10^{-6}Mcalcium ions in the absence of ion buffers. Electrodes with the octyl-based sensor, when calibrated at 25^0C with calcium-ion buffers, gave calcium-ion detection limits of 10^{-8} M. The E.M.F response of the electrodes in 10^{-3} M calcium chloride in 10^{-1} M sodium chloride was stable within the pH range from below 5 to about 9. All of the above electrodes showed good selectivity towards calcium ions over sodium, potassium and magnesium ions. A range of di-n-alkyl phenylphosphonate solvent mediators (alkyl =pentyl, hexyl, heptyl, octyl, nonyl, decyl or undecyl) can be used with calcium bis[di(4-octylphenyl)phosphate] sensor but electrodes from the pentyl and undecyl compounds degrade slightly more quickly than the others. Decan-1-ol solvent mediator leads to characteristic interference from magnesium ions. Electrodes with membranes containing the neutral carrier calcium-ion sensor NN'-di[(11-ethoxycarbonyl)undecyl]-NN'-4,5-tetramethyl-3,6-dioxaoctane diamide with 2-nitrophenyl octyl ether as solvent mediator were capable of near-Nernstian calibrations and calcium-ion detection limits of less than 10^{-6} M but quickly deteriorated **(Craggs *et al.*,1979)**.

A calcium ion-selective electrode (ISE) was characterized by bipolar pulse conductance measurements. The working curve showed that conductance is a function of calcium concentration (not activity), with detection limit of M Ca^{2+}. Interference levels were comparable to those obtained under potentlometric conditions. The results infer that divalent cations Interfere by displacing Ca^{2+} from the membrane surface, while monovalent cations enhance conductance by permeating the membrane. The conductometric response time to 90% total response was less than 10 ms, significantly shorter than the equivalent potentiometric response time of 4-5 s (**Powley *et al.,* 1980).**

Hobby *et al.,* (1983) attempted to utilize the covalent linking of organophosphates and phosphonates to the copolymer VAGH (a partially hydrolysed copolymer of vinyl chloride and vinyl acetate) and the phosphonate polystyrene obtained by Friedel - Crafts and free-radical processes, as calcium ion-selective electrode membranes. The authors inferred that the best membranes were all based on VAGH. Further studies using, VAGH PI, VAGH PI1 and VAGH PIII.A partially hydrolysed vinyl chloride - vinyl acetate copolymer (VAGH) were carried out grafting of organophosphate sensors and phosphonate solvent mediators. The studies inferred that of good response with grafted decylphosphate. A considerable loss of calcium ion selectivity, compared with free sensor was obtained when 4-(1,1,3,3-tetramethylbutyl)phosphate was grafted to VAGH copolymer. It was also noticed that VAGH copolymer linked to phenylphosphonate as some added dioctyl phenylphosphonate for functional membranes, the calcium bis(di[4-(1,I,3,3-tetramethylbutyl)phenyl]phosphate) sensor does not display its full calcium ion selectivity.

We measured the concentration of ionized calcium in human milk with a Radiometer ionized calcium analyzer at 37^0C. This instrument is designed to use simultaneous outputsfrom a pH electrode and a calcium ion-selective electrode to obtainthe trueionizedcalciumin blood plasma. Because both the ionic strength and the pH of human milk differ significantly from that of plasma, we used a calibration curve that corrected for these variables. No other substances in milk interfered with the electrode response to Ca^{2+}. The Ca^{2+} concentration of milk decreased and the pH increased as CO_2 was lost to the atmosphere. Samples collected in glass capillaries, which minimized CO_2 loss, contained 2.84 (SD 0.56) mmol of Ca^{2+} per liter at pH 6.98. We emphasize the importance of

maintaining physiological CO_2 concentrations when Ca^{2+} is being measured in milk (**Jonathan *et al*, 1983**).

Microelectrodes filled with neutral carrier selective to Ca^{2+} were used to measure the free intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in sheep cardiac tissue and frog skeletal muscle. Calibration of the electrodes was performed in the presence of a solution resembling the cationic composition of the cytoplasm. $[Ca^{2+}]_i$ at rest in normal physiological saline (20-22^oC) was 240 nM in Purkinje fibres, 270 nM in ventricular muscle, and 52 nM in skeletal muscle. In Purkinje fibres, elevation of $[Ca^{2+}]_o$ from 1.8 mM to 5.4 mM produced a 1.7-fold increase in $[Ca^{2+}]_i$.Elevation of $[Ca^{2+}]$o from 1.8 mM to 18 mM induced a 2.6-fold increase in $[Ca^{2+}]_i$. Exposure to Na^+-free solution (Li^+-substituted) gave rise to elevation of $[Ca^{2+}]$ by factors of 5.8 and 14 in ventricular muscle and Purkinje fibres, respectively. These latter changes in $[Ca^{2+}]_i$ were associated with the development of contractures which reached 34% and 172% of the corresponding twitch tension (**Robert Weingart and Peter Hess, 1984).**

The responses of biochemical materials towards calcium ions of calcium ion-selective electrodes have been studied, PVC matrix membrane electrodes based on calcium bis{di[4-(1,1,3,3-tetramethylbutyl)phenyllphosphate} sensor with dioctyl phenylphosphonate (I), tripentyl phosphate (11) or trioctyl phosphate (Ill) as solvent mediator were used for the present study. Experiments have also been carried out on electrodes made from commercial calcium ion-selective electrode membranes based on an ionophore sensor (electrode IV). E.M.F. changes of <0.5 mV were observed for up to 10^{-3} M starch, sucrose, uric acid, creatinine and bilirubin. Larger E.M.F. changes were observed for cholic acid, cholesterol, lecithin and vitamin D_2 (each of which were tested as solutions in ethanol or propan-1-01 added to the calcium ion containing solution in water or 0.15 M sodium chloride solution) (**Sadjedah *et al.,* 1985).**

Tyler and Comer (1985) studied a new ultra-sensitive fluoride – glass pH electrode differential cell and a calcium electrode monitor for the direct and simultaneous measurement of both fluoride and calcium in unbuffered acid solution. The results become increasingly independent of pH below pH 2. Linear deviation for a fluoride concentration of less than 0.005 pg ml^{-1} may be due to nanogram amounts of fluoride in the de-ionised water

and hydrochloric acid used in the experiments. The deviations were more pronounced at pH 0.1. The fast response of the fluoride differential cell together with the quantitative performance of the calcium electrode operating in an acid mode, provides a routine electrochemical technique for the simultaneous determination of fluoride and calcium in mineralised tissues.

This article reviews key advances in ion-selective electrode technology that have made potentiometric measurements of ionized calcium (Ca^{2+}) reliable and precise. Our use of two second-generation Ca^{2+} analyzers (Radiometer ICA1 and NOVA 8) made possible uninterrupted service as volume increased to 31 640 patient tests in 1985. The lower results on the NOVA 8 were adjusted upwards to match those of the ICA1 to give identical results. Both analyzers were evaluated under working conditions of high volumes and multiple operators to establish downtime, electrode life, and costs. We have classified all Ca^{2+} analyzers into first-, intermediate-, and second-generation instruments, the better to understand their differences. Results for large numbers of patients' sera were shown to be systematically different when any two analyzers were compared. These differences are the consequence of each manufacturer's unique choices of the following: (a) the matrix of the calcium calibration solutions, (b) the type and configuration of the reference electrode, and (C) the salt-bridge solution. Elimination of each analyzer's biases will require agreement on a reference system that defines the accuracy of Ca^{2+} measurements on serum, plasma, or whole blood. The sound analytical performance of today's second-generation Ca^{2+} analyzers has allowed us to exploit the inherent superiority of Ca^{2+} over total calcium ($Ca2^{+}$) measurements in the daily care of patients. We report on the preference of Ca^{2+} over Ca1 by physicians at our hospital since the introduction of second-generation Ca^{2+} analyzers. Therefore, we state unequivocally from our very satisfactory experience over the past five years that Ca^{2+} is a clinical laboratory test whose time has come (**George *et al.,* 1986)**.

A coated-wire ion-selective electrode for measuring ionic calcium has been developed, in collaboration with Teknekron Sensor development Corporation (TSDC), Menlo Park, CA. This coated wire electrode sensor makes use of advanced, ion-responsive polyvinyl chloride (PVC) membrane technology, whereby the electro active agent is incorporated into a polymeric film. The technology greatly simplifies conventional ion-

selective electrode measurement technology, and is envisioned to be used for real-time measurement of physiological and environmental ionic constituents, initially calcium. One of the main applications of this electrode is the real-time measurement of urinary and blood calcium changes during extended exposure to microgravity, during prolonged hospital or fracture immobilization and for osteoporosis research. Potential Advanced Life Support applications include monitoring of calcium and other ions, heavy metals, and related parameters in closed-loop water processing and management systems. The authors further state that this technology provided a much simplified ionic calcium measurement capability, suitable for both automated *in-vitro,* in-vivo, and in-situ measurement applications, which should be of great interest to the medical, scientific, chemical, and space life sciences communities. These results strongly indicate that the Calcium ion selective membrane is compatible with the commercial Radiometer electrodes (**Hines and Sara Arnaud, 1991)**.

The activity of Ca^{2+}, Na^{+}, K^{+}, and Cl^{-} with ion-selective electrodes after equilibrium dialysis of solutions with different albumin concentrations was measured. The investitigation reflected that the calculated Donnan ratio was the same for all ions in the same solution and increased with the albumin concentration, as predicted by the Donnan theory. The Donnan distribution ratio for Ca^{2+} was similar, as determined with instruments from three different manufacturers, For healthy subjects and patients with renal stone disease, no correlation was observed between serum concentrations of ionized calcium and albumin. In vitro influence of albumin on Ca^{2+} measurement was not supported by the data (**JorgenThode *et al.,* 1987**).

Neutral carrier based calcium and magnesium ion selective electrodes were developed for determination of calcium and magnesium without addition of any auxillary complexing agent such as acetylacetone. This approach worked well, even in situations where calcium-selective electrodes do not detect the second inflection point because the calcium/magnesium concentration ratio was too high or sodium concentration was high. The addition of acetylacetone the divalent electrode was used to determine the calcium concentrations and standard deviation obtained by this method was smaller. These findings were depicted with theoretical titration curves (**Magdalena *et al.,* 1989**).

A new optical Ion-sensor constructions (optodes) was developed using poly vinyl chloride) (PVC) membrane that incorporated a cation-selective neutral ionophore, a specially tailored H^+-selective neutral chromoionophore, and lipophillc anionic sites in the same plasticised PVC membrane. The investigated membrane was found to exhibit the theoretically expected absorbance response to Ca^{2+} activities in different pH-buffered samples **(Morf *et al.,* 1990).**

Teresa and Adelio (1990) studied the effect of temperature on the response characteristics of the calcium ISE. Electrodes with an internal reference ("type A") or with the membrane applied to a support made of electrically conductive silver - epoxy resin ("type B") were studied in parallel for comparison purposes. Isothermal measurements were carried out in the temperature range 10-50^{0}C and included calibrations at different temperatures for evaluation of operational temperature ranges and slope and standard potential variations, and also hysteresis curves. Isopotential points were determined from calibrations or hysteresis curves. The influence of the chloride ion concentration in the inner solution of type A electrodes was studied. This type of electrode responded more closely to the thermodynamic behavior than type B electrodes. The authors declared that the hysteresis increased with their utilization. The results confirmed that electrodes with a PVC membrane applied to an epoxy support are more difficult to equilibrate thermodynamically than electrodes with an internal solution. The investigated sensor system had an isopotential point activity *(5* X 10^6 M) well outside the operational activity range. Based on the studies the authors reflect that these electrodes should be used with strict control of the temperature and that they are less suitable for work involving measurements at different temperatures than the classical type. The authors also inferred that the temperature behaviour of these electrodes can be improved by changing the composition of their internal solution

Molecular imprints against calcium and magnesium ions, respectively, were prepared in divinylbenzene- based polymers. A vinylic, Ca^{2+} -selective, neutral ionophore N,N'-dimethyl-N,N'- bis- (4-vinylphenyl) -3-oxapentanediamide was synthesized and used as the ion-complexing monomer. In order to investigate the ion-complexing abilities of this neutral ionophore, this compound was tested as a neutral carrier in the plasticised PVC membrane of an ion-selective electrode. The resulting polymers were analysed for their

ability to extract calcium ions from methanolic water. The polymers prepared against calcium and magnesium ions were found to bind calcium ions with 6- and 1.7-times lower K^+-values, respectively, when compared with reference polymers prepared in the absence of metal ions. The authors imply that may be the increased binding strength due to the spatial arrangement of ionophore units in the resulting polymers by the template ions during the polymerization **(Thomas Rosatzin *et al.,* 1991).**

Lauver *et al.,* (1992) presented a method for the rapid and quantitative assessment of the transfer of calcium (Ca^{2+}) and hydrogen (H^+) ions between the surface of a leaf and simulated acidic rain. A small volume of pH 3-5 simulated ram solution was confined within a rigid teflon capillary on the foliar surface. A liquid membrane-type, neutral carrier-based, ion-selective microelectrode either for Ca^{2+} or for H^+ was placed within the capillary with the tip of the microelectrode at least 100//m from the foliar surface. This allowed spatial and temporal aspects of ion gradients generated by ion diffusion from the foliar surface to be studied. Results indicate that, throughout a 60-minute period after contact of simulated rain with the adaxial foliar surface of spinach (Spinaceaoleracea L. cv. Marathon) or Japanese pachysandra (Pachysandra terminalis Siebold & Zucc)., negligible amounts of foliar derived calcium diffused into the simulated rain solution, and there was virtually no neutralization of the pH 3-5 rain simulant present in the capillary.

A new experimental research to nullify the correction of anion interference in Ca^{2+} ISE with carrier PVC membrane was carried out. The authors stated that membrane characteristics could be changed in such a way that there is no need to correct the measurements when the electrodes are used during bicarbonate haemodialyses with 2 $mmol^{-1}$ acetate. This was achieved by optimizing the ratio of the concentration of synthetic, electrically neutral carrier and lipophilic anion and by using plasticizer effects. A special membrane was developed in the same way for an application in dialysis solutions with 35 $mmol^{-1}$ acetate **(Heidrieh *et al.,* 1993).**

Calcium, a critical nutrient for post harvest handling and quality of apples. Difficulties of analyse by atomic absorption spectrophotometry (AAS). This prompted an examination of the use of a “standard addition” procedure with an ion selective electrode (ISE) to estimate calcium in apple tissue after homogenization in water. However the results reflect that ISE estimates of calcium in apple homogenates were consistently lower than AAS estimates.

The error in ISE estimates was attributed to cell wall binding of calcium. Titration of isolated apple cell walls with calcium showed that the proportion of calcium bound was dependent on the free calcium concentration. An empirical relationship between the reciprocal of the square of bound calcium and the reciprocal of free calcium concentration was derived. Incorporating this relationship into the calculation of calcium in apple homogenates led to closer agreement between ISE and AAS analyses than with the uncorrected calculation (**Knee and Srivastava*, 1995*).**

A new ionic carrier N-ethyl-N-(2-tri methyl ammoniumethyl)-N'-heptyl-N'methyl-succinamide containing Nafion modified membrane based Ca-ISE was developed. This electrode showed a preferential selectivity towards alkaline metal cations and gave linear range down to pCa≥ 5, which have a equal response with Mg ion. The electrodes were also reflected to have long life time. This new ligand itself acted as quaternary ammonium cation and it interact with fixed ion – exchange sites as well as cation exchange sites of polymeric backbone. The authors report that the specialty of this ligand is, it does not require any plasticizer. By changing the nature, position and length of the hydrocarbon chain of the ligands, the selectivity towards calcium ions was found to increase **(Rondinini *et al.*, 1995).**

A new calixarene based Ca-ISE that has preferential selectivity to calcium ions was developed. Calixarene bearing phosphine oxide as liganding agent exhibited a contrasting behavior when compared to the well known tetra ester derivatives, which are selective for sodium against other alkali metals. The PVC based electrodes containing the above said ligands, displayed Nernstian slopes, exhibited excellent selectivity against interference such as magnesium and possessed very fast response times (**Mckittrick *et al.,* 1996**).

High adhesive one component room temperature vulcanizing-type silicone rubber (RTV-1-type SR) matrix based new calcium ion selective electrode was prepared by **(Bong Kyun Oh *et al.,* 1996)**. The membranes were formulated with 21.6 wt % bis(2-ethylhexyl) adipate, 0.3 wt % tetradodecylammonium tetrakis(*p*-chlorophenyl)-borate (ETH 500), 0.1 wt % potassium tetrakis(*p*-chlorophenyl) borate, and 0.8 wt % calcium-selective neutral carrier ETH 129 or ETH 1001**.** Decrease of Bulk membrane resistance was achieved by adding plasticizer on to the SR matrix because of this the solubility of electroactive components also increases without significantly deteriorating its adhesive strength. In SR matrix the

calcium selectivity was enhanced by lipophilic salt ETH 500. The selectivity coefficient value was evaluated by separate solution method. The selectivity coefficients for the optimized membranes were below 10-5. Potentiometric characteristics of planar-type Ag electrodes coated with optimized RTV-1-type SR membranes, e.g., response slope 29.0 (0.5 mV/decade, detection limit below 5.0 -10^{-7} M Ca^{2+}, and 2-3mV of potential drift per day, were virtually the same as those of the corresponding poly(vinyl chloride) membrane based conventional electrodes, but with greatly enhanced sensor-to-sensor reproducibility and lifetime (3-9 weeks).

A new photo cured calcium selective electrode was reported using a flow injection potentiometry method in multi sensor cell. Different compositions of four membranes were prepared in that an optimum selectivity and sensitivity membrane was selected for further studies. The electrode was prepared using *N*,*N*,*N*%,*N*%-tetracyclohexyl-3-oxapentanediamide ligand (ETH 129) as an ionophore, 2-nitrophenyl octyl ether as plasticizer and tetradodecyl ammonium tetrakis(4-chlorophenyl) borate was used as lipophilic additive. Thin membrane was coated on a silver wire as substrate transducer to produce the calcium sensor after photo-curing process was carried out. The photo curing process was faster (1 min) than previous methods and does not require a nitrogen atmosphere for reproducible production of membrane response characteristics. Four sensors constructed with the identical optimum membrane were shown to function reproducibly in a multi-sensor flow-through cell using the steady-state mode of flow measurement, and an average calibration slope of 28.590.4 mV change per activity decade was observed over a log-linear concentration range between 0.01 and 10 mM. The influence of pH on the electrode was noticed and it was found that the optimum pH of the electrode for calcium concentration was 8.3. The sensor was also used to determine calcium concentration in real samples in the FLB mode and the result was compared with atomic absorption spectroscopy and the accuracy was found to be 5-9% for different water samples **(Alexander *et al.,* 1997).**

A photo-cured membrane selective to calcium, based on the calcium bis [4-(l',l',3',3'-tetramethylbutyl)phenyl]phosphate ionophore with the lipophilic additive, potassium tetrakis(4-chlorophenyl)borate, that can tolerate up to 200 nM perchlorate ionic background

in the flow injection potentiometry mode was reported earlier. By adding anhydrous calcium chloride the selectivity, slope and sensitivity of the previously prepared electrode was improved. When calcium chloride was added to the calcium membrane, it dissolved easily with the aid of the DOPP plasticizer which was known to coordinate with calcium, and lipophilic calcium-phosphonyl oxygen complex was formed. Formation of phosphonyl complex in the calcium membrane lowered the membrane resistance compared with the photo-cured membrane of the Ca ISE. This incited Nernstian slope and selectivity of the electrode **(Di Benedetto *et al.,* 1997).**

Lipophilic anionic dichlorofluorescein ester was examined as a chromophore for the development of polymeric film based optical sensors for calcium. A calcium organophosphate salt and a neutral ionophore were employed as the active component for calcium sensing. The response pH, dynamic range, detection limit and selectivities were governed by the calcium camer as well as the membrane plasticizer.The membrane that uses the anionic chromophore and the calcium organophosphate salt responded to calcium in the range of 1.0 x to 0.2 M at pH 7.4 (**Enju Wang et al., 1997).**

An experimental optimization by the modified simplex method of a bipotentiometric flow injection analysis (FIA) system was carried out for the simultaneous determination of calcium and chloride in waters. This was constructed by placing two different ion selective electrodes (ISEs) placed in series. A weighted linear combination of three variables, as the response function was chosen to achieve maximization of the calcium electrode sensitivity, with minimal of interference of calcium signal on the chloride response. The results revealed that the optimized system was able to determine calcium in the range 5 3 1025–5 3 1023 mol l21 with a sensitivity of *ca*. 27 mV decade^{-1} and chloride in the range 2 3 1024–23 1022 mol l21, the maximum value tested, with a sensitivity of *ca*. 55 mV decade. The influence of the ionic strength on the results is discussed. The relative accuracy of the bipotentiometric FIA system was demonstrated by the repeatability obtained at 1.9 & 0.6% respectively (**Luis and Adelio, 1998)**.

Arslan and Tyson (1999) reported several procedures for the determination of Ca, Mg and Sr in soils have been compared on the basis of the accuracy of analysis of two NIST reference materials (Montana Soils SRM 2710 and SRM 2711). Samples were dissolved in a mixture of hydrofluoric and nitric acids in sealed vessels in a microwave oven

and in Teflon beakers on a hot plate. The digests obtained from both dissolution methods were evaporated to dryness in an attempt to remove silicon. Boric acid was added to prevent the precipitation of the lanthanum releasing agent (as lanthanum fluoride) and potassium was added as an ionization buffer. Determinations were made by flame atomic absorption spectrometry with both the nitrous oxide–acetylene flame and the air–acetylene flame, with calibration either by standard additions or against external standards matrix matched with respect to nitric acid, boric acid, lanthanum and potassium. The silicon remaining in the solution was also determined by external calibration. A single-line flow injection manifold was used to overcome any problems due to the presence of high dissolved solids. A volume of 300 ml was injected into a water carrier stream flowing at 8 ml min^{-1}. Calcium can be determined accurately with the nitrous oxide–acetylene flame without complete removal of silicon, while the removal of the silicon is necessary for the accurate determination in the air–acetylene flame. It is not necessary to remove the silicon to obtain accurate analyses for magnesium determination, both air–acetylene and nitrous oxide–acetylene flames can be used, but the former is twice as sensitive as the latter. Considering the problems associated with the operation and the lower precision, however, the use of fast-burning nitrous oxide–acetylene flame is not recommended for calcium or for magnesium determination. For the determination of strontium, it is concluded that the removal of silicon is not sufficient to perform accurate analysis in the air–acetylene flame despite the addition of lanthanum as a releasing agent and potassium as an ionization suppressor and so the nitrous oxide– acetylene flame should be used to eliminate the interferences.

Potentiometric cation sensors using a silicon transducer using Al_2O_3 layer as an insulating layer was studied. Ion-recognition element such as valinomycin, 18-crown-6 ether, bis[di(noctylphenyl) phosphato]calcium(II) and ETH1117 were immobilized in the matrix of poly(vinyl chloride) and embedded on the surface of Al_2O_3 layer . Potassium tetrakis (p-chlorophenyl) borate was used as a lipophilic salt. The results revealed that K^+, Ca^{2+} and Mg^{2+}. Sensitive - silicon electrodes showed responses similar to those of the corresponding ion-selective electrode. The investigations revealed that the presence of lipophilic anion increased the slope (**Seki *et al.,* 1999**).

A new calcium electrode was prepared and influence of montmorillonite on the prepared electrode was studied by combining and immobilization of ionophore and montmorillonite into a polymer membrane. Two types of electrodes were prepared for the determination of calcium ion and the results were compared. The results obtained better performance of the montmorillonite modified electrode than without montmorillonite. The relationship between the potential and calcium ion concentration was obtained and the testing range of calcium ion concentration was found to be from 461076±1071 M. Comparing selectivity, reproducibility, and stability of both electrodes, montmorillonite containing electrode exhibited better result than without montmorillonite because the cation exchange property of montmorillonite and its compact structure stabilized the modified membrane. The optimal amount of the montmorillonite for the electrode preparation was found to be 24.8 wt% **(Wang and Chou, 1999).**

A new solid state Ca-ISE was prepared using soluble, electrically conducting polyaniline (PANI), di(2-ethylhexyl)phosphate (H^+DEHP^-) and Ca^{2+}-ionophore ETH1001 was developed. The consequence of incorporated lipophilic salt and plasticizer potassium tetrakis(4-chlorophenyl)borate ($KB(ClPh)_4$) on the electrode was studied. Using H^+DEHP^- , the non conducting PANI was changed to conducting form of PANI, it also acted as a complexing agent for calcium ions. The electrode membrane was easily prepared in a single step using solution –casting method, where PANI was dissolved in THF with other membrane components on glassy carbon. PANI played a vital role to facilitate charge transfer at the substrate/ membrane interface. It was shown that incorporation of ETH1001 and $KB(ClPh)_4$ improved the Ca^{2+}-sensitivity of the electrodes studied. The best Ca^{2+}-sensitivity, 28.6±1.1 mV/log a_{Ca} (10^{-1}–10^{-3}M$CaCl_2$) in 0.1M NaCl, was obtained for an electrode membrane containing 40% (m/m) PANI, 30% (m/m) ETH1001 and 30%(m/m) $KB(ClPh)_4$.No redox sensitivity was observed for a PANI electrode membrane consisting of 30% ETH1001 and 5% KB(ClPh)4 in a solution of 10mM $Fe(CN)_6^{3-/4-}$ with 10^{-1}–10^{-3}M $CaCl_2$ as the ionic background. The selectivity coefficient ($logK_{Ca:J}^{pot}$) of this electrode was −1.8 ±0.1, −1.7±0.1, −1.7±0.1 and approximately −2 towards *j*=Na^+, K^+, Li^+ and Mg^{2+}, respectively. In this the redox sensitivity of PANI membrane was suppressed and at the same time sensitivity of Ca^{2+} ion was improved. It was very useful for analytical purposes. In this type no PVC was used but gave similar advantage like PVC membrane ie., (i) Ca^{2+} selectivity (ii) no inner reference solution was required (iii) redox sensitivity was suppressed

or even eliminated and (iv) the electrodes was easily miniaturized and prepared by solution-casting. But the major disadvantage was that the electrode can work only at a constant pH (**Lindfors and Ivasku, 2000**).

Electrically soluble PANI based novel calcium electrode was prepared using di(2-ethylhexyl)phosphate (H^+DEHP^-) and tetraoctyl ammonium chloride (TOA^+Cl^-). The electrode membrane was prepared using TOA^+Cl^- to the solution by drop casting method on glassy carbon substrate. 0–40% (m/m) TOA^+Cl^- was used to make PANI membranes. Incorporation of 20–30% (m/m) TOA^+Cl^- was used to improve the calcium sensitivity on the PANI membranes. The best Ca^{2+}-sensitivity, 27.0±0.4 mV/log a_{Ca} (10^{-1}–10^{-3}M $CaCl_2$, n=3, LOD=10^{-4} M) in 0.1M NaCl, was obtained with an electrode membrane containing 25% TOA^+Cl^- (PANI25). The three identical PANI25 electrodes show good reproducibility. The selectivity coefficient ($logK_{Ca:j}^{pot}$) of this electrode towards j=Na^+, K^+ and Li^+ was found to be−1.6. It was found that Mg ions reflected drastic interference in the determination of calcium ions. No redox sensitivity was detected in the case of 0.1 M $CaCl_2$ ionic backgrounds but it was found to give small redox sensitivity in the case of 10^{-3} $CaCl_2$ ionic background. Better response was observed at the pH range of 4.5 – 9.7 to give better response. The electrochemical impedance and cyclic voltammogram of PANI revealed that TOA^+Cl^- improved the ionic mobility within the PANI membrane. The working mechanism of the PANI electrode membrane was explained with the charge carrier model, which was usually applied to PVC-based ion-selective electrodes (**Lindfors *and* Ivasku, 2000**).

Two types of polypyrrole (PPy)-based calcium sensors were constucted, one sensor with PPy–calcion film as the active part and the other sensor with PPy–calcion as a solid-state contact coated with a conventional membrane selective towards calcium ions. It was shown that the PPy–calcion film, due to the complexing properties of calcion ensuring high loading of the film with calcium, was sufficiently selective to be used as the active part or as a mediating layer of the indicator electrode. The electrode, with PPy–calcion film as the active part, was used as the indicator electrode in potentiometric titrations of calcium in mixed solvents, where conventional PVC-based electrode cannot be used (**Blaz *et al.,* 2000**).

A new Ca^{2+} selective polyaniline (PANI)-based membrane material consisting of electrically conducting PANI, bis[4-(1,1,3,3-tetramethylbutyl)phenyl]phosphoric acid (DTMBP-PO_4H), dioctyl phenylphosphonate (DOPP) and cationic (tridodecylmethylammonium chloride, TDMACl) or anionic (potassium tetrakis(4-chlorophenyl) borate, KTpClPB) lipophilic additives was prepared and suitability for all-solid-state sensor applications were studied. PANI functions as the membrane matrix and transformed the ionic response to an electronic signal. PANI membranes with three different plasticizer (z) and additive contents(s) were prepared by drop-casting on glassy carbon (GC) substrates (the molar ratios between the plasticizer or additive and DTMBP-PO_4H were denoted as *z* and *s*, respectively). Depending upon the sensitivity and selectivity of the electrode, the best performance was obtained for PANI membranes prepared with *z* = 0.90 and *s* = 0.10, containing KTpClPB as ionic additive. The Ca^{2+}-sensitivity of this membrane was 27.8 ± 0.2mV/log aCa^{2+} (10^{-1} to 10^{-4} M $CaCl_2$,LOD = 8×10^{-7} M) in 0.1M NaCl, and the selectivity coefficients ($log^{pot}_{Ca,j}$) towards j = Na^+, K^+, Li^+, NH_4^+ and Mg^{2+},determined with the fixed interference method, were − 3.9 ± 0.1, −4.0 ± 0.1, −3.5 ± 0.1, −3.5 ± 0.1 and −3.6 ± 0.1, respectively (**Lindfors and Ivaska, 2001**).

Va´zquez *et al.,* (2001) prepared a new ion-selective electrode and optimized for the determination of Na^+, K^+ and Ca^{2+} in wood pulp suspension. The potentiometric results obtained by the different ISEs (Orion, VOLTA, ProSens) both in wet ‘‘native’’ samples (pulp suspension) by direct potentiometry and in the corresponding filtrates by direct potentiometry and standard additions, were close to one another for the same sample. This shows that the results obtained by ISEs are reproducible. In addition, the commercially available glass electrodes for pH, as well as a solid-state membrane electrode for Cl^- were used. The free ion concentration of pulp samples obtained by ion-selective electrodes was compared to the total concentrations obtained by ICP-AES and XRF.

A calcium selective electrode was prepared using ETH1001 as an ionophore and effect of the plasticizer on the extended linear calibration curve and on the selectivity of a calcium selective electrode was studied. To study the effect of plasticizer, (2-Ethylhexyl) sebacate (DOS) and o-nitrophenyloctyl ether (o-NPOE) were used as plasticizers and the PVC membrane contains potassium tetrakis (4- chlorophenyl) borate. It

was found that the plasticizer o-NPOE based electrode gave linear part of the calibration curve and the detection limit was lower compared to DOS based electrode. The optimal activity of free Ca^{2+} and Na^{+} in the internal reference solution was 10^{-4} and 10^{-1} for the membrane with DOS and 10^{-6} and 10^{-1} for the membrane with o-NPOE, respectively. Both the plasticizers gave same lowest detection limit in case of reproducibility of the response of electrodes. The selectivity coefficients value were also determined using activity of calcium ion in the internal solution ranging from 10^{-2} to 10^{-10}. The unbiased selectivity coefficients were obtained for electrodes with lower activity of the primary ion in the internal solution and significant depletion of the primary ion in the diffusion layer at the membrane/ sam-sample interface. The properties of the electrodes and transport properties of the membranes were correlated **(Bedlechowicz *et al.,* 2002).**

A PVC based new potentiometric sensor was developed for determination of various ions. A series of ion selective electrodes consisting of Ca^{2+}, $Mg^{2+,}$ NH_4^{+}, K^{+}, Na^{+}, Li^{+}, and H^{+} electrodes were prepared for the determination of calcium and total hardness in natural waters. The selectivity of the calcium and magnesium ISEs was not fully achieved as other cation species interfered with the analysis. The proposed sensor array device can overcome this drawback since it can take advantage of the cross-selectivities of cation species towards each ISE. A high accuracy of analytical information was obtained by using multivariate data by the quantification of calcium and total hardness in the water samples by means of chemometric methods. The standard complexometry value was correlated with the obtained result using the present method. In order to develop a method extracting multi analyte information other components of interest such as heavy metals, anion species was also analyzed with similar sensor array devices extract rather than calcium and hardness measurement **(Saurina *et al.,* 2002).**

The determination of calcium and total hardness in natural waters was carried out with a potentiometric sensor array which consists of a series of ion-selective electrodes (ISEs) for Ca^{2+}, Mg^{2+}, NH^{4+}, K^{+}, Na^{+}, Li^{+}, and H^{+}. The selectivity of the calcium and magnesium ISEs was not fully achieved as other cation species may interfere with the analysis. The authors stated that the proposed PVC-based electrodes for the construction of sensor arrays can overcome this drawback since it can take advantage of the cross-selectivities of cation species towards each ISE. The multivariate data generated by the

sensor array allowed the quantification of calcium and total hardness in the water samples by means of chemometric methods. Results obtained are in reasonable concordance with those given by the standard method based on complexometry (**Javier *et al.,* 2002**).

Milk is a heterogeneous fluid in which the colloidal phase is homogeneously dispersed in the aqueous phase. Calcium is partitioned between the colloidal and aqueous phases and is in complex electrochemical equilibrium with several major milk components. In human and bovine milk, calcium is mainly distributed between the aqueous and casein micelle in the colloidal phases (Holt & Jenness, 1984; Neville *et al.,*. 1994). Caseins form a complex micelle structure that contains approximately 25 000 phosphorylated monomers that react with calcium phosphate complexes in the milk to bind 20–40 mole calcium per mole casein (Holt & Jenness, 1984; Neville *et al.,*. 1994). Thus, the distribution of calcium between the colloidal and aqueous phases appears to be governed by the level of casein in the milk (Holt & Jenness, 1984; Neville *et al.,*. 1994). In human milk, the casein level is low; 25% of calcium is associated with casein, whereas in cows and goats the corresponding figure is higher at y65%. In rats, casein levels are among the highest in mammals and 95% of calcium is associated with casein (Neville *et al.,*. 1994). In the aqueous phase, calcium is divided among ionic calcium (Ca^{2+}), calcium citrate and calcium phosphate. Calcium citrate and calcium phosphate constitute most of the aqueous calcium in bovine milk in contrast to less than 50% in human milk (Holt & Jenness, 1984; Neville *et al.,* 1994). As the concentration of Ca^{2+} in milk appears to be essential in preserving the integrity of the mammary tight junctions during lactation (Neville & Peaker, 1981), it is important to follow its concentration precisely in mammary secretions in different stages of the reproduction cycle. The concentration of Ca^{2+} in milk can be determined with a calcium-selective electrode (Allen & Neville, 1983). The presence of a metal-chelator complex in the solution affects the ionic activity of the soluble ion and, consequently, the response of an ion-selective electrode is not proportional to ion concentration (Kim & Padilla, 1978; Schoenmakers *et al.,* 1992). Determination of ion concentration in solutions that contain chelators requires therefore, special correction procedures (e.g. Kim & Padilla, 1978) or elimination of the chelator from the solution. As noted above, caseins are powerful biological chelators. Nevertheless, to the best of our knowledge, potential effects of caseins on determination of Ca^{2+} concentration in milk have not been considered. The aim of this study was to verify that caseins in the milk of various mammals interfere with the

determination of Ca^{2+} concentration with an ion-selective electrode, and to evaluate several ways of correcting for the interference (**Silanikove *et al.,* 2003**).

Thick film metallization process was used to develop solid-state thick film calcium ion-selective electrode. Calcium ion-selective membrane was prepared by Silicone rubber or photoresist combined with ionophore (ETH 129) and the membrane was coated on the different electrode surfaces. Silicone rubber-based membrane gave a Super-Nernstian equilibrium relationship between the phase boundary potentials and calcium ion concentrations in10^{-7} M $CaCl_2$ with montmorillonite. Manufacturing process was easily carried out because of the use of photoresist. The memory effect of the silicone rubber-based membrane was found to be eliminated when the membrane was conditioned with 0.1 M $CaCl_2$. The response time of the electrode with silicone rubber and montmorillonite modified silicone rubber were less than 20 s and the one with photoresist was less than 3min. The sensitivity of these calcium ion-selective electrodes was around 30mV per decade. Fixed primary ion method was used to calculate selectivity and it was found in the range from −2.9 to −3.8 for Na+, K+ and Mg^{2+} ions **(Wang *et al.,* 2003).**

A pulsed galvanostatic technique was presented to distinguish free and total levels of calcium with a single membrane electrode by varying the magnitude of the applied current. Pulsed chronopotentiometry created the possibility of accurate control of ion fluxes across ion-selective plasticized polymeric membranes without ion-exchanger properties. During a constant current pulse ions, were forced to extract from the aqueous sample into the contacting membrane phase. Each current pulse was followed by a constant potential pulse to remove the extracted ions from the membrane. The induced concentration gradients were reproducible from pulse to pulse. At relatively small applied currents and in the presence of labile complexes in the sample, the sensor responds to the ion activity, in analogy to a conventional ISE. If a larger current was applied, the flux of complexed ions dominated the sensor response because of its increased magnitude, and the observed potential was now a function of the total ionic concentration. This approach allowed to detect, with the same sensor, the levels of free and total ionic concentration by varying the magnitude of the applied current. The technique utilized gave much more stable signals than with earlier work demonstrating the principle with zero-current potentiometry. This

concept was illustrated with calcium selective membranes based on the ionophore ETH 5234 **(Shvarev *and Bakker,* 2004).**

According to **Kumar and Mittal, (2004),** a new calcium ion selective electrode was developed based on PVC membrane modified by means of new ionophore dibenzo-18-crown-6 (DB18C6). The electrode was prepared using the ratio of ionophore (7%), PVC (85%) and plasticizer (8%). The sensor exhibited a near Nernstian response over a concentration range of 1.0 x 10^{-5} M to 1.0x10^{-1} M with a slope of 28 mv/decade having detection limit of 4x10^{-6}M. Potentiometric signals were obtained within a short period of <30s and could be used for 5 month without any considerable divergence in potentials. It gave preferential selectivity towards alkali, alkaline earth and transition metal ions. The pH range of the sensor was found to be 3-11. The electrode was also successfully tried as an indicator electrode in potentiometric titration of Ca ions with EDTA. The advantage of this electrode over the other available electrodes includes ionophore is inexpensive, easily available and low interference from heavy metal ions and it gives significantly very fast response time.

A new cation selective all-solid-state electrode was prepared and effect of spontaneous charging/discharging of conducting polymer ion-to-electron transducer on potentiometric responses was studied by **Michalska and Maksymiuk, 2005**. Ca-selective all-solid-state electrodes was studied with a plastic, solvent polymeric membrane and anion exchanging conducting poly(pyrrole) film doped with chlorides used as the contact phase. The selection of composition and pH of conditioning solution resulted in substantially different detection limits of the sensor. The sensor was optimized for linear responses within an activity range from 0.1 to 10^{-9} M $CaCl_2$ or to achieve unbiased selectivity coefficients. The $log^{pot}_{Ca,j}$ values obtained were equal to -9.3 for K^+, -7.6 for Li^+ and -9.6 for Mg^{2+}, respectively, more favorable values than those reported for tailored internal solution electrodes. The analytical performance of all-solid state electrodes with CP contact was improved by conditioning in such a way that the interferent ion will be accumulated predominantly in the transducer with only some amount of primary ions. The results showed that a significant improvement in selectivity, were obtained for all-solid-state sensors, using the transducer phase, an anion-exchanging conducting polymer layer, which was not designed to interact specifically with calcium ions.

Industrially and environmental useful ion selective electrodes were prepared by **Bedlechowicz *et al*., (2005)**. Ion selective electrodes were useful analytical tool and significant advantage like insensitivity to sample color, viscosity or suspended solids, rapid response to changes in determinant concentration, and possible use in a very wide concentration range. A further advantage of analytical method involving ISE is that they are relatively simple, and cheap to develop, set up and run. The detection limit of ISE was improved by control the ion transport through the ion-selective membrane with a galvanostatic current. Passing nanoampere-level direct currents across the membrane can control ion fluxes through the membrane, which can ambiguous the determination at low concentration level. A sudden change in the sample composition was found to affect even the well balanced system. This causes inevitable induce concentration gradients and concomitant ion fluxes in either direction. Applying an external current presented an easy and straight forward experimental control, surface cleaning or pretreatment was done by external current. Finally it was found that the ion selective electrode membrane was influenced by plasticizer also.

Malon and Maj-Zurawska, (2005) reported that clinical potentiometric analyser can be used to determine inter cellular ionized magnesium, calcium, sodium and potassium with the help of ion selective electrodes. They reported the reliability of the new method that is comparison with other methods such as nuclear magnetic resonance spectroscopy (NMR), atomic absorption spectrometry (AAS), flame emission spectrometry, zero-point titration did not differ significantly. An appropriate composition of ion selective membrane for intercellular calcium determination was found and also ionized magnesium determination in erythrocytes was successfully used for the treatment of medical analysis. They also reported that this new method was accurate, reproducible and cheap for the cytosolic free cation concentration measurements.

Calcium-selective electrodes (Ca-SEs) based on the geometric isomers of di-(1,2,3,6-tetrahydrobenzo)-crown esters bearing four ester fragments in the macrocyclic ring were prepared, and their electrochemical properties were studied. It was demonstrated that these electrodes offer promise for medical and biological studies and clinical medicine. The

electrodes exhibit high selectivity at crown compound concentrations, ensuring the formation of a 1:1 Ca–ionophore complex in the membrane (**Shabanov *et al.,* 2005).**

Potentiometric calcium selective sensor was developed using Tetronasin as ionophoric antibiotic besides its interacting tendency with sodium ion. As expected from mixed hydrogen–calcium equilibrium, the response was super-Nernstian and dependent on pH, with optimal response conditions at pH 5.0. The selectivity pattern to common inorganic cations was also determined. The studies with primary ion and interferents, validate an optimal formulation of a PVC membrane for Ca^{2+} which employs 1% ionophore, 0.2% anionic sites, 66% *o*-nitrophenyloctylether and 33% PVC. Using electrochemical impedance study. Ca^{2+} interaction with the membranes through an evaluation of the mechanisms and responses were obtained. Results in the range 50 KHz–0.05 Hz indicated an electrochemical system not impeded by charge transfer or slow diffusion. The single semicircle observed on the corresponding Nyquist plots was translated into a bulk resistivity lower than 700KΩcm, with a geometric capacitance of 98 pF **(Calvo *et al.*, 2006).**

A simultaneous determination of calcium and potassium in coconut water samples using a flow-injection system with tubular ion-selective electrodes (ISEs) in series was developed. The samples were injected into a 0.1 mol L-1 HEPES (pH ¼ 6.0) carrier solution, using an injection volume of 100 mL and a flow of 2.0mLmin-1 in the FIA system. The electrodes developed exhibited nernstian response for calcium and potassium in the concentration range between 1.0x10-5 and 1.0x10-1 mol L-1 with detection limits of 5.6x10-6 mol L-1 for calcium and 9.5x10-6 mol L-1 for potassium. And no significant interference between both ions was observed. The flow-injection analysis (FIA) system with tubular ISEs was suitable for the simultaneous calcium and potassium on-line monitoring. The determination of potassium presented good results when compared to the reference method. And the recovery results were 95±1% for calcium and 102±2% for potassium, showing a good evidence of the accuracy of the method **(Chumbimuni-Torres and Kubota**, **2006**).

Self-plasticising acrylic matrices (cross-linked poly(n-butyl acrylate) (nBA)) successfully employed as membrane materials for solid-state calcium ion sensors was developed by **Lee Yook and Elizabeth, (2006).** The self plasticizing**,** photocurability as

well as good adhesive characteristics of these polymer matrices enable workable solid-state calcium ion sensors to be fabricated by simple photocure procedures employing the calcium ionophore ETH 5234 and a lipophilic additive as ion sensing components. Thus, they are a good alternative to the existing membrane materials for solid-state calcium ion sensors. An optimum amount of the cross-linker 2,2-hexanedioldiacrylate (HDDA) and the incorporation of n-heptyl acrylate (nHA) led to improvement in the calcium ion-selectivity. The best calcium ion selectivity was obtained from a copolymer membrane with composition: nBA = 74 wt-%, nHA = 20 wt-% and HDDA = 0.1 wt-%. The selectivity coefficients of calcium over major cations were: ($logK^{pot}_{Ca:Na}$) = -4.4, ($logK^{pot}_{Ca:K}$) = -3.6, ($logK^{pot}_{Ca:Li}$)= -5.9, ($logK^{pot}_{Ca:Mg}$)= -4.4 with a Nernstian slope (29.1 ± 0.8 mV/decade) under buffered conditionss and it has satisfied the requirements for the assay of calcium ions in blood or serum.

Dimethyl 1-(4-nitrobenzoyl)-8-oxo-2,8-dihydro-1H-pyrazolo[5,1-a]isoindole-2,3-dicarboxylate was used as an ionophore and o-nitrophenyloctyl ether as a plasticizer in order to develop a poly(vinyl chloride)-based membrane electrode for calcium ion detection (**Hassan Ali Zamani *et al.,* 2006**). The sensor exhibited significantly enhanced response towards calcium (II) ions over the concentration range 8.0 x10^{-7} 1.0x10^{-1} M at pH 3.0-11 with a lower detection limit of 5.0 x10^{-7} M. The sensor displayed Nernstian slope of 29.5 ± 0.5 mV per decade for Ca(II) ions. The sensor found to have a fast response time within 10 s over the entire concentration range and can be used for at least 2 months without any divergence in potentials. The proposed electrode revealed good selectivity and response for Ca^{2+} over a wide variety of other metal ions. The selectivity of the sensor was comparable with those reported for other such electrodes. The proposed sensor was successfully applied as an indicator electrode for the potentiometric titration of a Ca (II) solution, with EDTA.

Acrylic acid grafted poly vinyl chloride based PVC membrane ion selective electrode was developed by **Nanda *et al.,* (2007)**. The prepared electrode was advantageous over the other electrodes, as it doesn't require internal solution. In this DBP was used as plasticizer and sodium tetra phenyl borate was used an anion excluder and it gave near Nernstian response to Mg^{2+} and Cu^{2+}, and showed super Nernstian response to Calcium ions in the concentration range of 1x10^{-1} to 1x 10^{-5} molL^{-1}. To restrict the interference of metal ions EDTA was used. The electrode was kept at 10^{-2} molL^{-1} concentration to give

good result. For the determination of calcium concentration and hardness measurement in river water, the response time was found to be ≤20s for this electrode, because of grafting process the ion exchange group was covalently bonded with PVC matrix instead of physical adsorption, which was not in the case of other electrodes.

A solid-state ion-selective potentiometric sensor based on temperature, moisture was developed with the objective of monitoring the behavior of H^+ and Ca^{2+} ions in soil and in real conditions. According to the authors, the evaluation of the sensorial system to pH monitoring revealed a good correlation between the results and the standard methodology. With regard to calcium, the sensor system also presented an agreement between its results and those obtained by flame atomic absorption spectrometry, using a calibration model based on multiple linear regressions that allows the correct determination of Ca^{2+} concentrations in soil depths **(Sherlan *et al.,* 2007).**

Linear Nernstian response was obtained for a neutral ionophore-based Ca^{2+}-selective electrode down to 10^{-10} M $CaCl_2$ by means of galvanostatic polarization. The densities of the applied cathodic current were tuned for particular concentrations of Ca^{2+}. The procedure included recording the potential at zero current, followed by measurements when current was passed through the electrode, and then again at zero current. The respective chronopotentiometric curves included negative ohmic drop immediately after turning the current on, the polarization domain, and positive ohmic drop when the current was turned off, followed with the relaxation domain. The potentials immediately after the positive ohmic drops were used as analytical signals. These potentials were found to make a straight line with Nernstian slope when currents were tuned (optimized) for each particular concentration (**Peshkova *et al.,* 2008**).

A new PVC-based membrane using p-isopropylcalix [6] arene (I) as an ionophore has been developed as a calcium-ion-selective sensor. Among various membranes prepared with and without plasticizer, the best performance was shown by a membrane with a composition (mg) I : NaTPB : PVC (2 : 2 : 120). This sensor exhibits a good potentiometric response to Ca^{2+} ion over a wide concentration range ($3.9x10^{-6}$ to $1x10^{-1}$ M) with a near Nernstian slope (30mVper decade of activity) and response time of 15 s. The sensor can be used for a period of 3 months without any drift in potential. The selectivity coefficient values are in the order of$1.0x10^{-3}$ for mono-, bi-, and trivalent cations, which indicates a good

selectivity for Ca^{2+} ion. The useful pH range for the electrode was found to be 2.5–6.0, and it works well in mixtures with non-aqueous content up to 25% (v/v). The sensor can also be used successfully as an indicator electrode in the potentiometric titration of Ca^{2+} against EDTA (**Ajay *et al*., 2008**).

Calcium content in the black liquor was determined using solid state Ca ISE. The sensitivity and selectivity of Ca^{2+} ion electrode remained unchanged even after using the electrode in black liquor. LA-ICP-MS experiments showed that there was a sorption of sodium from the black liquor into the ion selective membrane of the Ca^{2+}-ISE. Calcium was found to be replaced by sodium inside the membranes while the surface concentration of calcium remained almost unaltered. (**Granholm *et al.*, 2009**).

An electropolymerized melatonin modified glassy carbon electrode (EPMT/GCE) was developed by **Wu, (2009)** using electrochemically polymerizing of melatonin in a 0.04 mol l^{-1} perchloric acid solution. In KCl solution, Ca^{2+} demonstrated the selective electrochemical response on EPMT/GCE, that was related to the complex behavior and adsorptive property like sharp complex adsorption wave of Ca^{2+} with polymeric melatonin film. The developed sensor was used for determination of Ca^{2+} in the cerebrospinal fluid and rabbit serum samples with satisfactory results.

A new biosensor based on the activation of catalyse enzyme using calicium ion for the investigation of effect of calcium ion on the activity of enzyme was developed by **Akyilmaz and Kozgus, (2009)**. The authors stated that these biosensors developed cannot only used for food saples but also clinical purposes since it is fast accurate precise and has low operative cost.

Calcium glyxolate complex doped PVC based calcium ion selective electrodes was developed by (**Ismail and Al-Hitti *et al.,* 2010).** It gave a stable Nernstian response 29.97mV/decade with a linear range of (10^{-5} - 10^{-1}) M, detection limit of 10^{-5} M, response time of (20 - 60 sec) and the life time of the electrode was found to be four months. The working pH range was found to be 5-9 and small influence of temperature was also found on the efficiency of the electrode. Lower potentiometric selectivity coefficient value implied high selectivity of the electrode to calcium ions in the presence of many cations. Little interferences were found for cations but anions severely interfered, which were exploited for

determination of either the anions or calcium ions. This new electrode was significantly used to determine calcium ions in drinking water and blood serums.

Variations in calcium ion (Ca^{2+}) concentrations play a crucial role in cell–cell communication; periodic changes act as cellular signals according to **Park *et al.,* (2010)**. A solid-state calcium ion-selective electrode (Ca^{2+}ISE) integrated in a microchannel to measure extracellular Ca^{2+} variations was developed with high detection sensitivity (as low as 10^{-9} M) and selectivity against potassium ions (K+). The sensor was used to monitor the cellular activities of HepG2/C3As cultured in a microchannel. A periodically oscillating extracellular Ca^{2+} concentration that was superimposed on a gradual increase in the Ca^{2+} concentration after potassium chloride (KCl) stimulation as measured. The oscillation period was in the range of 30–50 s, which was in good agreement with the literature.

Urinary stone formation has evolved as a widespread disease during the recent years. The reasons for the formation of urinary stones are little crystals, mostly composed of calcium oxalate, which are formed in human kidneys. The early diagnosis of the risk for urinary stone formation of patients can be determined by the “Bonn-Risk-Index” method based on the potentiometric detection of the Ca^{2+}-ion concentration and an optical determination of the triggered crystallisation of calcium oxalate in unprocessed urine. In this work, miniaturised capacitive field-effect EMIS (electrolyte-membrane-insulator-semiconductor) sensors have been developed for the determination of the Ca^{2+}-ion concentration in human native urine. The Ca^{2+}-sensitive EMIS sensors have been systematically characterised by impedance spectroscopy, capacitance–voltage and constant–capacitance method in terms of sensitivity, signal stability and response time in both $CaCl_2$ solutions and in native urine. The obtained results demonstrate the suitability of EMIS sensors for the measurement of the Ca^{2+}-ion concentration in native urine of patients (**Beging *et al.,* 2010**).

A new reduced size solid state ion selective disposable electrode with carbon nano tube transducer layer was used to determine calcium ion in sap to overcome the difficulties obtained by using commercial ion selective electrodes. In this method the samples were used directly without any pretreatment. This new method was found to provide good Nernstian slope, outstanding stability, good Selectivity co efficient value and excellent linearity range and detection limit. On comparision with standard ISE this method was more

reliable and maintenance free and smaller limitation in temperature and pressure **(Rafael *et al.,* 2010).**

Calcium ion activity in simulated milk ultrafiltrate (SMUF) and milk was determined using Calcium ion selective electrode (Ca-ISE). It was shown that the ionic compositional difference between conventional calibration solutions and milk type samples had a significant effect on the single Ca^{2+} activity coefficient, which generates the erroneous estimate of Ca^{2+} activities in SMUF and milk. The authors tested new standards with ionic profiles similar to SMUF and the generated error were play down using the compositional difference between conventional standards and milk samples. It was found that a new significant Ca^{2+} activity and Ca^{2+} activity coefficient standard was developed over the predictable methods. The systematic error was reduced from 20% to 5% for SMUF and from 44% to 15% for milk. These method also helpful for other systems with a complex ion composition **(Gao *et al*., 2011).**

A new calcium ion selective electrode was prepared and used to measure solutions containing $CaCl_2$ and sodium dodecylsulfate (NaDS). It was revealed that the electrode based on ionophores ETH 1001 and ETH 129 cannot be used as Ca^{2+} ion sensors in these solutions because of strong anion interference from DS^- anion. Compared with other formulations it was found that the electrode that based on calcium bis (tetramethylbutylphenyl) phosphate in tri(2-ethylhexyl)phosphate appeared most promising. The interpretation of the ISE response in solutions under study relied on a novel approach that considered three forms of calcium: Ca^{2+} free ions, Ca in $Ca(DS)_2$ precipitate, and Ca^{2+} bound by the DS^- micelles. Data needed for the respective calculations were obtained by DS^- selective electrode based on tetradecylammonium, and Na+ selective glass electrode. Finally it was concluded that (tetramethylbutylphenyl) phosphate may be present in membranes in two forms: HTMPP acid, which plays a role of a neutral ionophore and $CaTMBPP_2$ salt – charged ionophore **(Ivanova *et al.,* 2011).**

A hyphenated method based on FTIR-ATR and electrochemical impedance spectroscopy has been applied to simultaneously measure the water uptake, changes in the bulk resistance and potential of plasticized poly(vinyl chloride) (PVC) based Ca^{2+}-selective coated-wire (CaCWE) and solid-contact electrodes (CaSCISEs). Most of the water uptake

of the ion-selective membranes (ISMs) used in both electrode types took place within the first 9 h in 10^{-3} M $CaCl_2$ showing good correlation with the stabilization of the individual electrode potentials. The bulk resistance of the ISMs of the CaCWEs and the CaSCISEs with poly(3-octylthiophene) (POT) as the solid-contact (SC) increased most during the first 18 h in 10^{-3} M $CaCl_2$. The increase in the resistance was found to be related to the exchange of K^+ for Ca^{2+} in the ISM and the formation of the Ca^{2+}-ionophore (ETH 5234) complex having a lower diffusivity than the free K^+ ions. In contrary to previously published results on silicone rubber based SCISEs and poly(methyl methacrylate):poly(n-decyl methacrylate) membranes containing POT, the plasticized PVC-based CaSCISEs with POT as the SC had a higher water uptake than the CaCWEs. The CaSCISEs had a detection limit of $2x10^{-8}$M Ca^{2+} and a good potential reproducibility of 148.9±1.0 mV in 10^{-4} M $CaCl_2$ (**Lindfors et al, 2011**).

A simple and efficient montmorillonite-calcium modified carbon paste electrode (MMT-Ca modified CPE) was constructed for simultaneous trace determination of Cd(II), Pb(II), Cu(II) and Hg(II). The MMT-Ca modified CPE significantly enhances the voltammetric stripping peak current magnitudes of the investigated metal ions compared to the bare CPE due to the large cation-exchange capacity and the strong adsorptive property of montmorillonite-Ca clay. A fully validated simple, sensitive, selective and precise square-wave anodic stripping voltammetric method was developed for the simultaneous trace determination of Cd(II), Pb(II), Cu(II) and Hg(II) in various water samples using a fabricated 10% (w/w) MMT-Ca modified CPE. The achieved limits of detection of Cd (II) 0.54 mgL^{-1},Pb(II)0.30 mgL^{-1}, Cu(II) 0.75 mgL^{-1} and Hg(II) 1.05 mgL^{-1} indicating the high sensitivity of the described SW-AS voltammetry method for the assay of these metal ions in aqueous solutions. The method was successfully applied for analysis of tap water, bottled natural water and seawater samples (**Amr *et al.,* 2011**).

An all-solid-state sensor consisting of a thick film calcium ion-selective electrode and a pseudo-reference electrode for rapid blood calcium test was studied. Poly (3,4-ethylenedioxy thiophene) (PEDOT) doped with poly(styrene sulfonate) (PSS) was screen-printed on carbon paste electrodes as internal solid contacts for both the ion-selective and reference electrodes. PVC membranes with and without neutral carrier ETH129 were coated on the PEDOT (PSS) layer to form calcium ion-selective and pseudo-reference

electrodes, respectively. The properties of screen-printed PEDOT (PSS) film on carbon paste electrode were studied by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) methods. The response time of the calcium sensors was 15 s while less than 15μL sample was applied. And the sensitivity was 30.0 ± 1.0 mV per decade, while the potentiometric log selectivity coefficients were −3.80, −3.35 and −4.60 for Na^+, K^+ and Mg^{2+}. Other properties of the calcium sensors were evaluated, including the temperature effect, repeatability, reproducibility and stability. The testing results with the calcium sensors were compared with those with a routine hospital electrolyte analyzer. The clinical trial results reflect that the all-solid-state calcium sensor was promising for the point of care testing (**Wang *et al.,* 2012**).

A new calcium ion sensor was developed using new conducting polyaniline polyindole-camphorsulfonic acid (PIn-CSA) composite. The composite was developed by homogenizing equimolar ratio of chemically synthesized PIn and CSA in tetrahydrofuran. Then the mixture was spread over Pt disc electrode under ambient conditions. The prepared composite was characterized using UV–vis, SEM, EDX, and cyclic voltammetric measurements. The ion-sensor exhibits near Nernstian response for Ca^{2+} over a concentration range of 2 x 10^{-5} to 1 x10^{-2} M with a response time of 8 s and the sensor was successfully used over a period of 3 month without any considerable change in drift potential. The working pH of the electrode was found to be 4- 8. The selectivity of the sensor was studied and it was found that the negligible interference was observed when other cations were less than 0.01M concentration. The slope of the present ion sensor was calculated to be 25.4 ± 0.21 mV per decade. The limit of detection for present sensor was found to be 5 μM **(Prem *et al.,* 2012).**

An analytical system based on ion-selective field effect transistors (ISFETs) with Ca^{2+} ion selective photocurable membranes was developed to offer a semiautomatic analysis of serum calcium concentration of ruminants. Enhancement of the performance and the lifetime of calcium ion sensitive polymer membranes were tested by synthesizing & investigating different types of copolymerisable plasticizers. Optimisation of ion-sensitive membrane composition containing different copolymerised plasticizers was performed to minimize the effect of a highly liphophilic samples on sensors characteristics. The system was used to analyse the total calcium concentration in bovine sera. The precision of the ion

determination was higher than reported for double charge ions with a standard deviation of about 3–7%. The results revealed that the presence of coagulant in blood serum samples does not affect the determined total calcium concentration (**Abramov *et al.,* 2013**).

An all-solid-state miniature calcium ion selective electrode (ISE) based on poly(3,4-ethylenedioxythiophene) doped with poly(styrene sulfonate) (PEDOT(PSS)) for continuous *in situ* measurement in seawater was studied. The electrode substrate was a platinum (Pt) wire of 0.5 mm diameter and PEDOT(PSS) was electropolymerized on one end of the Pt wire to act as the solid contact of this calcium ISE. The PEDOT (PSS) layer was covered with a calcium-selective poly(vinyl chloride) membrane, which contained ETH129 as calcium ionophore, potassium tetrakis-(p-chlorophenyl)borate as lipophilic anion and bis(2-ethylhexyl) sebacate as the plasticizer. Experiments using electrochemical impedance spectroscopy and reversed chronopotentiometry illustrated that electropolymerized PEDOT (PSS) decreased the resistance and improved the stability of the electrode. The sensors can work stably in the calcium ion concentration range of 10^{-6}–10^{-1} mol L^{-1} with the slope of 27.7 mV/decade. Also Na^{+}, K^{+} and Mg^{2+} can hardly interfere with the performance of the electrode. This electrode was applied to measure the calcium ion concentration of seawater samples. The experimental data showed that the electrode can resist the corrosion of seawater and its reproducibility was good (SD < 0.1 mM kg^{-1}). The lifetime of such an electrode was at least six months. Because of the wire-shape and the small size of such a liquid junction free calcium electrode, it is pressure-resistant and easy to package and seal, therefore it is suitable for use in underwater equipment for *in situ* seawater measurement **(Hui *et al*., 2013)**.