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THE UNIVERSITY OF

SUSSEX

Towards Biomedical Intelligent Sensors

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The University of Sussex

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Towards Biomedical Intelligent Sensors

Summary

Potassium, sodium, and calcium are crucial electrolytes of human blood. Among the functions of the electrolytes are maintenance of osmotic pressure and water distribution in the various body fluid compartments, maintenance of the proper pH, regulation of the proper function of the heart and other muscles, involvement in oxidation-reduction or electron transfer reactions, and participation in catalysis as cofactors for enzymes. Therefore, abnormal levels of electrolytes may be either the cause or the consequence of a variety of disorders, and thus the determination of electrolytes in different body fluids in general and in whole human blood particularly is one of the most important functions of the clinical laboratory.

Serum or plasma is the usual specimen employed for assay of potassium, sodium, and calcium but not whole human blood. This research intended to create a sensor-array capable of analysing potassium, sodium, and calcium ion-activity in whole human blood based on miniaturised ion-sensors and zero-current direct potentiometry. This research dealt with **sensing-side of the sensor-array** hoping for integration of these sensors with microelectronics or discrete parts based electronics in due time. Clinics use single electrodes for K^-, Na^-, and Ca^{2+} sensing in body fluids. However, single sensing is costly and in emergency cases time consuming as well. Therefore a portable, battery-operated cost- and time-effective sensor array is needed for **multi-sensing of K^+, Na^+, and Ca^{2+}** either in emergency cases in field or for patient near bed-side measurement/monitoring. The approach of this thesis to the problem is as follows:

This thesis has five phases, all equally important. The first phase, a theoretical phase, mainly deals with the determination of the technique which fits best with chemical sensors and integration with microelectronics, and the decision among many alternative chemicals, whether natural or man-made, available for multi-sensing realisation, by taking into account all the best efforts devoted to development of chemical-sensors. The second phase is the miniaturisation process of the electrochemical sensor-array, either ion-sensors or reference electrode, as much as possible. The third phase is multi-sensing application of the sensor-array for sensing K^+, Na^+, and Ca^{2+} with binary and mixed electrolytes and with artificial and whole human blood respectively, the fourth phase is the sensor-array response with only millilitre or microlitre volumes of whole human blood and the fifth phase is the integration of a sensor array with discrete, parts based, electronic circuitry.

So, as a **new application a disposable reference electrode** has been realised which reasonably fits with the sensor array for the analysis of potassium, sodium, and calcium in whole human blood. Since all the single sensing of these cations have been realised by using a conventional reference electrode, either silver/silver chloride or calomel electrode, which is a bulky and expensive alternative, this research prepared, tested, and implemented **a new disposable reference electrode** for the sensor-array based on a *poly (vinyl alcohol) matrix incorporating a proper amount of KCl*. Having a successfully working miniaturised reference electrode, *valinomycin*, *ETH 157*, and *ETH 1001* ionophores have been used as the selective materials for potassium, sodium, and calcium in whole human blood, respectively. **Multi-sensing applications have only used solutions as testing medium whereas the work**

described in this thesis goes one step further and uses artificial and whole human blood with fairly reasonable responses.

Acknowledgements

The life span of human being is deadly short although what has to be done is much that clearly requires perfect leadership, sincere co-operation between individuals as well as organisations; mental, spiritual and materialistic peace in individual mind, family life and community relations; strong desire to succeed in what is targeted for, and absolute determination.

During my life so far many individuals and institutions have been contributing via different methods with different intentions. Today I need to remember the hero and heroes of my life for whom I have always been a hope to prosperity, better life, good education, a door to bread and butter, a new and powerful dawn upon their future expectations, and somebody to whom investment has been and must be done.

I shall put into the first group who helped me to survive a better life by providing daily human spiritual and materialistic needs, among them are my grandfather (Salih) who was reminding my mother about the care she was putting on me and for **his absolute goodwill** of me telling my mother, "O lady daughter, take care of my grandson. he is going to be a great man!", and my mother (Hanim) who fed me while starving herself. father (Kemal) who appreciated the importance of education and not saw it as a sin. uncle (Cemal) who is known as a wise man, sister and brothers (Murside, Atilla and Yalcin) in the first place, and then Mehmet Behcet, Ekrem Ak, Hasan Kalyoncu, Ismail Ilmi Kideys. Abdulvahap Demir. Nurettin Uzungol. and Ismail Senturk in chronological order.

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And finally I would like to thank to **the University of Inonu-Malatya** for its funding of my research here at the University of Sussex during the unforgettable years.

Fikret Yalcinkaya
1997 - Brighton

DECLARATION

I hereby declare that this thesis has not been submitted, either in the same or different form, to this or any other university for a degree.

Fikret Yalcinkaya

Figures

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dedication

This thesis is dedicated wholeheartedly to the three suns, my wife Mrs. Atiye Yalcinkaya, and beloved sons Ahmet and Ali respectively, who have risen to my life giving it taste and meaning.

Fikret Yalcinkaya

ithaf

her ne kadar bu kalitesiz çalişma o kalite karakterlere adanmaya liyakat kesbedememişse de bana ödünç verilmiş iyi niyetlerini bir kez daha bencil kullanmaya cesaret ederek **Dünyama doğmuş üç güneşe** (Atiye Yalçınkaya, Ahmet Yalçınkaya ve Ali Yalçınkaya'ya) adıyorum.

Fikret Yalçınkaya

Chapter One

Introduction

Summary

First of all, this chapter briefly mentions the roles of potassium, sodium, and calcium ions in the human body, their importance and different techniques of detecting them. It defines the problem tackled and the approach to the problem in different phases of the thesis and gives a literature survey for single- and multi-sensing of subject cations, and chip-design approaches for potassium, sodium, and calcium sensors. The chapter ends up with an overview of the thesis.

1. Introduction

1.1 The roles of K^+, Na^+, and Ca^{2+} in the human body

1.1.1 The role of potassium

Potassium is the major intracellular and extracellular cation. A decrease of extracellular K^- is characterized by muscle weakness, irritability, and paralysis, fast heart rate and specific conduction effects apparent by electrocardiographic examination; and eventual cardiac arrest. Abnormally high extracellular potassium produces symptoms of mental confusion; weakness, numbness, and tingling of the extremities; weakness of the respiratory muscles, flaccid paralysis of the extremities, a slowed heart rate, typical conduction defects on the electrocardiogram, and eventually peripheral vascular collapse and cardiac arrest[1.1].

1.1.2 Diseases due to potassium

Decreased plasma potassium concentration is known as **hypokalemia**. The causes of hypokalemia can be grouped into decreased intake, redistribution of extracellular K^+ into intracellular fluid, and increased loss of potassium-rich body fluids. **Hyperkalemia** is increased plasma potassium concentration and may be precipitated by intravenous infusion of K^+ at a rate in excess of 20 mmol/h or by treatment using K^+ solutions with concentrations >40 mmol/l or doses of >80 mmol/day.

1.1.3 Methods for the determination of potassium

Potassium may be determined by atomic absorption spectrophotometry (AAS), flame emission spectrophotometry (FES), or electrochemically with an ion-selective electrode (ISE)[1.1]. The latter has two methods of detection. One is called indirect (the sample is introduced into the system with a large volume of diluent of high ionic strength) while the other is called the direct method (the sample is presented to the sensors without dilution). Both methods, FES and ISE are in current use.

Single-use, thin-film ion-selective electrodes for sodium, potassium, and calcium determinations are unique applications of the direct ISE method. The direct method, particularly as applied to whole blood, gives the advantage of more rapid determinations; the analytical approach is also nondestructive of the sample, i.e. the sample can be recovered, essentially unchanged by the direct ISE measurement process, for additional testing. There are important differences in the applications of potentiometry between the direct and indirect methods that cause significant differences in analytical results.

1.1.4 The role of sodium

Sodium is the major cation of extracellular fluid since it represents almost 90 % of the 154 mmol of inorganic cations per litre of plasma. Sodium is responsible for nearly half of the osmolality of the plasma and therefore plays a central role in maintaining the normal distribution of water and osmotic pressure in the extracellular fluid compartment. Since the body requirement is only 1-2 mmol/day, the excess is excreted by the kidneys, which are therefore the ultimate regulators of the amount of sodium and potassium in the body[1.1].

1.1.5 Diseases due to sodium

Hyponatremia is a sign of decreased plasma sodium concentration and is a predictable consequence of decreased intake of sodium, particularly that precipitated or complicated by unusual losses of sodium from the gastrointestinal tract, kidneys, or sweat glands. **Hypernatremia** is a sign of increased plasma sodium concentration and is often attributable to excessive loss of sodium-poor body fluids, i.e., to water loss disproportionate to sodium loss, as may occur in profuse sweating, prolonged hyperpnea, vomiting, diarrhoea, or polyuria.

1.1.6 Methods for the determination of sodium

Sodium may be determined by atomic absorption spectrophotometry (AAS), flame emission spectrophotometry (FES), or electrochemically with an ion-selective electrode (ISE)[1.1]. Single-use, thin-film ion-selective electrodes for sodium, potassium, and calcium determinations are unique applications of direct ISE method as described in section 1.1.3.

1.1.7 The role of calcium

Calcium is the main inorganic form of matter that exits in the mineral phase of hard tissues, for example, bone, dentine, and enamel. The concentration of calcium in plasma is dependent partially on the balance between bone mineral deposition and bone resorption; at the same time, the concentration of calcium has an important bearing on the mineralization process itself.

Intracellularly, calcium is the prime inorganic messenger for regulation of cell functions. It determines the activity of enzymes, notably adenylate cyclase and phosphodiesterase, through reversible combination with calmodulin. Calcium also functions at the plasma membrane itself. It serves to regulate membrane permeability, to cause neurotransmitter release, and to diminish neuromuscular excitability. Calcium is involved in cell-to-cell adhesion and possibly communication. The ion plays a vital role in blood coagulation. As a principal constituent of hydroxyapatite, it serves to confer rigidity to the extracellular protein matrices of bones and teeth[1.1].

1.1.8 Diseases due to calcium

Calcium is the fifth most common element in the body, practically all of the 25 mol of calcium (nearly 1 kg) in the human adult body is extracellular, 99 % of it is in the hard tissues, the extracellular fluid contains nearly 25 mmol (1 g) calcium; blood contains 7.5 mmol (300 mg), virtually all of which is in the plasma.

Calcium exits in plasma in three physicochemical states: protein-bound (~45%); complexed with small diffusible ligands (~10%); and ionized (~45%). Under normal conditions, the concentration of total calcium in human plasma (or serum) is nearly 2.5 mmol/l (10 mg/dl), and that of the ionized fraction is almost 1.125 mmol/l (4.5 mg/dl). The ionized fraction is the physiological active form[1.1].

1.1.9 Methods for the determination of calcium

Calcium in biological fluids has traditionally been difficult to measure accurately and precisely. Of the many methods that have been introduced are atomic absorption spectrophotometry (AAS), and spectrophotometry of calcium-dye complexes and calcium ion-sensitive electrodes[1.1].

Hence, the physicians need instruments capable of identifying the mono- and di-valent cation concentration, separately or together, in real whole human blood in emergency cases and/or near the bedside. They need to be simple, cheap and readily useable. Therefore, a disposable, miniaturized, multi-sensing sensor-array capability will greatly help the physicians in emergency cases, bedside treatment and tests.

In hospitals and related departments, single sensing has been successfully carried out for potassium, sodium, and calcium. It was thought that a multi-sensor sensor array will help to reduce waiting time threefold and contribute to patient's well-being by giving the medical experts a quick outcome. Otherwise the experts might have to wait until test results have been received from a different department, or even a regional hospital, leading to undue delays in the treatment.

Another advantage of a multi-sensor sensor array is that it is intended to be disposable and mass-producible.

1.2 Definition of the problem

Physicians need equipment which helps the understanding of patient status in emergency and critical care or for near bedside monitoring. Potassium, sodium, and calcium are crucial elements of human blood, the concentration of which give clear and comprehensive information about some diseases and the functionality of human blood and/or body as well. There are a lot of *roles of electrolytes* in human body. There are almost no metabolic processes either not affected or dependant on the electrolyte balance of the human body system. Among the functions of the electrolyes are maintenance of osmotic pressure and water distribution in the various body fluid compartments, maintenance of the proper pH, regulation of the proper function of the heart and other muscles, involvement in oxidation-reduction or electron transfer reactions, and participation in catalysis as cofactors for enzymes. Therefore, it is clear that abnormal levels of electrolytes may be either the cause or the consequence of a variety of disorders. The fact is that the determination of electrolytes in different body fluids in general and in whole human blood particularly is one of the most important functions of the clinical laboratory as well as providing clinical evidence leading to proper treatment of the patient.

Blood, collected by venipuncture into an evacuated tube, is the usual specimen employed for assay of potassium, sodium, and calcium. Either serum or plasma is appropriate for assay and *with ion-selective electrodes whole blood may also be used*[1.1]. The use of plasma has the advantage of expediting the assay and shortening turnaround time in emergency situations.

Therefore it is quite desirable to have a sensor array appropriate for analysis of potassium, sodium, and calcium ion-concentration or ion-activity in whole human blood for critical care, emergency or near bedside monitoring. This research intends to deal with the sensor array side of the problem hoping for integration of these sensors with microelectronics in due time. A portable, miniaturized, integrated, and disposable sensor array will undoubtedly help the physicians very much by obtaining the information they urgently require in a very short time with full confidence.

Such a sensor or sensor array under normal conditions is expected to obey the Nernst Equation (see Chapter Four for more detail). With the assumption that the junction potential is negligible, the hydrogen cell may thus be used for the estimation of hydrogen ion activity (*or pH*) in solutions with unknown concentration to confirm. Therefore, the relationship between the potential differences obtained and activity of hydrogen ions is defined by the Nernst equation[1.1a].

$$E = E_{reference} - E_{unknown} = (RT/nF)\log_e(H^+_{reference} / H^+_{unknown})$$

where R=the gas constant (*8.315 J/mol/degree*), T=the absolute temperature, n=the valency charge (for potassium and sodium it is equal to unity whereas for calcium it is two), and F=Faraday's constant (*96500 coulombs*).

If the reference and second half cell solutions are *1 mol (M)* and *0.1 mol (M)* for hydrogen ions respectively, then at 25°C:

$$E = [(8.315 \times T) / (1 \times 96500)] \times 2.303 \times \log_{10}(H^+_{reference} / H^+_{unknown})$$

$$= 0.0001983 \times T \times (\log_{10}1 - \log_{10}10^{-1}) = 0.0541 \times (0+1) = 54.1\ mV$$

i.e. a *10-fold* (decade) difference in activity for a monovalent ion (for example, potassium and sodium) at *25°C* will produce a potential difference of *54.1 mV* for a perfect electrode. This relationship applies at all concentrations at which the Nernst equation can be applied to the response of the electrode. The Nernst equation in that form is only applicable to an ideal cell, in which there are only two sources of potential. In actual fact, there are other potentials which must be incorporated into the equation, one of which is the junction potential is caused by the introduction of the salt bridge.

For calcium the numerical value for the potential *E* of the Nernst equation will be *54.1/2=27.05 mV* due to the valency charge which is equal to two for calcium ions.

A perfect electrode obeys the Nernst equation, but in reality there are few ion sensors obeying the Nernst principle since there are a lot of factors which can not be controlled perfectly. As a result of that, sometimes it becomes necessary to claim that the ion sensors are responding in a manner described in the literature as a near-Nernstian response, i.e. if the slope of the calibration curve is not *54.1 mV/decade* for monovalent and *27 mV/decade* for divalent ions.

Clinics use single electrodes for K^+, Na^+, and Ca^{2+} sensing in body fluids, specifically in human blood since their levels, either in terms of concentration or ion-activity, help medical doctors to diagnose illness.

Single sensing is costly and in emergency cases time consuming as well Therefore a portable, cost- and time-effective sensor array is needed for multi-sensing of K^+, Na^+, and Ca^{2+} either in emergency cases in the field or bedside measurement/monitoring.

The experimental set-up used during this research period for such a sensor array which measures potassium, sodium, and calcium in binary solutions, mixed solutions, artificial blood, and finally in real whole human blood is given in **Fig.1.1**.

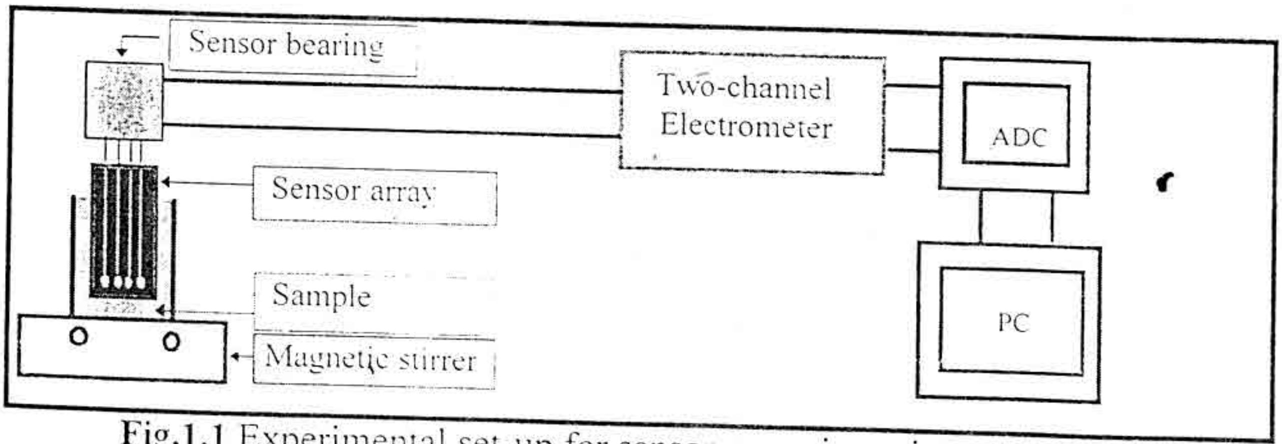


Fig.1.1 Experimental set-up for sensor-array in various samples

Electrolytes	Ranges	
K^-	3.60 - 5.50	mmol/l
Na^+	135 - 155	mmol/l
Ca^{2+}	1.14 - 1.31	mmol/l

Table 1.1 The ions (or electrolytes) and their normal ranges in blood

The cation- and anion-ions in the whole human blood are K^-, Na^+, Ca^{2+}, Mg^{2+} and Cl^-, HCO_3^-, HPO_4^{2-}, and SO_4^{2-} respectively. Since this research is looking for ion measurements in whole human blood, it is necessary to give the subject electrolytes and their normal ranges in blood as seen in **Table 1.1**.

In clinical practice indirect potentiometry is being used. Direct potentiometry is not preferred due to the heritage of flame photometry. Still from time to time clinicians try to justify ion-selective electrodes based readings with flame photometry. Another reason for not using direct potentiometry is because it reads 40% less than indirect potentiometry.

Clinical practice is predominantly not based on a millivolt scale for analysis but instead ion concentration is used. Ion activity is expressed in terms of ion concentration not in terms of millivolts. The reason ion concentration is being used is because at low levels, ion activity is considered to be equal to ion concentration. So it makes easier reading in terms of ion concentration.

In this research it was preferred to make measurements in millivolts because it gets rid of the need for programming converting ion activity into ion concentration and it uses direct potentiometry as it directly converts ion activity into voltage (mV). This research has been specifically concentrated on getting repeatability of measurements right and putting a special care to differentiate properly between various ion concentrations tested. Hence, although accuracy was not the first priority to look at but clinical practice uses ±2% or ±5% limits for stable measurements.

There are mainly two different ways of reporting the results, one is giving the calibration curve in terms of ion activity versus millivolts and the other is reporting in terms of the slope of Nernstian equation and statistical analysis of data. Research groups who have done thousands of tests measuring ion activity have preferred to report in terms of the latter method as it makes it more simple to present the results. The latter technique requires a well established background in the field as well whereas the former technique is more research oriented.

Different research groups report various results and different responses for potassium, sodium, and calcium[1.1b]. By using ISFETs, which are a new version of ion-selective electrodes, response curves have been reported as 0, 35, 80, 120, 170, 230 millivolts for $log(K^{-})$ = -6, -5, -4, -3, -2, -1 mmol/l, respectively. The same paper reports the results for calcium as: 0, 25, 50, 80, 105 millivolts for $log(Ca^{2+})$ = -5, -4, -3, -2, -1 mmol/l, respectively. The ISFET membrane is photolithographically deposited for this specific research. For sodium the reported values are [1.1c] 20, 60, 120, 170, 190, 200 millivolts for $log(Na^{+})$ = -6, -5, -4, -3, -2, -1 mmol/l, respectively. Another paper[1.1d] reports sodium, potassium and calcium levels in millivolts and slope (mV/decade) as 350.6, 151.9, 322.8 millivolts, respectively. The slopes are 55.2, 54.9, 26.3 mV/decade, respectively. The test results of this research are detailed in Chapter Five, Chapter Six, and Chapter Seven in terms of ion-activity versus millivolts. The discrete parts based electronic circuit design for this specific research is also given in **Fig.1.2** in a block diagram form:

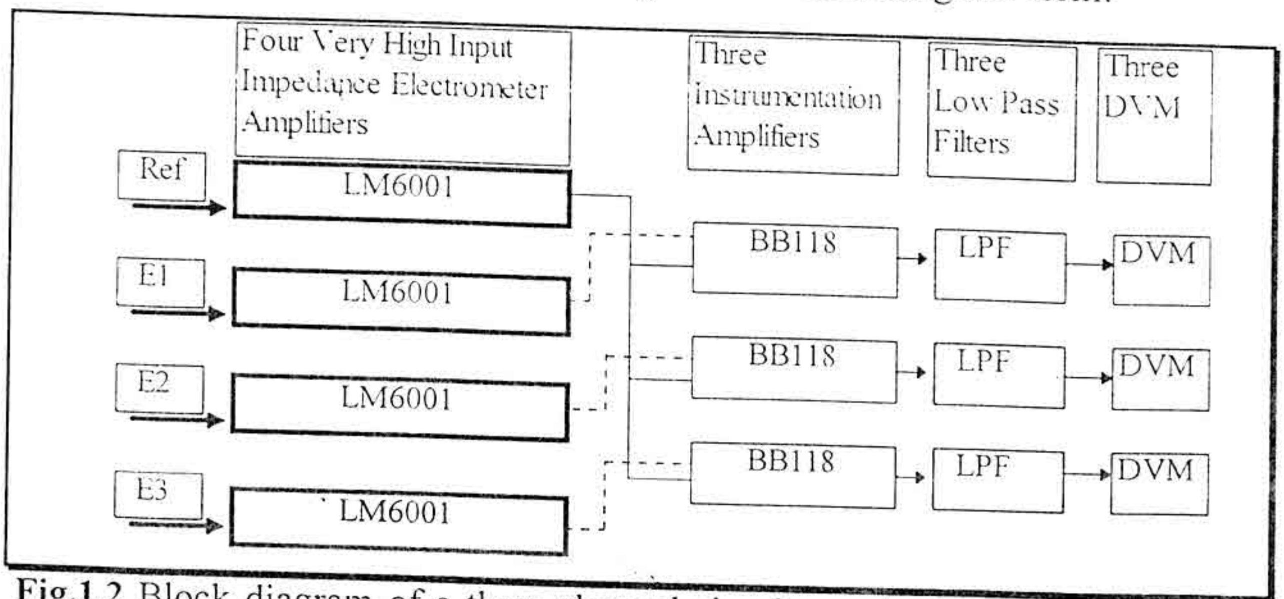


Fig.1.2 Block diagram of a three channel simultanously measuring electronic circuit for potassium, sodium, and calcium sensor-array

1.3 The approach to the problem

1st-Phase: Theoretical phase which deals mainly with methodology,

2nd-Phase: Miniaturization of ion sensors and reference electrode as much as possible,

3rd-Phase: Multi-sensing application of the sensor array for sensing K^+, Na^+, and Ca^{2+} with binary and mixed electrolytes in the first instance but with artificial and whole human blood at the last stage,

4th-Phase: Reasonable results with only a few drops of blood.

5th-Phase: Integration of sensor-array with traditional circuits, surface mount and microelectronics circuit design, respectively.

This thesis is based on five phases which all equally have been important during the course of the research. The first phase is the **theoretical phase** which mainly deals with the determination of the technique which best fits with chemical sensors, integration with microelectronics, together with the decision made among many **alternative chemicals available for multi-sensing and the realization of multi-sensing**, whether natural or man-made. It takes into account all the best efforts devoted to development of chemicals for sensors that could be integrated and miniatured. The second phase is **the miniaturization process of the electrochemical sensor-array of either ion sensors or reference electrodes**. The third phase is multi-sensing application of the sensor array for sensing K^+, Na^+, and Ca^{2+} with binary and mixed electrolytes in the first instance but with artificial and whole human blood at the last stage. The fourth phase is to be able to get reasonable results with the sensor array by using only a few drops of blood, whilst the fifth phase considers the integration of a sensor array with traditional circuits, surface mount and microelectronics circuit design, respectively. Discrete parts based design has been realised as in **Fig.1.2** given above.

Firstly it was intended to realize a disposable reference electrode which will perfectly fit with the sensor array for the analysis of potassium, sodium, and calcium of whole human blood. Since all the single sensing of these cations have been realized by using a conventional reference electrode, either silver/silver chloride or calomel electrode, which is a bulky and expensive alternative, it was desirable to prepare and implement a new disposable reference electrode[1.2] for the sensor-array based on *poly (vinyl alcohol) incorporating a proper amount of KCl.*

Having successfully prepared and implemented the subject reference electrode which acts as the half-cell of the sensor-array system under consideration, a search was undertaken to find out proper chemicals for ion-sensors[1.3]. Finally, *valinomycin, ETH 157*, and *ETH 1001* ionophores have been used as the selective materials for potassium, sodium, and calcium respectively.

Fig. 1.3 shows the structure of the sensor array that has been used for this research. This design was produced following detailed studies of single and multi-sensor practice[1.3a-1.3b] and discussion with analysts in hospital biology and pathology laboratories. The substrate is a glass laminate used in hospitals for blood group analysis onto which a very thin layer (*8.3 μm*) of pure silver has been deposited by using a vapour deposition technique in the laboratories of the Physics department at the University of Sussex. The figure shows the actual dimensions of the glass laminate for sensor array deposition of K^+, Na^+, and Ca^{2+} ions.

A future intention is to integrate these sensors with microelectronics by using VHDL and/or VeriLOG hardware description languages to produce a portable miniaturized diagnostic instrument/tool for near bedside treatment or patient treatment in emergency and critical care.

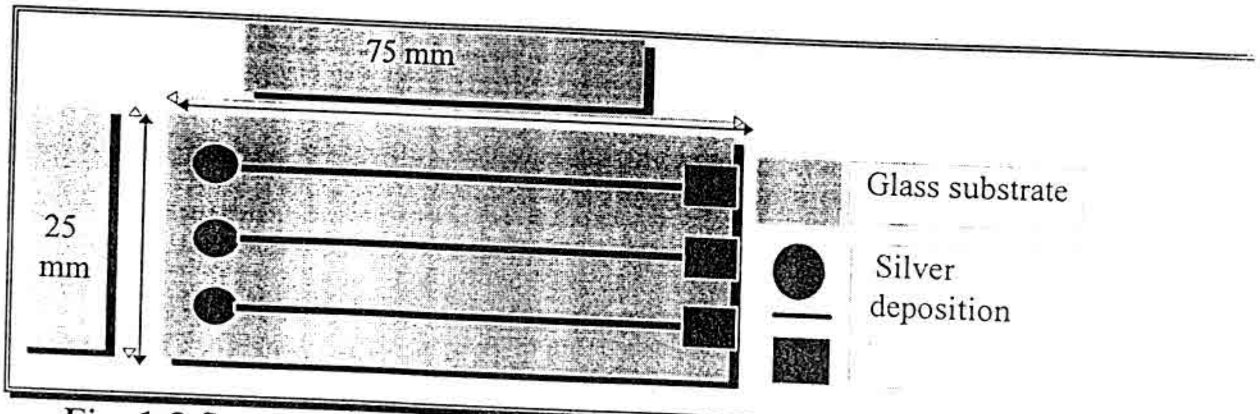


Fig. 1.3 Sensor-array structure of K^+, Na^+, Ca^{2+} and the reference electrode

1.4 Literature survey

1.4.1 Single sensing of K^+, Na^+, and Ca^{+2}

Coated wire sodium- and potassium-selective electrodes based on bis(crown ether) compounds[1.4] have been prepared and manufactured showing excellent electrode properties, i.e. near-Nernstian response (i.e. if the slope of the calibration curve is not 54.1 mV/decade for monovalent and 27 mV/decade for divalent ions) in a wide concentration range of calibration plots and stable emf in a wide pH range.

Potassium ion responsive coated wire electrodes based on valinomycin have also been prepared and manufactured. The application of this type of electrode to *whole blood* (**sheep blood**) *and sea water* has been investigated[1.5] leading to verified results. H. Freiser and R.W. Cattrall have developed coated wire electrodes (CWEs) for calcium as well[1.6], although it is widely accepted that CWEs do suffer from drift and poor reproducibility[1.7]. The observed drift might originate from the absorption of water by the membrane or leaching out of the electroactive component.

A new type of membrane matrix for the preparation of ion-selective coated wire electrodes has been developed[1.8]. It uses a solid solvent membrane composed of an organic compound with a low melting point, such as stearyl alcohol (octadecan-1-ol) or triphenyl phosphate, in which are dissolved ion-exchanging materials for nitrate, p-toluenesulfonate, chloride, calcium, and potassium. The coated wire electrode has been prepared by dipping its tip into a molten ion exchanger. A calcium-selective solid solvent membrane electrode (SSME) has been used to determine calcium in water samples.

Poly(vinyl chloride) membrane-based calcium ion-selective electrodes have been constructed[1.9] with calcium bis(2-ethylhexyl) phosphate as a sensing material and tetrahydrofuran as solvent.

A disposable single-sensing sodium sensor in double matrix membrane technology has been described[1.10]. This sensor has been prepared from filter paper with an evaporated silver conducting line on one side. For insulation the sensor is laminated with a pre-perforated heat-sealing film. A defined volume of an ion-sensitive polymer matrix membrane cocktail is filled into one hole so. an ion-sensitive coated film sensor in double matrix membrane technology is produced. The double matrix membrane is formed by the polymer matrix membrane and by the additional filter paper matrix. The response behaviour of the sodium electrode is comparable with conventional macro ion-selective electrodes. By this technology mass production of low cost sensors has been shown possible.

The construction of an ion-selective electrode for potassium. employing **valinomycin as a carrier** in a poly(vinyl chloride) membrane and a poly(vinyl ferrocene) solid contact between the membrane and the metallic substrate has been described[1.11]. The performance of this electrode is compared to electrodes in the coated-wire configuration and the conventional electrode with an aqueous internal reference electrolyte. Nernstian responses (i.e. the slope of the calibration curve is equal to *54 mV/decade* for monovalent and *27 mV/decade* for divalent ions) have been obtained for the three types of electrodes and the selectivities and the detection limits are very similar for all electrodes. The stabilities of these electrodes have been investigated over the first 20 days of use which shows that the solid-contact electrode showed a significant improvement over the coated-wire electrodes and its performance approaches that of the conventional one.

All-solid-state potassium-selective electrodes have been constructed on a platinum disc electrode using a double-layer film of polypyrrole/poly(4-styrenesulfonate) (PPy/PSS) composite covered with a plasticized poly(vinyl chloride) (PVC) membrane containing valinomycin[1.12].

It is reported that the lower composite layer was formed by electropolymerization of pyrrole in an aqueous solution containing poly(sodium 4-styrenesulfonate), and the upper PVC layer was formed by casting. The double-layer film electrode using this composite shows no overshoot and lower drift of the standard potential than both a single PVC-coated electrode and a double-layer electrode using polypyrrole doped with ClO_4^-. This is due to both the highly electrochemical activity of the lower PPy/PSS and the cation-exchange ability between the PPy/PSS and the potassium-selective membrane[1.12].

A potassium optical-sensing layer based on reflectance change measurements of the Takagi reagent (4'-picryl-3'-nitrobenzo-18-crown-6) immobilized on a plasticized PVC membrane has been developed[1.13]. A very limited operational life time for the sensing layer using a PVC membrane has been observed due to leaching of the reagent and water absorption in the membrane. The same optrode type sensor has been further developed for the determination of potassium in urine, milk, and sea water[1.13].

A new optical membrane sensor which is composed of cellulose triacetate polymer as a membrane support, o-nitophenyloctyl ether as a membrane plasticizer and sym-(decyl)(2-hydroxy-5-nitrobenzyloxy)dibenzo-16-crown-5(1) as a Na^+ ion-selective chromoionophore has been developed[1.14].

ISFETs have been developed for K^+, Na^+, Ca^{2+}, H^+, and NH_4^+ and have been used *in vivo*[1.15].

D. Ammann *et al* have developed a Ca^{2+}-selective electrode[1.16] based on a neutral carrier. This ISE uses an electrically uncharged Ca^{2+}-selective complexing agent, such as *ETH 1001*, which is dissolved in the plasticiser, for instance o-nitrophenyl octyl ether or bis (2-ethylhexyl) sebacate, in a PVC membrane. For such applications, the ionophore and plasticiser must be as hydrophobic as possible to avoid being leached out of the membrane by samples. ETH-1001 and related ionophores are selective for calcium because they provide an environment of conformation and atomic interaction into which the ion reversibly fits, rather as a substrate fits the active site of an enzyme. The mobility of the ionophore in the liquid membrane allows the ions to permeate under the influence of the prevailing electrochemical gradient. The membrane performance of such systems is usually optimised by the presence of further additives which may serve a number of functions. For example, potassium tetrakis (p-chlorophenyl) borate is frequently added to lower the electrical impedance of neutral carrier membranes and its large lipophilic anion serves to prevent entry of sample anions into the membrane.

There is a huge amount of quite old, 60s and 70s, but well-established literature for potassium, sodium, and calcium sensing separately. Therefore, it is not possible to mention it all, and that is why the author in this thesis has preferred to refer to a few researchers who have used this knowledge for contemporary biomedical applications. Another reason for this approach in this thesis is that one aim is to use in a multi-sense mode the elements mentioned.

1.4.2 Multi-sensing of K^+, Na^+, and Ca^{+2}

A multi-sensor array for pH, potassium, sodium, and calcium measurements based on coated-film electrodes has successfully been realized[1.17]. This sensor-array has only been *tested with binary solutions but not with serum, plasma, artificial or whole human blood.*

ISFETs for measuring more than one ion have also been produced[1.18]. Janata has described a dual FET which may be incorporated into an arterial catheter[1.19], Esashi and Matsuo have developed a very interesting sodium and potassium FET sensor in the form of a needle for basic physiological research[1.20], and Sibbald and co-workers have developed a four-function ISFET[1.21] that may be attached to a blood sampling cannula inserted into a patient's artery or vein, for measuring H^+, Na^+, K^+ and Ca^{2-}.

Recent improvements to the methods of making ISFETs have been reported in which ionophore doping techniques are used[1.22], clearly a good indication of the crucial role of ionophores in making chemical and medical sensors.

Poly(vinyl acetate)-based membrane materials for *thick-film* liquid sensors have also been developed for amperometric and potentiometric multisensor applications. A four-channel potentiometric sensor[1.23] for Li^-, Na^+, K^+, and NH_4^+ has been realized in which three electrodes form one channel. One of them is coated with a membrane layer containing plasticizer and ionophore and another one with a layer of the same membrane mixture but without carrier. A third electrode in the middle between the two coated electrodes serves as a solution contact. The differential voltage between the two membrane-carrying electrodes is measured.

J. Janata *et al* have developed a combination of flow-injection analysis and ion-sensitive FETs for the determination of *pH*, K^+ and Ca^{2+} ions[1.24] with an improved signal-to-noise ratio, quick response, and reduced detector dispersion.

A monolithically integrated SOS/FET multi-biosensor[1.25] has been realized by J. Kimura and his team which has four ISFETs, and can detect urea, glucose and potassium simultaneously. Minute amounts of enzyme or ionophore have been used as sensitive elements for this four-function sensor array.

1.4.3 Chip design

A monolithic single-chip sensor array of individually addressable microelectrodes has been developed and fabricated[1.26] for measuring chemical and biochemical parameters in a modified standard CMOS process. The array can be used for amperometric or potentiometric measurements in order to get a concentration-dependent spatial profile of the detected analyte. In addition to 400 working electrodes and two reference electrodes, the chip contains integrated read-out amplifiers, digital circuits for addressing the individual components and test circuits. The on-chip signal processing allows detection of chemical and biochemical analytes without any interference by the external measurement equipment. High electrode impedances and low power consumption have been achieved by using CMOS technology. Chemical modification of the electrodes is possible by applying an external voltage to the electrodes. In an advantage to non-integrated solutions, the system can be used in a wide field of applications, especially for measurements in narrow passages because of the total chip size of only 14 x 14 mm.

A rapid determination of different analytically relevant parameters is of increasing interest in medical and environmental applications. A simple and easy-to-handle instrument replaces more and more complex laboratory analytical systems. The advantages of such portable instruments with integrated disposable sensors are the flexibility for measuring on the spot, fast response time given by the miniaturized sensors and low costs for procuring and running. This research[1.27] presents a portable compact battery-operated instrument with interchangeable chemical sensors for the direct potentiometric determination of different analytically relevant *inorganic ions*, such as nitrate and *potassium*. The battery operated instrument consists of an 8-bit microprocessor with a 12-kbyte EPROM on chip and a 12-bit sampling A/D converter with internal reference. The data are transferred by an SPI serial interface. The software program is crucial for this application. Before measuring the sample a two-point calibration has to be carried out, the data being stored in the microcomputer. The transducers for the ion-selective planar sensor strips are manufactured using thick-film technology with integrated receptor and reference electrodes on the chip. The reference electrode shows a high long-term stability as disturbing ions have to diffuse through a reference channel filled with a hydrogel in order to reach the recessed miniaturized electrode.

Microelectronic ion sensors based on monolithic silicon integrated circuit (IC) and hybrid circuit technologies have been the subject of considerable research and development over the past three decades[1.28]. The integration of various types of semiconductor devices with particular electrochemically active *thin films*[1.29] makes possible a new generation of integrated-circuit chemical microtransducers which have many important and unique advantages over their conventional antecedents, including small size, robust solid-state nature and the potential for low-cost mass-fabrication; attributes which are particularly appropriate for biomedical usage. The prime semiconductor device for such applications is the MIS transistor, first modified into an ion-sensitive (chemical-sensitive) field-effect transistor (ISFET and

ChemFET) in 1970 by P. Bergveld. A. Sibbald reviews the development of ChemFET devices since their inception, and describes the important aspects of their theory, structure and design with particular emphasis on the practical features of device operation and gives as an illustrative example, *a novel triple-function chemical-sensing IC for the simultaneous measurement of potassium, hydrogen, and calcium in aqueous solutions*[1.30].

1.5 An overview of the thesis

Chapter One is the introductory chapter which gives a definition of the problem, the reason why this thesis has tackled this problem, the method suggested, the literature review concerning the subject under discussion, and an overview of the thesis. **Chapter Two** gives a comprehensive background of intelligent and integrated sensors. **Chapter Three** gives the system side of the intelligent and integrated sensors. **Chapter Four** is a very comprehensive literature survey of the chemistry side of the sensor array under development. **Chapter Five** fully documents the reference electrode which has been prepared, tested, and implemented as the half-cell of the ion-sensors of this thesis. **Chapter Six** presents the test results of this complete sensor array with binary solutions and mixed electrolytes. **Chapter Seven** presents the test results of the sensor array with artificial and whole human blood. **Chapter Eight** is the discussion and suggestions for future work resulting from the thesis.

1.6 Conclusions

This thesis describes the design and implemention of a biomedical sensor array based on potentiometry and neutral charge carriers (ionophores) for sensing of potassium, sodium, and calcium free-ion activity in whole human blood. The literature survey shows that multi-sensing of relevant clinical parameters is very desirable since it is the way forward for clinical practice. Additionally being able to use the smallest possible substrates for clinical analysis is another motivation leading the research. During the experimental course of this research, both solutions (binary or mixed), artificial and whole human blood have been used as the test samples with quantities of $50\ ml$ and $20\ \mu l$, respectively.

Miniaturization of ion sensors and reference electrodes to the extent possible is another strong motivation among researchers and there is quite a strong interest in achieving that goal.

During the 60s and 70s, the reference electrodes used for measurements have always been conventional ones which was not backing the intention to integrate sensor-array systems with electronics. This is one of the main reasons and major motivations behind the strong desire to miniaturize the electrochemical sensor-arrays, especially the reference electrodes.

The first attempt therefore must be to create a new miniaturized reference electrode leading readily to integration with electronic circuit design and strong enough to be used with, for example, all types of body fluids, especially with human whole blood. That is why this thesis presents this problem first [Chapter Five].

The literature of the 80s and 90s mainly looked at the possible miniaturization through the integration of silicon surface- or bulk-micromachining of electrochemical sensor arrays with microelectronic circuit design, principally in the field of semiconductor sensors. Although there has been a big achievement in the circuit design there are still problems with encapsulation of sensor systems and their related characteristics.

Chapter Two

From Basic Sensors to intelligent Sensors

Summary

This chapter briefly discusses the need and the roles of sensors in traditional measurement and control systems. It also considers the breakthrough brought about by intelligent sensors to traditional sensor systems as well as to measurement and control systems; the concept of an integrated sensor and an intelligent sensor; the most important restrictions imposed on smart-sensor electronics due to their nature; the importance of intelligent sensors for a microsystem, an electrochemical microanalytical system and, finally, the intelligent sensor approach of the automotive industry. Some practical examples of different sensor systems are also briefly discussed putting attention primarily on definitions and examples.

2.1 Human senses and sensors

Although human senses are mainly receivers and processors of information from nature and the environment, they have restrictions naturally imposed on them. That is why practical sensors must be an extension of our senses.

An infrared sensor is required to see beyond the human colour spectrum; an ultrasonic sensor to discover sounds higher than that the human ear can cope with, and an intelligent sensor may be used to examine more effectively what components and interrelationship complexity there are, for example, in human blood.

Often sensor devices need to operate in severe conditions, such as in underwater exploration, the inspection of nuclear reactors and biomedical applications. In such cases, the device often produces a poor output, that is data with a low signal-to-noise ratio (SNR)[2.1] which may require amplification, filtering and processing prior to further use and/or interpretation. At this point measurement and control systems assume greater significance.

2.2 Sensors in measurement and control systems

Measurement and control systems have always been important components in any application. Sensors usually provide analogue information on the system being monitored through signal conditioning circuits connected to a µP-based controller by a digital bus. The processor interprets the information, makes appropriate decisions most likely in conjunction with higher level control, and implements those decisions via actuators.

Organization of a prototypical measurement and control system is shown in **Fig.2.1** to which smart sensors bring extraordinary changes[2.2].

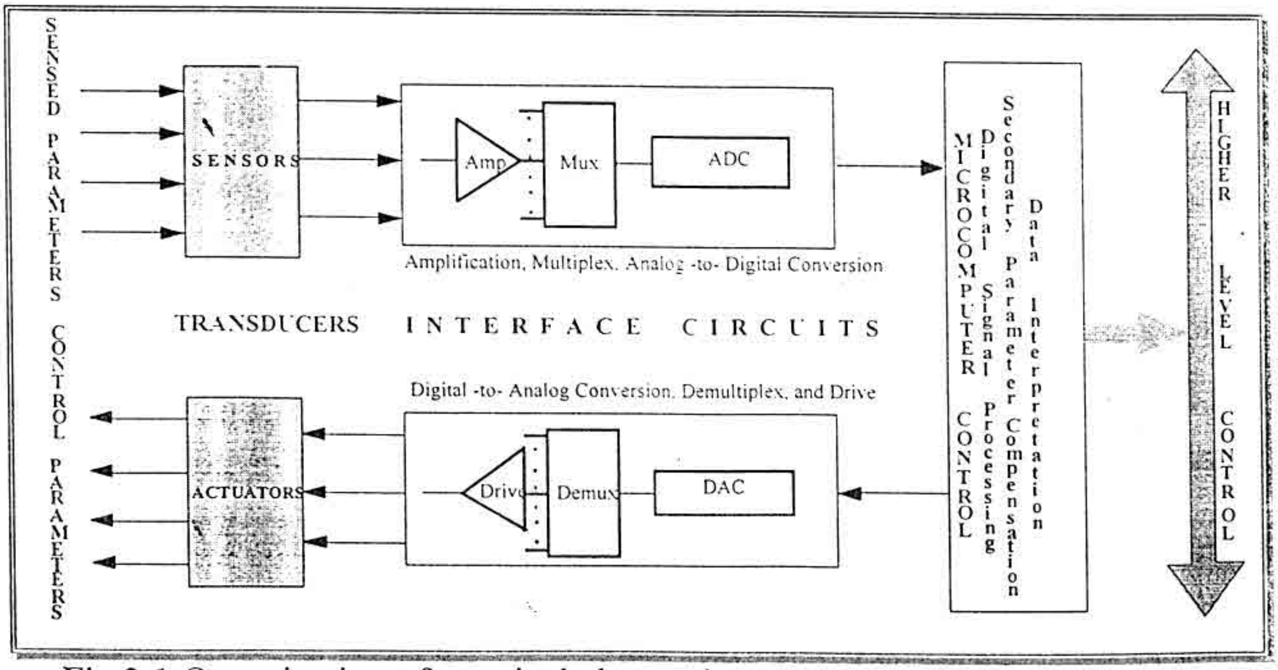


Fig.2.1 Organization of a typical electronic measurement and control system

2.3 Intelligent sensors

Prior to the discussion of the kinds of breakthrough brought about by smart sensors to the traditional sensors as well as to the measurement and control systems, consider current techniques and practice.

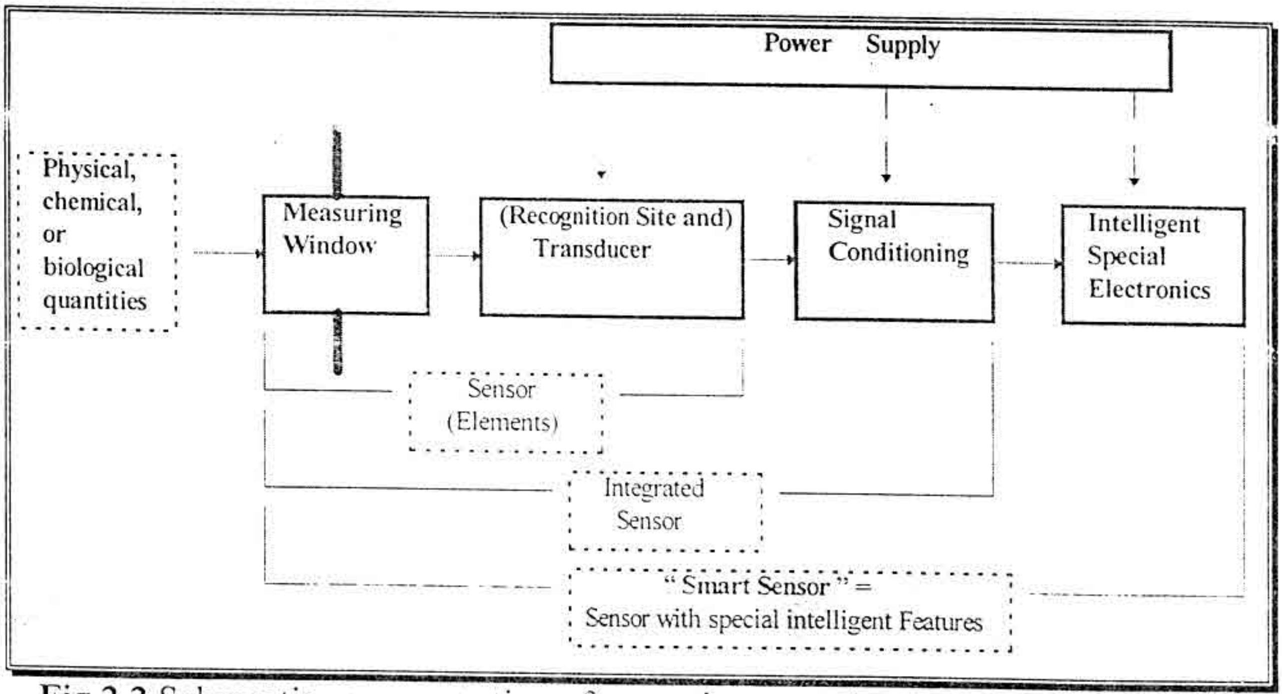


Fig.2.2 Schematic representation of general sensor elements and sensors[2.3]

In the literature, there are several general and many more application specific definitions given for sensors [2.4-2.11] but the core remains unchanged which is clearly the integration of electronics with the sensor or sensor-system.

In the general description of sensors for physical, chemical or biological quantities, usually a distinction is made between sensor elements; sensors **without** special intelligent features ("integrated sensors"), and smart sensors **with** special intelligent features. Smart sensors may include the measuring window, transducer, signal conditioning, intelligent special electronics, and power supply as shown in **Fig.2.2**.

A systematic treatment of sensors requires a five-dimensional coordinate system to be considered. The coordinates describe **the quantities to be monitored,** the detection principle, the physics as well as physical

chemistry background of the sensor, the technology of the sensor manufacturing principle, and the applications.

For this specific research, the five-dimensional coordinate system is as follows:

a) the quantities to be monitored/measured are K^+, Na^+, and Ca^{2+} in whole human blood,

b) the detection principle is a membrane mechanism leading to a potential difference based on zero-current potentiometry,

c) the physics as well as physical chemistry background is the *ISEs* and *ISFETs* theory and practice,

d) the technology of the sensor manufacturing principle is the thin-film based on vapor deposition, and

e) the application is the practice that this research introduces.

The sensor consists of the transducer, which transfers physical, chemical, or biological quantities through the measuring window to the sensor element. The latter transforms non-electrical quantity, p_i , into electrical signals, x_i' , which are conditioned subsequently to x_i. Further processing with intelligent special electronics is done in "smart sensors"[1.12]. For this specific research, the sensing site is the ionophores used (*Valinomycin, ETH 157, and ETH 1001 for potassium, sodium, and calcium respectively*) and the transducing site is the membrane mechanism of the ion-selective electrodes, in other words for this research the sensing site and transducing site are combined together in the nature of the system.

In its simplest term, a resistor in an electrical circuit could be a temperature sensor and an ammeter a transducer. However in terms of instrumentation, a sensor is defined as a measuring device that shows a characteristic of an electrical nature when it is exposed to a phenomenon that is not electric. A sensor recognizes the phenomenon, in a specific way if possible, and translates it into a quantifiable property which is then transformed into an electrical signal by a transducer.

2.4 The Concept of an "intelligent" sensor system for a microsystem

In comparison with conventional systems, in combination with data processing elements the sensor elements for such systems are equipped with local intelligence. Microsystems allow one to realize more complex functions in the same or similar volumes. High-precision sensors are needed as part of intelligent microsystems.

Though the cost of microelectronics has decreased dramatically, it is necessary to keep the costs of hardware and software as low as possible. The correction for the deviation of linearity of a sensor with a resolution of 1.5×10^{-5} needs a table consisting of a memory which has a capacity of 128 kbyte (16 bit address input, 16 bit data output). If a temperature compensation is additionally needed, a temperature sensor and a memory of 2 Mbyte are required. Perhaps these requirements can be reduced by using interpolation algorithms; anyway, the calibration of the system becomes complex and, therefore, expensive. This illustrates that highly linear and thermally insensitive sensors are the basis of intelligent and fast microsystem[2.13].

By means of data processing which may be integrated into a microsystem together with one or more sensor elements, a further improvement of the acquired data may be achieved resulting in an intelligent microsystems.

The analog output signal is adapted to the full input range of the analog-to-digital converter (ADC) by an amplifier, in order to use the full resolution of the ADC. After multiplexing (MUX) and digitizing the analog data into digital values, the microcontroller (μC) is used to process the sensor raw data since smart sensors are distinguished by a microcontroller which handles measured data and transmits them usually by means of a serial interface[2.14]. This is done by a program stored in resident memory (EEPROM). The processed data are put into random access memory (RAM). The serial communication port together with the local intelligence offers the possibility of transferring as much information as needed in the most minimal form possible to the host.

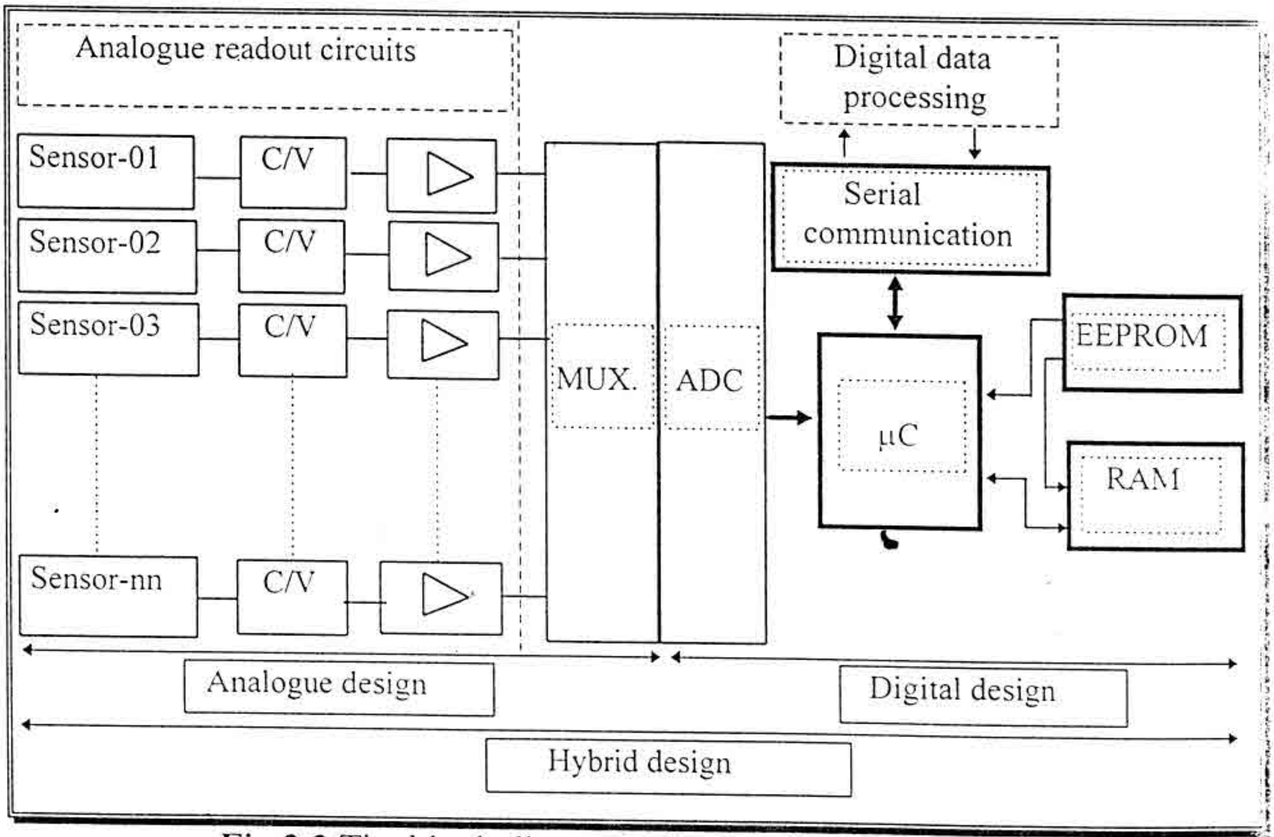


Fig.2.3 The block diagram of an intelligent sensor system

In the first stage of data processing, the sensor raw data may be pre-processed in order to obtain more reliable and precise data than from the sensor alone. In order to obtain information about the sensor validity, different sensor tests are conceivable which are called the plausibility test giving information about the validity of the sensor signals and the sensor self-test.

The accuracy of such a system is improved by the analysis of several sensors in a sensor array. The LIGA process, which is the German acronym for *lithographie*(lithography), *galvanoformung*(electroplating), *and abformung* (moulding), allows one to fabricate sensor arrays with identical sensors and also sensor arrays with graded sensitivities[2.15].

2.5 Integrated sensors

Integrated sensors offer many advantages, including high reliability, small size, low cost, high performance and system compatibility. **The reason behind these advantages is the ability to amplify and pre-process the transducer signals before presenting them through cables to microcomputers or a display unit, for example, a pH meter. They have the ability to use small signals from the transduction element. This property is one of the reasons behind the efforts to integrate electronics on the same substrate as the sensor.** Integrated electronics offers excellent opportunities, such as the elimination of interconnections, the reduction of the overall transduction element and electronics circuit land area. Real life applications require more sensitive and maintenance-free properties which are clearly the characteristics of the integrated sensors. Integrated sensors must offer an improved temperature performance, long-term stability and sensitivity. Below are some examples of different kinds of integrated sensors designed for different signal domains[2.16]:

Signal domain	Sensor
1. Radiant signal domain	1. Strip detector for nuclear particles
2. Mechanical signal domain	2. Position-sensitive detector for light beams
3. Thermal signal domain	3. Silicon thermopile temperature sensor
4. Magnetic signal domain	4. Magnetic field vector sensor
5. Chemical signal domain	5. Polymer-based capacitive gas sensor

2.6 Smart sensors electronics

Smart sensors are becoming integral parts of systems performing functions that previously could not be performed or were not economically practicable. **The natural extension of integrating electronics is to convert the simple sensor into a smart sensor.** However, designing integrated electronics for use on a smart sensor chip is often multi-faceted, compared to other applications. The design is influenced by unusual demands, the special smart-sensor environment, and intelligent-sensor-specific restrictions. The most important restrictions imposed on smart sensor electronics are[2.17]:

1. **The output signals of the smart sensor are usually very small,** so electronic circuits should often be able to handle signals in the microvolt range to obtain tangible sensitivity.

2. **The output signals of the smart sensor are often slowly varying,** so circuitry where accuracy and resolution can be traded off against speed is very suitable.

3. **The smart sensor often consumes considerable chip space** which restricts the chip space available for the electronic circuits.

4. **Special process steps that are needed for the smart sensor** can aggravate the specification of normal integrated components and can even restrict the availability of some types of components.

5. **The conversion of smart sensor signals into a digital form means the presence of digital signals on the same chip.** Digital interference on the sensor analogue electronics may cause large errors and vice versa.

It has easily been predicted that the most difficult task in discussing smart sensors is to obtain a definition since there exits no standardization regarding intelligent sensors. There are many different as well as common definitions of intelligent sensors. Four of them are dominant: the first definition is that **any sensor with integrated electronics** is smart. The second definition is that **only those sensors with integrated microprocessors** are smart. The third definition is that **those sensors that include some logic functions and/or make some type of decisions** are smart. The fourth definition is that a sensor with an off-chip μP used to communicate to the user and modify its response is considered to be smart.

From the smart sensor literature it may be seen that **a smart sensor must do one of the following**[2.18]: (i) perform a logic function, (ii) perform two-way communication, or (iii) make a decision. In addition to these three functions, smart sensors enhance the following applications as well: (i) self-calibration, (ii) computation, (iii) communication, and/or (iv) multisensing.

Offset and gain are the two parameters of sensors mostly adjusted during fabrication. These adjustments usually change in time for various reasons, requiring the device to be recalibrated. Smart sensors are a solution to such cases where sensors are very expensive to service. The key to this problem is a built-in μP that has the correction functions in its memory. Smart sensors can compensate for the changes that have occurred in time. These sensors also include the capability to take factory calibration data stored in memory and to calculate the appropriate compensation for the existing temperature or, in the case of differential sensors, for static pressure. Diagnosis is a sub-category of self calibration. This is the inherent ability of a smart sensor to determine if it operates properly. With system complexity steadily increasing, it is crucial that malfunctioning components be identified as quickly as possible.

Computational ability which needs computational statistics to be applied to the sensing-element signals is another very important characteristic of smart sensors. Here are two examples of computationally smart sensors[2.18]:

1. A pH sensor consists of ten sensing elements on a single silicon chip

and allows the average, variance and standard deviation for a set of measurements to be obtained.

2. An Olfactory sensor utilizes the varying sensitivities of different sensing elements to a group of measurands. The sensor is composed of a set of thick film sensing elements and electronics, including a microcomputer. The μC identifies the scent on the basis of similarities calculated by comparing standard patterns stored in memory and a sample pattern developed by the sensing elements.

A smart sensor must have the ability to communicate with its environment. In industrial electronics there is an increasing demand for sensors capable of communicating with a microcontroller. There is great benefit in solid state sensors involving the capability of communication with a microcomputer[2.19].

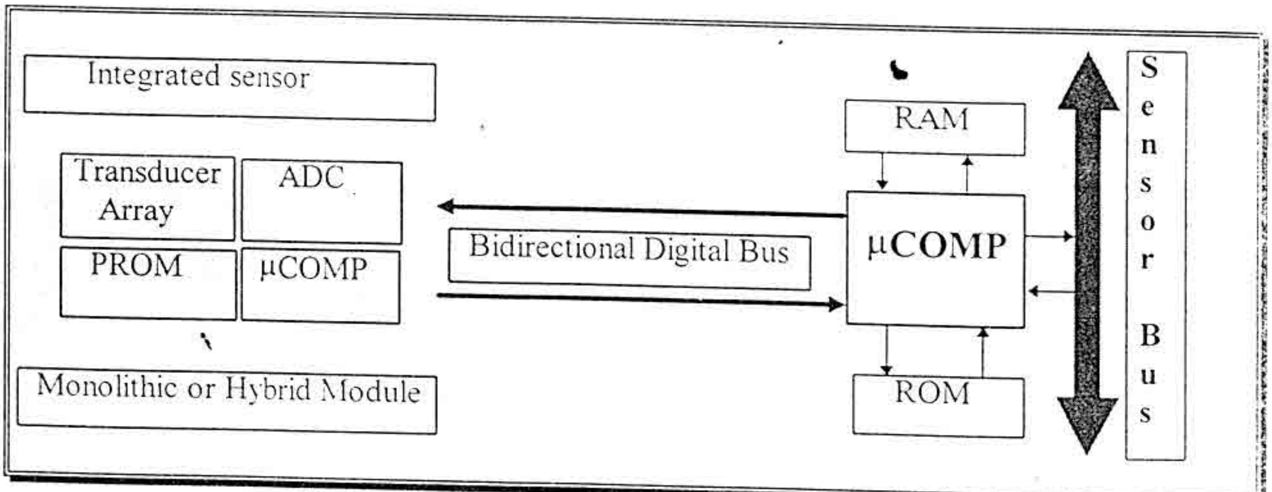


Fig.2.4 Sixth generation solid-state sensors

This will inevitably create a breakthrough in traditional and even modern measurement and control systems. Installing a memory element on an intelligent sensor will undoubtedly help further improvements in measurement and control systems. This looks to be the core intent of intelligent sensor researchers and designers at the present time.

A smart sensor is expected, depending on the application, of course, **to have the ability of measuring more than one physical or chemical variable simultaneously**[2.18]. An example of such a smart sensor is a chip which contains conventional MOS devices for signal conditioning together with the following on-chip sensors: a gas flow sensor, an infrared sensing array, a chemical reaction array, cantilever-beam accelerometers, surface-acoustic-wave vapour sensors, a tactile sensor array and an infrared charge-coupled device imager.

Table-2.1 Comparison of two integrated smart sensors

	LAPP	PASIC
1. Technology	3 μm CMOS	1.2 μm CMOS
2. Number of sensors	128 x 1	256 x 256
3. Pixel size	50 x 50 μm^2	32 x 32 μm^2
4. Number of A/D	128	256
5. A/D resolution	1 bit	8 bits
6. Number of Pad	128	256
7. ALU type	binary logic	bit-serial ALU
8. On-chip RAM	128 x 14	256 x 128
9. Chip size	5 x 7 mm^2	10 x 11.2 mm^2

A reasonably new and intelligent smart sensor is a single-chip based image sensor with a digital processor array. Indeed, this chip contains two intelligent sensors, one is LAPP(Linear Array Picture Processor) and the other is PASIC(A Processor-A/D Converter-Sensor Integrated Circuit). The basic idea behind the two designs is to integrate an image sensor array with a digital processor array in a single chip. The integrated camera-and-processor eliminates the bottleneck of the sequential image read-out that characterizes conventional systems. They provide fast, compact and economic solutions for tasks such as industrial inspection, optical character recognition and robot vision. The comparison of two integrated smart sensors[2.20] is given in **Table-2.1**.

2.7 Basic functions of smart sensors

The four basic functions of smart sensors are measurement, configuration, verification, and communication. Each function may have more than one sub-function as well.

Although all four functions are very important and they individually play a crucial role in design of intelligent sensors, **the functions which largely contribute to the smartness of the intelligent sensor are verification and measurement functions**. The verification function offers services, such as continuous supervision of the intelligent sensor's behaviour using a set of supervisory equipment implemented in the intelligent sensor. Results of continuous supervision can be stored in a database systematically updated (FIFO structure) and available for maintenance purposes. It offers the necessary services for diagnosis, allowing the user to locate faults whenever they are detected.

The communication function allows bi-directional communication between the intelligent sensor and other equipment to which it is connected by a field bus, for example, by a control area network (CAN).

In a broad sense, smart sensors have mainly four basic functions which could diagrammatically be pictured out as shown in **Fig.2.5**[2.21].

Briefly, intelligent sensors can be grouped into (a) sensors with processing circuits and active devices to improve signal-to-noise ratio; (b) sensors with electronic circuits for signal processing and decision making to convert signals to various forms ready for further manipulation[2.22].

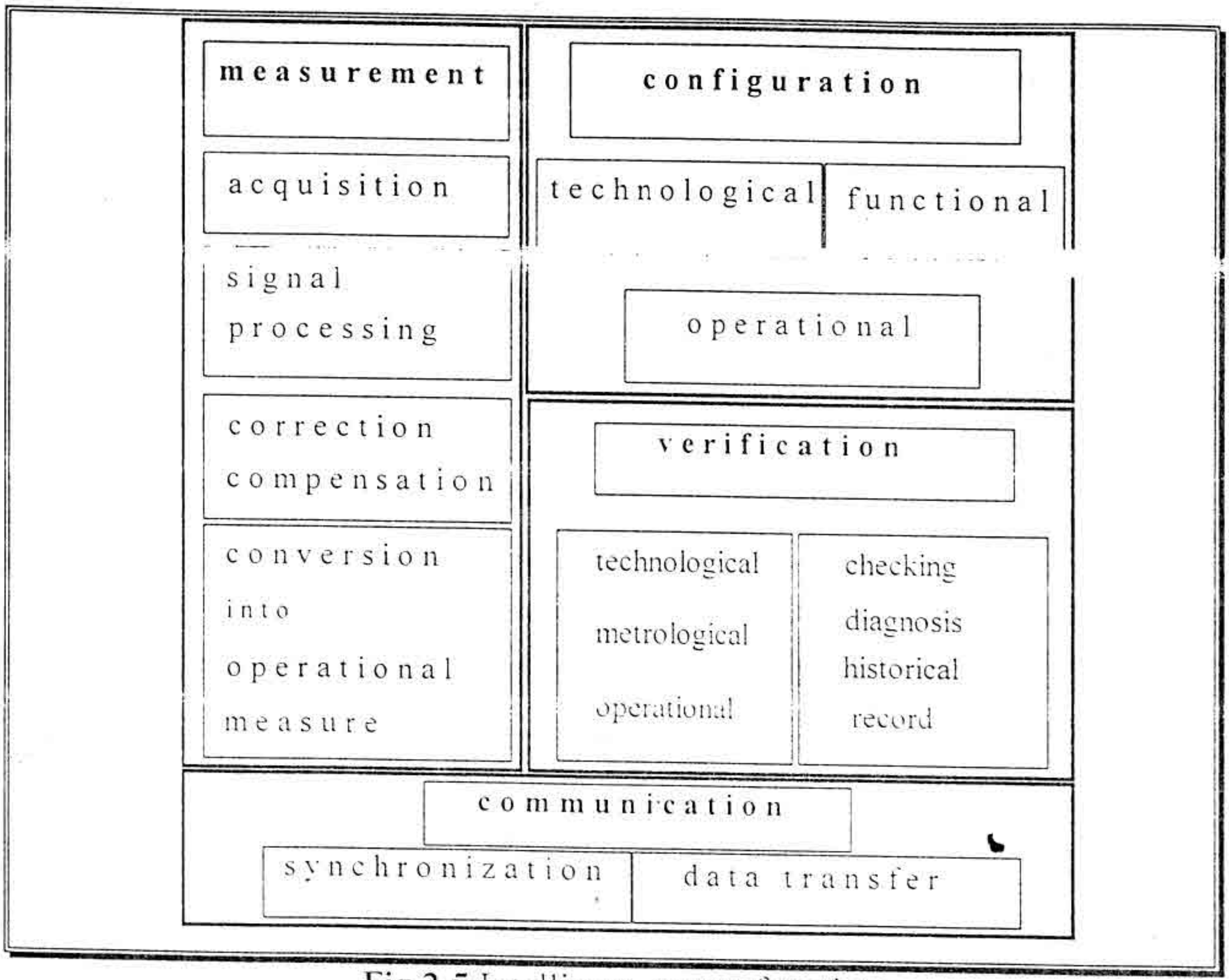


Fig.2.5 Intelligent sensor functions

2.8 Electrochemical microanalytical system (ELMAS)

The continuously rising demand for more chemical information in a broad analytical field from chemical process control to environmental protection, medical diagnostics, and health care has resulted in the development of numerous chemical microsensor devices[2.23]. In many cases these sensors can be manufactured with small geometric dimensions relative to widespread conventional macrosensors, and at low cost because of the use of mass production technologies which are available from the microelectronics industry.

However, in spite of the evident and very promising advantages, chemical microsensors are not dominant on the market and they have had no practical significance in widely used analytical techniques so far. Problems concerning the reliability and long-term stability and, in some cases, bad selectivity of the sensor signals are the most serious drawbacks.

Therefore, attempts have been started in some laboratories for integrating sensors, actuators, and microelectronic components onto complete miniaturized chemical analytical systems based on microsystem technologies. The basic idea involves self-testing and self-calibration of the system by recording signals alternatively from sample solutions and from calibration solutions, in a similar way as is done in the flow injection analysis technique. Drift phenomena of the sensors can be effectively compensated and the reliability can be improved by using feedback mechanisms.

Conditioning of the sample to take advantage of the optimum sensor performance and cleaning and preconditioning of the sensor itself are further challenges which can be met by sensor/actuator systems. The creation of so-called "total micro-analytical systems" is envisaged as the final goal of all these development efforts. It means that the essential functions of modern automated laboratory analyzers have to be scaled down to small-sized devices.

In such systems, electrochemical sensors are favoured. They convert directly the chemical signal (kind and concentration of electrochemically active species, eg, ions in the analyte) into an electronic signal[2.13, 2.24]. In the case of ion-selective electrodes (ISEs) and ion-sensitive field effect transistors (ISFETs), **Fig.2.6**, an electrochemical potential established at the solution/sensor interface is controlled by the ion concentration of the solution. A thin ion-sensitive membrane (ISM) which is placed at this interface has a determining influence on the basic characteristics of these potentiometric sensors. Any distortion of this sensitive interface will disturb the sensor function, thus demanding self-control and self-calibration.

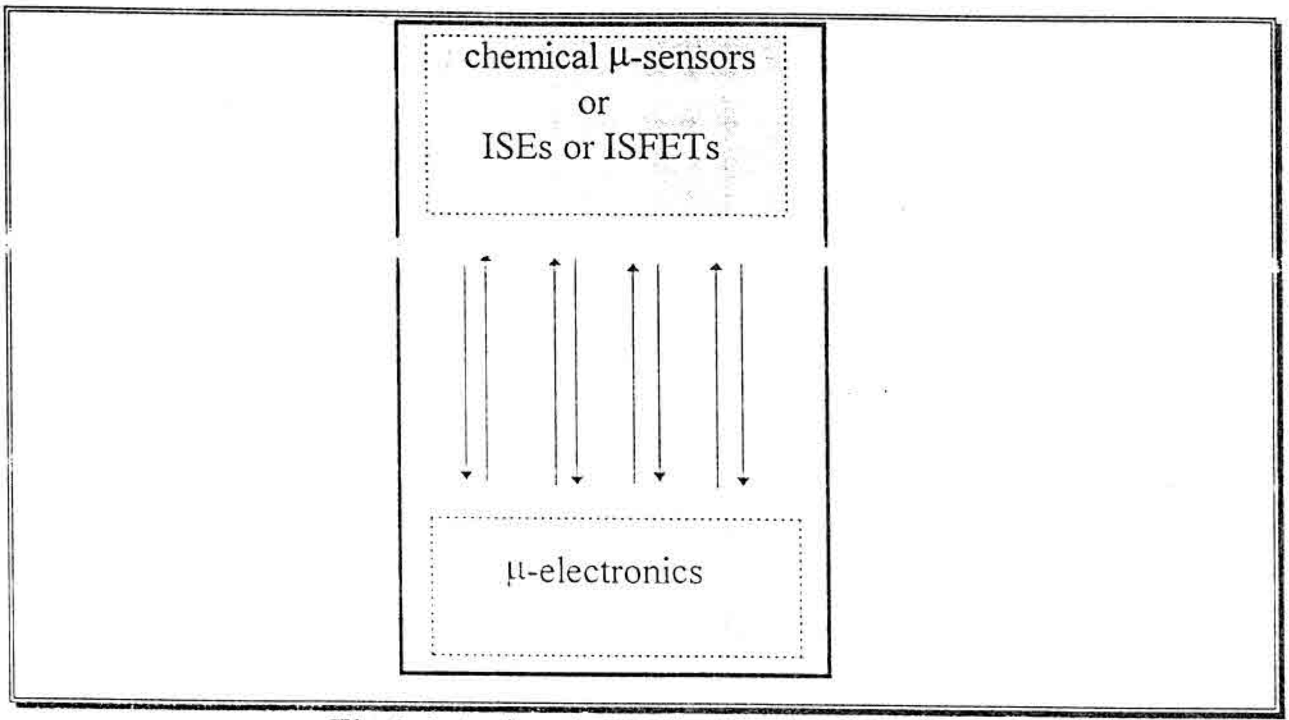


Fig.2.6 A chemical microsensor array

2.9 Integrated, smart and intelligent sensors

In order to define more carefully the different types of intelligent sensors, it is necessary to subdivide the processor into a signal preprocessor (converter) and the main processing unit, e.g. a microprocessor. The signal preprocessor or converter carries out low level tasks such as amplification, filtering, or analogue-to-digital conversion. Here are the three levels of integration which make up sensor systems.

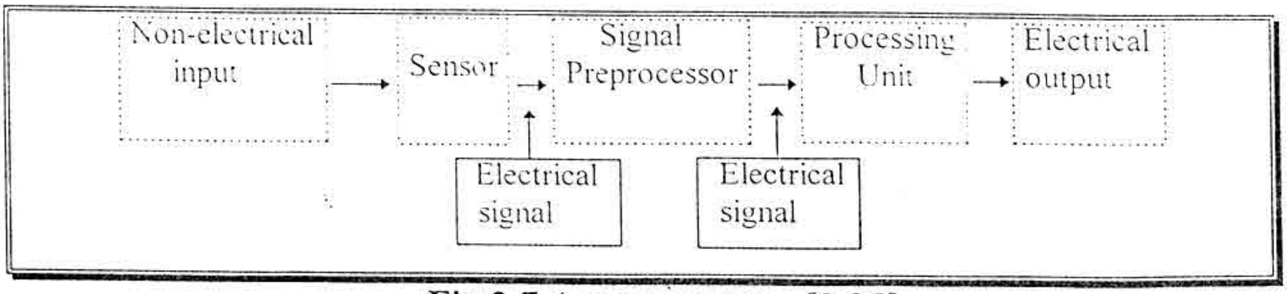


Fig.2.7 A sensor system[2.25]

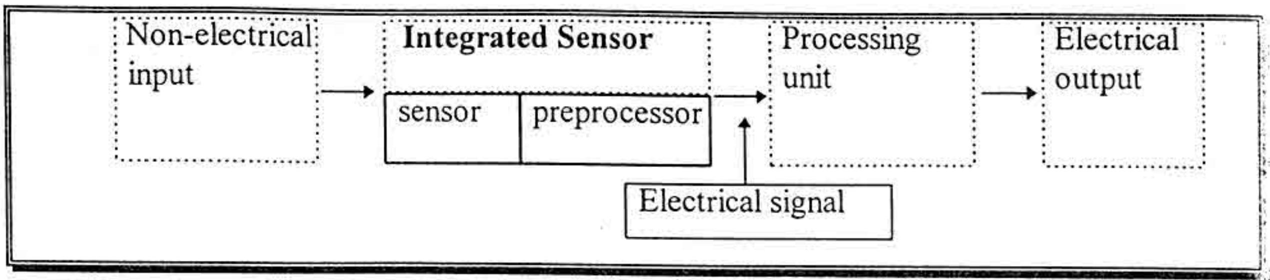


Fig.2.8 An integrated sensor in a sensor system[2.25]

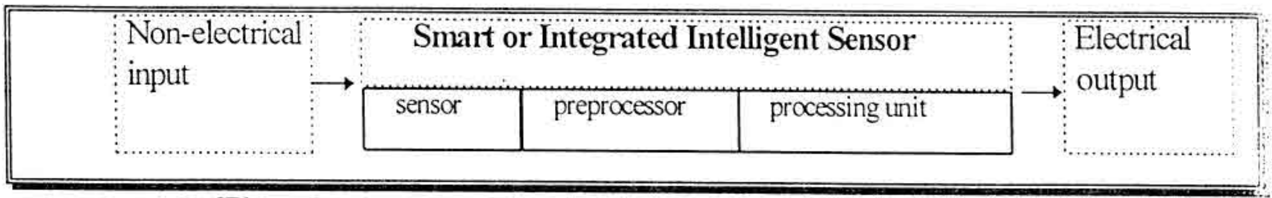


Fig.2.9 A smart sensor or integrated intelligent sensor[2.25]

There are different views at present over the precise definition of a smart sensor. One proposes that a device onto which at least one sensing element and signal processing has been integrated is a smart sensor. Actually such a sensor should be called an *integrated* sensor since it has a low level of intelligence due to integrated preprocessor. The term *smart* is reserved to denote the integration, in part or full, of the main processing unit which adds intelligence.

There is little confusion in this practical definition because all smart sensors must be integrated and intelligent, while any sensor that has significant intelligence which has not been integrated can be called an intelligent sensor. The only definition remaining is that of an intelligent sensor. The definition proposed by Breckenbridge and Husson[2.26] takes some account of work in artificial intelligence and runs as follows:

"*The sensor itself has a data processing function and automatic calibration/automatic compensation function, in which the sensor detects and eliminates abnormal values or exceptional values. It incorporates an algorithm, which is capable of being altered, and has a certain degree of memory function. Further desirable characteristics are that the sensor is coupled to other sensors, adapts to changes in environmental conditions, and has a discrimination function*".

This definition is rather long but does incorporate the essential functions required to define intelligence in a sensor, especially for space-oriented research and applications.

The integration of the processing unit with the sensor to make a smart sensor then requires a significant electronic design effort, perhaps through the use of Application Specific Integrated Circuit (ASIC) technology[2.27].

The ability of a sensor to communicate, i.e. exchange information with another device, is particularly useful. An intelligent sensor may be able to communicate with its operator and so provide valuable information about problems, etc. Alternatively, an intelligent sensor may communicate with another device and so modify its own behaviour. This type of intelligence can, in its simplest form, provide a warning of abnormal operating conditions, or more cleverly provide a feedback control mechanism. An intelligent sensor may have some form of high level adaptive control strategy which permits the control parameters to be automatically updated with time. The implementation of a sensor which can warn its user, or adapt to environmental conditions, requires some decision-making capability. Traditionally, sensors use parametric data to make a decision. However, the more intelligent sensors of the future may use non-parametric methods such as artificial neural networks or a so-called expert system which relies upon fuzzy data.

The definition of an intelligent sensor used here includes any sensor system that contains a discrete microprocessor unit.

2.10 Smart sensors approach by automotive industry

The most basic form of sensor is sensitive not only to the intended variable but also to other perturbing effects, of which the most significant is usually temperature. Thus its output signal will be a function of several variables.

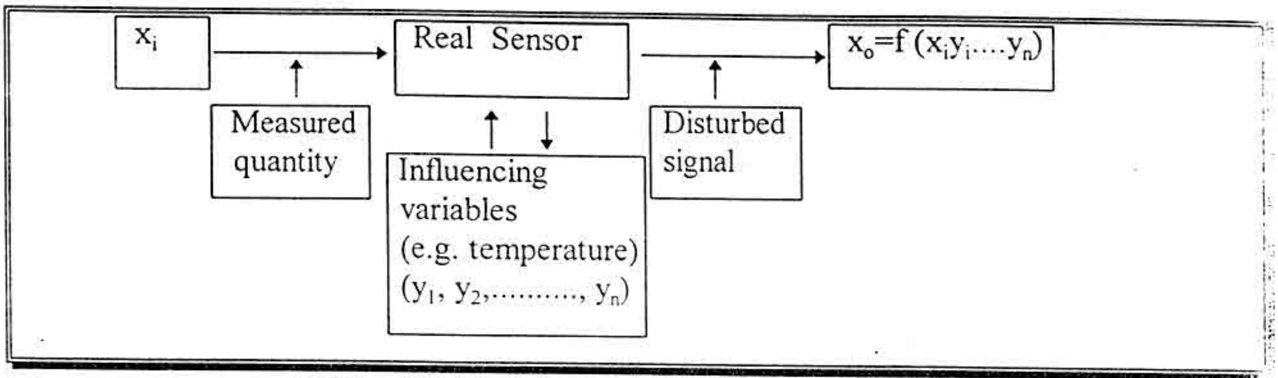


Fig.2.10 Conventional sensor model[2.28]

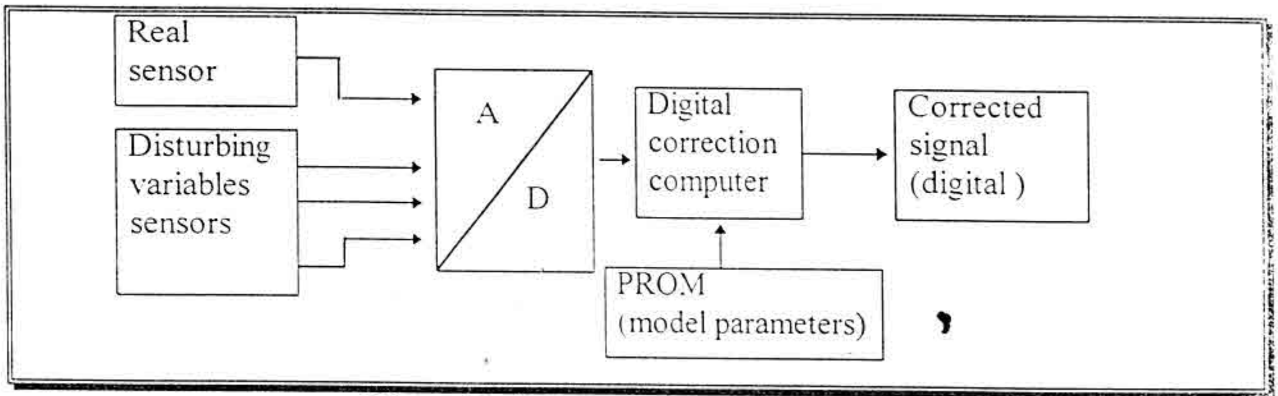


Fig.2.11 Smart sensor model[2.28]

In an environment such as that of a road vehicle it is not practical to maintain these perturbing variables constant, as the temperature can vary over a range of up to 200°C, so some form of compensation must be provided. In cases where the function relating the output to the perturbing variable are simple this can be done by analogue means. For instance, a temperature sensing element can be incorporated in the sensor package and used to apply a measure of correction. In the case where there are a number of perturbing variables, and if the transfer functions for these variables are nonlinear, digital techniques must be applied if a "true" signal is to be obtained from the device.

The concept of a fully "Smart" sensor for the automotive industry is shown in **Fig.2.11**. The signal to be measured is obtained by a sensor whose output, togerher with outputs from sensors for the various perturbing variables is passed via an analogue to digital converter to a correction computer. This is programmed with a set of parameters for the individual sensor, which describe the transfer functions for each variable, obtained during initial calibration. In this way a "true" digital signal is obtained at the output. As an application of this sensor concept, a smart sensor for methanol concentration in fuel which uses a microprocessor to correct for temperature variations in a capacitive sensor has been realized[2.28].

Smart sensors are the heart of system integration as well in the automotive industry, for example. since the increasing complexity of vehicle systems and subsystems. it becomes apparent that the output of a sensor may be required by several subsystems. It thus becomes attractive to integrate the subsystems together in a "bus" architecture. and sensors having digital outputs can communicate directly with the bus and avoid sensor duplication[2.28].

2.11 Conclusion

There is no standard definition of intelligent sensors. Different research groups define it differently depending on their own experiences and research activities. Even then various concepts are used, for example, intelligent sensors, smart sensors or sometimes integrated sensors.

Different definitions have been made on different bases[2.24, 2.29-2.30]. here are some examples, self-validating sensor systems, home automation systems, μP-based systems, μC-based systems, intelligent materials or chemical based systems, multi-sensing systems, built-in-self-test systems, decision-making systems, logical decision-making systems (one or zero), selfcalibrating systems, compensating systems, VLSI based designs, silicon micromachining based systems, and fault-detecting systems.

The biggest difficulty is to be able to find a way of putting sensor arrays or even a single sensor into an intelligent form. Some systems just do not need to be in an intelligent form due to their nature whereas a few clinical applications need to be in an intelligent form, and the automotive industry has a vast area of practice requiring intelligent sensors and sensor systems.

Many scientists and engineers who are confronted with real life sensor problems agree that a better solution will only be found when an intelligent sensor can be produced. An intelligent sensor should be designed in such a way that its application should only be as difficult or easy to use as a usual microelectronic component. The intelligent sensor should have a standard output and the **ability to compensate unwanted cross-sensitivities**. In addition, **offset, drift and non-linearity should be minimized**. Some kind of **self-testing or calibration** should periodically be performed so that the system will be cheaply maintained. The way to realize this concept is to combine a sensor device with a number of microelectronic components into a single sensor package often referred to as an intelligent sensor. The smart sensors must be 'care-free', a prime requirement. If a smart sensor is fabricated with, for example, the help of glass fibre technology, it is clear that a hybrid package containing the glass fibre device and the electronic circuits is required. Although we are, in general, still far from having a complete family of smart sensors for biomedical applications, yet it makes sense to tread the road of smart sensors as far as possible, *since partial solutions can already bring important improvements*[2.30].

So, it is quite appropriate for this specific application to have a multi-sensing sensor-array based on ISEs integrated with traditional electronics or microelectronics leading to a portable bed side battery operated diagnostic tool since electrochemical sensors convert directly the chemical signal (kind and concentration of electrochemically active species, e.g., ions in the analyte) into an electrical signal[2.20]. This property is great help in designing integrated sensors for medical applications.

In actual fact, clinical research in general does not need intelligent sensors. Any sensor able to measure any clinical parameter in a better way is preferable to more sophisticated but less reliable sensor systems.

To save resources, either money, time or any other, in clinical analysis it is true that multi-sensing systems are the way forward and this is a major reason behind the intention of this research.

Chapter Three

Intelligent Sensors and/or Integrated Sensors -Structure and System-

Summary

This chapter discusses a modern measurement and control system, the hardware and software structure of an intelligent sensor, an intelligent transducer, the differences and similarities between intelligent sensors and intelligent transducers, functions that could be carried out by intelligent transducers, how an ASIC system becomes an intelligent sensor, what is the relationship between VLSI design and intelligent sensors, and finally gives very brief information about other methods used to manufacture intelligent sensors other than μC-based sensors.

3.1 A modern measurement and control system

A modern measurement and control system[3.1] could be set up as in **Fig.3.1**. In such a system, a great number of sensors collect analogue information *in situ* about the biological, chemical or physical processes in the system being monitored. Although sensors are employed in many different kinds of applications with different degree of requirements, the fact is the more complex the system being monitored becomes the more powerful, comprehensive or intelligent the sensors are required to be.

In the digital sensor bus, a central computer is connected to a number of input and output devices. It picks up information from different sensor systems via a digital bus and either does some signal processing before sending them out or directly sends them to actuators having done some basic conversion operations, such as D/A conversion and amplification.

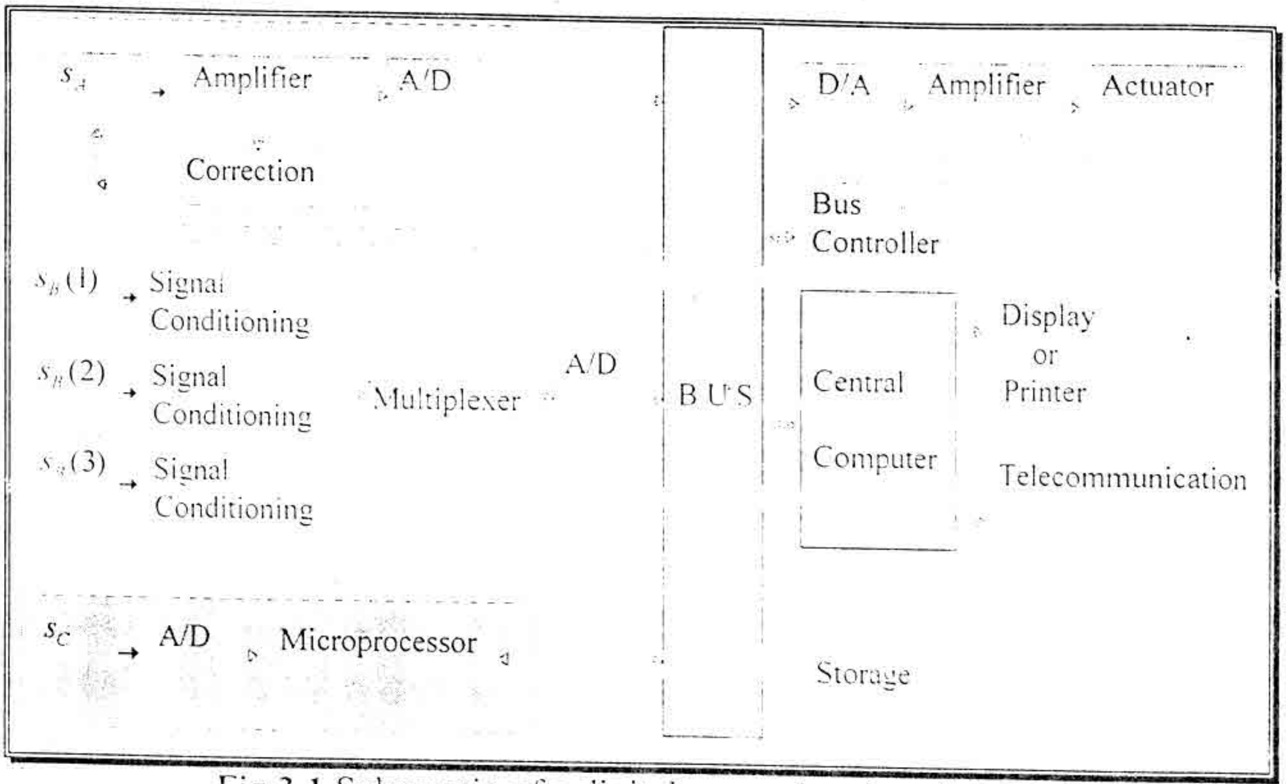


Fig.3.1 Schematic of a digital sensor bus interface structure

In Fig.3.1. the output signal of sensor S_A is first amplified and corrected for offset. non-linearity, and other types of sensor errors before it is transfered to the bus system. In the second group of sensors $S_B(1)$, $S_B(2)$, and $S_B(3)$, the signals are preferably treated in separate signal-conditioning circuits for offset. non-linearity. temperature sensitivity, and so on. Then, a single multiplexing circuit feeds these signals one after the other into a single A/D converter and from there the signals are sent to the digital bus. This second approach is the preferable solution because several sensors are positioned fairly closely to form one unit only.

In the last example of the sensor system, the sensor S_C is connected by an A/D converter to a microprocessor. The microprocessor has stored the sensor's specific data in its memory, ROM, and based on this and the sensor signal, the microprocessor transfers a corrected signal to the bus. An interesting property of this sub-system is that the microprocessor also permits the central computer to send data back to the sensor, which can be used to change the measurement range, to effect a recalibration or to adjust the offset. In such a sub-system, each sensor or group of sensors also contains a circuit that can recognise addresses and this means the circuit can detect when communication between sensor and central computer is desired. Today, most of the described components of a bus system are still separate and have their own housing. However, in the future the integration of these components on a single chip will certainly be pursued. This is indicated by the dashed lines in **Fig.3.1**.

3.2 Intelligent sensors /intelligent transducers

In existing sensor systems, intelligence is still centralised but the more the systems become decentralised the more the intelligence will be distributed and hence the more the systems will be efficient and effective. This will need what is called an intelligent sensor localizing intelligence. A modern microprocessor-based intelligent sensor[3.1] could be composed of (i) a sensing element, (ii) a signal-processing part, and (iii) a μP (microprocessor) as shown in **Fig.3.2**.

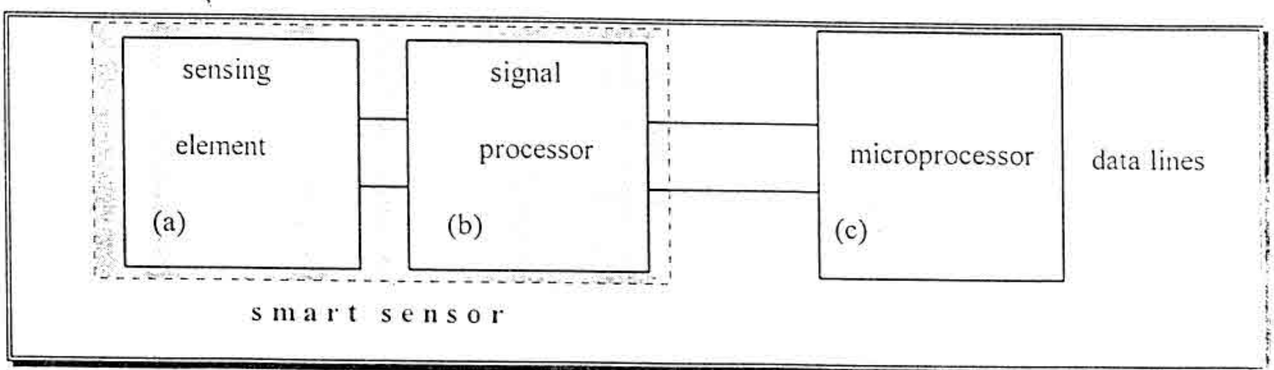


Fig.3.2 Schematic diagram of a intelligent sensor with microprocessor

If we combine a sensing element with a signal processor on a single chip then what we get is an intelligent sensor by some definitions. In the near future it will be possible to put an intelligent sensor on a μP, but a lot of local signal conditioning can be taken over via a μP used in a system.

3.3 Intelligent transducers

Intelligent transducers are a natural result of developments that have occurred in LSI and VLSI technology. Transducers can be integrated with signal-processing circuits on the same chip to perform active functions. These are defined as intelligent transducers[3.2], in a broad sense, and this is shown in **Fig.3.3**.

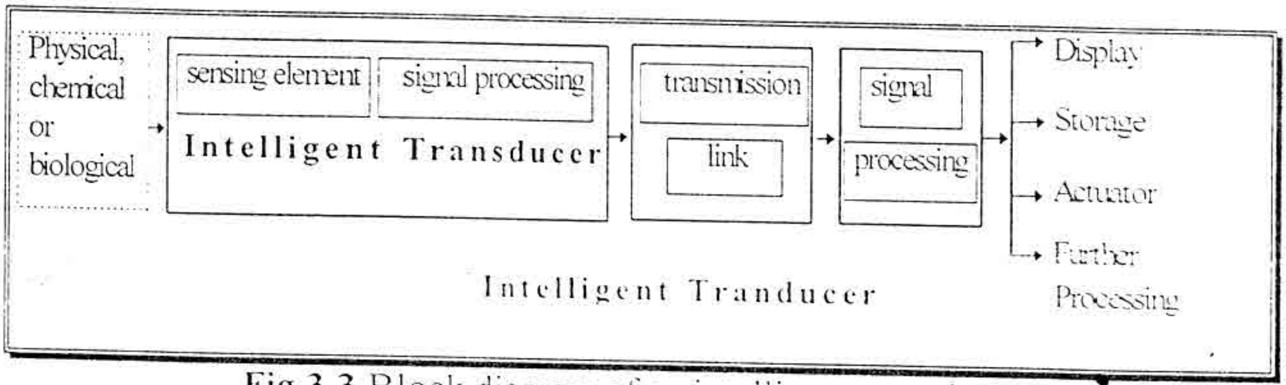


Fig.3.3 Block diagram of an intelligent transducer

Actually an intelligent transducer is nothing other than an intelligent sensor as it can easily be seen in **Fig.3.2** and **Fig.3.3**. The some functions performed via intelligent transducers[3.2] could be tabulated as in **Table-3.1**.

Signal improvement, for example, includes impedance transformation and amplification. Examples of signal improvement with impedance transformation and signal amplification have been reported. One example is the capacitive pressure transducer where the oscillator and a low output impedance amplifier are incorporated with the capacitive pressure transducer[3.2]. **Pre-processing of signals**, for example, includes compensation for temperature and power supply variations, offset balance. filtering of unwanted signals and some kind of non-linearity corrections. **Signal processing**, for example, includes coding and modulation of transducer output signals to get a better signal-to-noise ratio, signal averaging and redundancy, and self-checking and fault detection systems to detect any malfunctioning of the transducers. A second example is the ten pH sensors with an on-chip multiplexing circuit to achieve the redundancy functions[3.2]. Additional circuitry can be added that will provide a single output with an averaged pH value from ten sensors. The ten sensors' output pH value will be more accurate and reliable than that from a single sensor.

Table-3.1 **Functions of intelligent transducers**

Signal improvement	Pre-processing of signals	Signal processing	Logic and decision-making functions
z-transformation	temperature & supply compensation	coding	correlation
amplification	offset balance	redundancy	trend
	filtering	self-checking	multivariable calculation
	non-linearity correction	fault detection alarm	signal recognition or rejection

A very important function of intelligent transducers is **logic and decision-making functions**. These functions, for example, include functions such as the trend of the signal and correlation between several transducer variables, multiple variable calculations which need to measure n quantities through m transducers such that each of them will be a function of X_n variables, but through calculation, the output will be a set of accurate X_n

values, or the desired final output quantity, Z_1, Z_2 ,................, Z_p . The last logic and decision-making function is signal recognition and rejection function which compares the signals with the features stored in the memory of the system to perform recognition and rejection functions.

Different means of power reduction might be used for low signal bandwidth transducers to reduce the power consumption, for instance, time-sharing between different sensors, or operating sensors at low duty cycles. Intelligent transducers have the ability to integrate different system parameters and decide what to do. The status of any system could be controlled and determined by instrumentation or control systems by measuring several parameters of the system being monitored. The parameters measured may be directly or indirectly related to the status of the system, or the desired output, in a deterministic or statistical manner. An intelligent transducer could be designed to translate some quantities into manageable information that could be displayed or used as an input to control some other systems with complex processing circuitry and complicated intelligence[3.2]. For example, pressures, temperatures, engine speeds and exhaust gases are measured to determine the combustion status in order to control an automobile engine's performance. The current trend is that future transducers will not only report the values of pressure, temperature, gas, etc., but, with built-in intelligence, will also produce an output indicating how complete the combustion is or what needs to be done to improve the efficiency of the engine.

Briefly, the most significant features of intelligent transducers are: (i) improved signal quality from sensors, with wider margin for noise and interference; (ii) improved reliability and accuracy; (iii) compatibility with computer and other system blocks, and (iv) reduction in size and cost of large quantity lots[3.2].

3.4 Elements of an intelligent sensor system

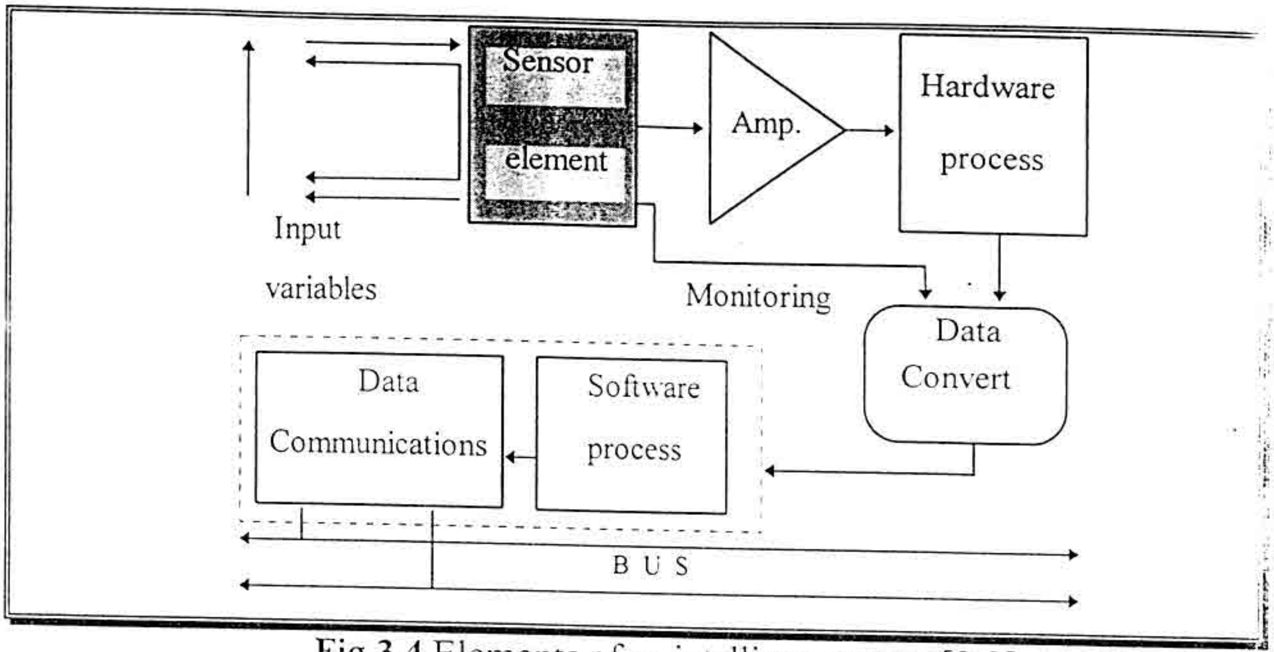


Fig.3.4 Elements of an intelligent sensor[3.3]

An intelligent sensor is a very complex system which needs a range of disciplines be applied all together in the design, implementation and realisation phases. In addition an intelligent sensor combines a number of both analogue and digital sub-systems which make the job more difficult. Sensors largely operate on signals that need very complicated digital and analogue signal processing. The main sub-systems within the structure of an intelligent sensor are[3.3]:

1. A principal sensing element.
2. Excitation control,
3. Amplification (possibly variable gain),
4. Analogue filtering,
5. Data conversion,
6. Compensation,
7. Digital information processing,
8. Digital communication processing.

The principal sensing element is the heart of any intelligent sensor design and implementation. Unless it works properly, no benefit could be obtained from intelligent sensor design. For this research the sensing elements are ion-sensors based on different ionophores whereas the sensing mechanisms are based on membrane mechanisms. **Excitation control** may take different forms depending on the application. **Amplification** of the output of the principal sensing element is nearly perpetually a fundamental requirement. **Analogue filtering** is a fundamental requirement at least to block aliasing effects in the conversion stage. It is also becoming an attractive requirement where and when digital filtering is most likely going to take up too much of the real-time processing power available. **Data conversion** is a conversion stage of analogue signals into discrete signals where a digital processor becomes a must. At this stage it is important to bear in mind that the process of A/D conversion is an approximation and represents a potential gross distortion of the incoming information. This stage may need very important software which will control the conversion process as a part of a control loop or accomplish some sort of auto-ranging for the intelligent system under investigation. **Compensation** is central to the philosophy and a main reason for the existence of the intelligent sensor: also its needs may affect the basic design of the system. An intelligent sensor may have the ability to compensate for any parameter of the system being monitored which is not steady in the acceptable range. **Information processing** is, without doubt, unique to the intelligent form of sensors. Although there might be some overlap between compensation and information processing, but there are some areas where they become fairly independent of each other. An intelligent sensor system needs compressed information and data to check the integrity of incoming and outgoing information to make sure that they are physically reasonable. Information processing stages may destroy information instead of creating it or even introduce false information to the system. The final, but extremely important, element of an intelligent sensor is **communication processing**. Often it requires a processor of its own although it could be realised as part of the main processor chip. A multi-drop bus system is accepted as the natural form of communication for the intelligent sensor processor. Addressability and integrity of information are another two important attributes of the intelligent systems. As a last point, such an intelligent sensor system is expected to be capable of[3.4]: (i) converting analogue sensor data to digital values, (ii) correcting the value for non-linearity and temperature drifts, (iii) sending the corrected values over a IIC (Inter-Integrated Circuit) serial bus when so requested, (iv) self-calibration and, (v) for economic reasons, it is highly desirable for the chip size of the complete unit to be as small as possible.

3.5 VLSI and intelligent sensors

The thrust of VLSI design has been predominantly in the digital area. It is only in recent times that VLSI techniques are being applied to analogue circuits for intelligent sensors. It is well known that most instrumentation and control systems employ a digital microprocessor. despite the fact that sensors are usually analogue. Thus, analogue interfacing systems are required. consisting of gain stages, multiplexers, analogue to digital converters and a communication system connecting sensors to the processor. It would be more convenient if the sensor outputs were digital and communication via a serial bus, such as the Intel/Philips IIC system[3.4]. The form of such a sensor is shown in **Fig.3.5** (a) and consists of a single chip containing the sensor proper, a conversion block, correction/precondition stage and finally a communications block. Most of the communication and signal processing is readily undertaken by the simple microprocessor. Since the speed of operation is not critical and a serial communication link is employed, a simple serial processor is adequate. It is possible, depending upon our application, to put more than one sensor on the same chip and even sometimes it could be possible to use one sensor as a measurement element as well as a correcting element. One basic and one more general VLSI-based intelligent sensor are shown in **Fig.3.5** (a) and **Fig.3.5** (b).

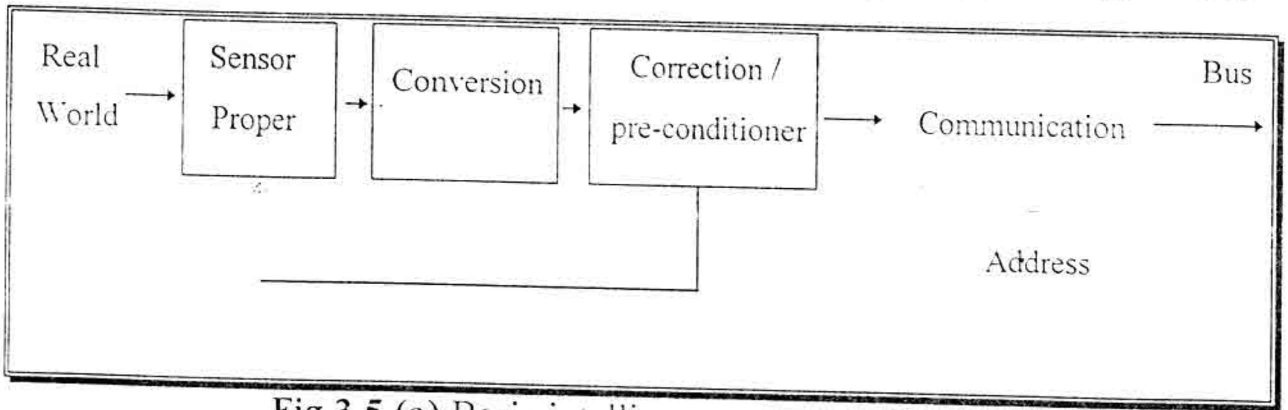


Fig.3.5 (a) Basic intelligent sensor configuration

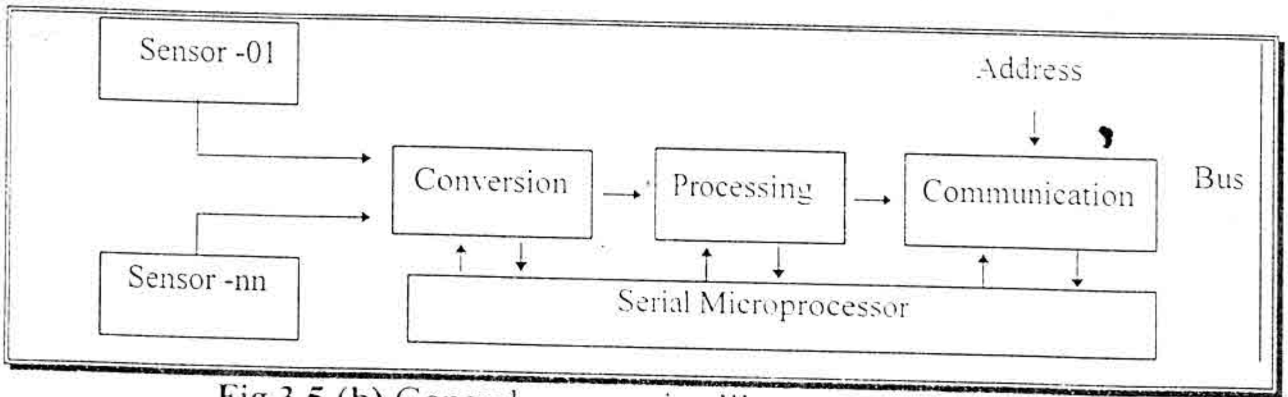


Fig.3.5 (b) General purpose intelligent sensor system

3.6 A Generalized system approach to intelligent sensors

Both of the following figures will help to understand more easily what is meant by the system approach to intelligent sensors[3.5]. Actually an intelligent sensing system generally has a hierarchical structure as human sensory system having multi-layer as shown in **Fig.3.6**.

The top layer acts like the brain of the intelligent system where the most intelligent information processing is being conducted. Processing is centralized in the top layer. Processed information is not dependent on the operating principle and physical structure of sensors. In contrast, information processing in **the lower layer** is strongly dependent on the sensor's underlying principles and structures.

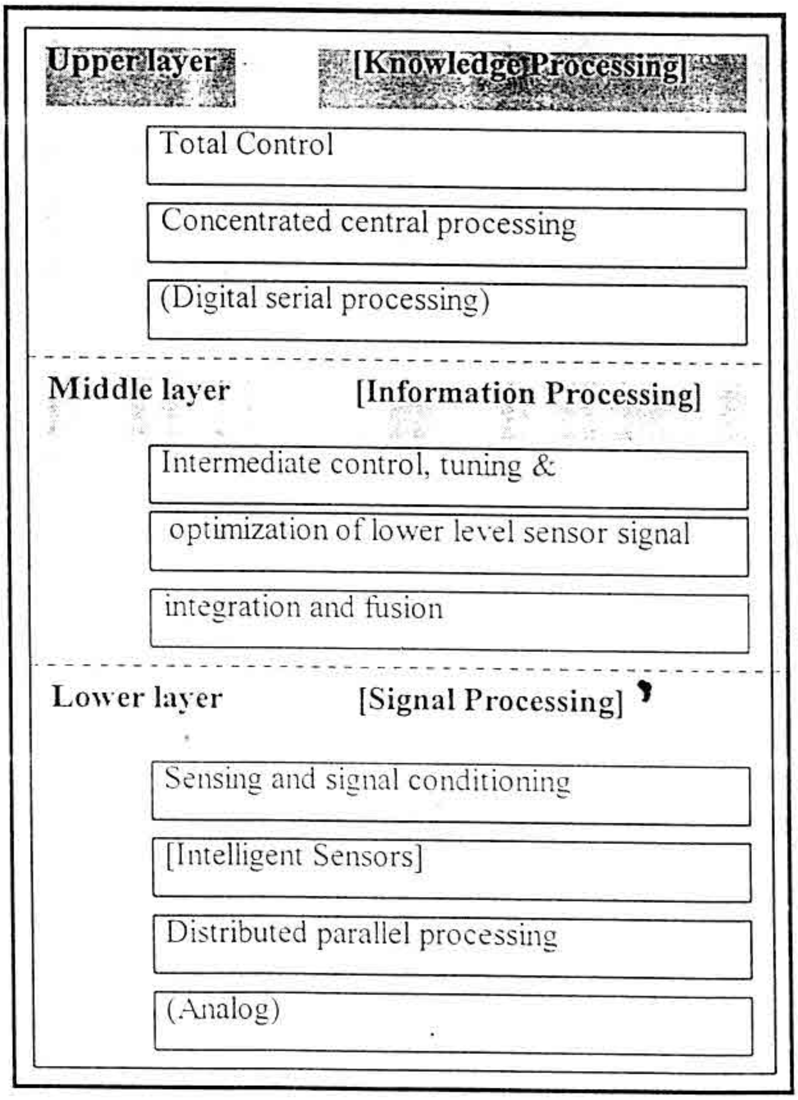


Fig.3.6 Structure of an intelligent sensing system

A group of intelligent sensors or ordinary sensors on the lower layer collects information from the external objects like our distributed sensory organs. Signal processing of these intelligent sensors is done in a distributed and parallel manner. The role of sensor intelligence in the lower layer is limited within signal processing of signal selectivity and characteristic compensation of individual sensor devices. This layer is much more hardware dependent. **In**

the middle layer, there are intermediate signal processing functions. One function of the intermediate signal processing is the integration of signals from multiple sensors. When the signals come from different types of sensors, the function is referred to as sensor signal fusion. Another function is tuning of the parameters of the sensors to optimize the total system performance. Finally, in general, **the property of information processing done in the lower layer is more directly related to hardware and less hardware related in the higher layer. For the same reason, the algorithm of information processing is more flexible and more knowledge oriented in the higher layer and less flexible and less knowledge related in the lower layer**. Processing in each layer can be characterized as follows: signal processing in the lower layer, information processing in the middle layer and knowledge processing in the upper layer[3.5].

The most popular concept of an intelligent sensor is an integrated monolithic device combining a sensor with a microcomputer within one chip. However, such a device has not yet been realized. Development stages of such intelligent sensors[3.6-3.8] are illustrated in **Fig.3.7**.

Technology is now in the second stage of development which means that technology is able to integrate some signal conditioning circuitry on the sensor element itself while at the same time integrating A/D on the microprocessor. A final goal is to integrate the sensor element with signal conditioning circuitry and A/D converter or put the sensor element itself on the microprocessor. Without doubt, this final stage will have a revolutionary effect on the intelligent sensors as well as on the measurement and control systems.

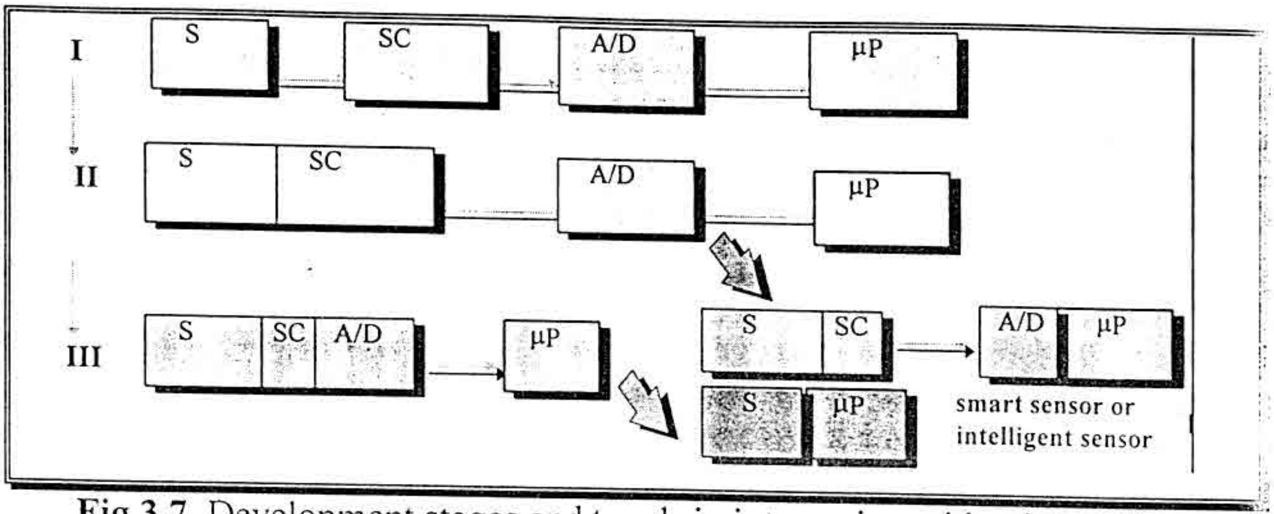


Fig.3.7 Development stages and trends in integration with microprocessor

[S:Sensor element. SC:Signal Conditioning Circiutry, A/D:Analog-to-Digital Converter, μP:Microprocessor]

3.7 The Role of intelligence in smart sensors and sensor systems

The roles of intelligent sensors are to enhance design flexibility and realize new sensing functions, and additional roles are to reduce loads on central processing units and signal transmission lines by distributed information processing in the lower layer of the system.

The sensor intelligence performs distributed signal processing at the lower layer in the bottom-up structure of the sensing system. The role of signal processing in intelligent sensors system can be summarized as;

1. Signal improvement for extraction of useful features of the objects/signals.

2. Fortification of intrinsic attributes of sensor device.

The important role of the sensor intelligence is to improve the signal selectivity of individual sensors. This includes simple operations of output from multiple sensor devices for feature extraction. However, this does not include optimization of device parameters or signal integration from multiple sensor devices, because this requires knowledge of the sensor devices and the objects.

Mainly there are three roles of intelligence in the middle layer of intelligent sensing systems: The first role of intelligence is to extract essential features of the object or signal while the second role is to organize multiple outputs from the lowest layer and generate an average output. The middle layer combines or integrates the coming-out signals from multiple sensors. The extracted features are utilized for recognition of the situation by upper layer intelligence.

Signals from sensors of different measurands are combined and the results give new, useful information. Ambiguity or imperfection in the signal of a measurand can be compensated by another measurand. This is called sensor signal fusion. This feature of processing creates a new phase of information.

The third role of the middle layer is parameter tuning of the sensors to optimize the total system performance. Optimization is done based upon the extracted feature and knowledge about target signal. The knowledge is given by the upmost layer as a form of optimization algorithm.

3.8 Different approaches to create intelligent sensor systems

There are four different approaches to realize intelligence in a sensor system:

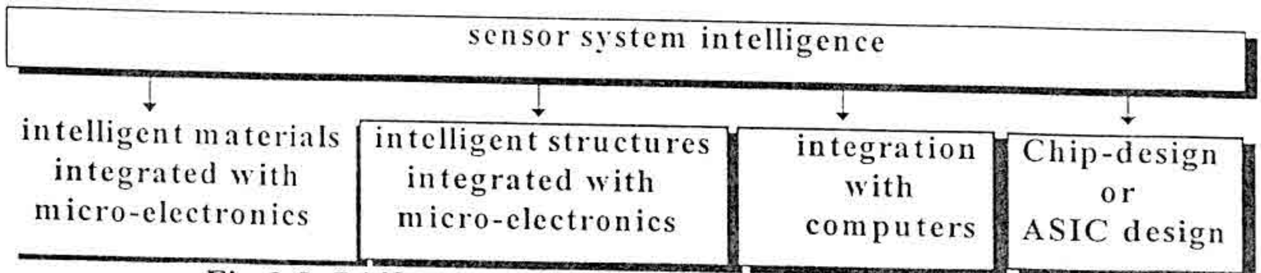


Fig.3.8 Different methods of sensor system intelligence

The first approach is the use of specific functional materials, so-called **intelligent materials**. Intelligent materials are vitally important for intelligent sensor design. It needs a very deep understanding of chemistry, physics and mathematical formulation of the facts at hand. Intelligent materials are called intelligent because they choose only the signal required, they suppress the noise and undesirable effects, they fit very well the object material with sensor material to realize nearly ideal signal selectivity. These materials will be very useful especially for biosensor applications.

The second approach is the use of functional mechanical structures, so-called **intelligent structures**. They are named as intelligent because they have the ability to choose only the signal required, and to suppress noise or undesirable effects. This method needs silicon technology and silicon micromachining of a high technology nature. This approach requires VLSI (very large scale integration) technology as well.

The third approach is **integration with microcomputers or microprocessors**. This method is a widely used and preferred technique. The reason behind this tendency is the availability of the systems that will be needed for design and implementation. It is most popular and is usually represented as the integration of sensor devices and microprocessors. The algorithm is programmable and it can be changed even after it is designed.

The fourth method is **stand alone chip design**[3.9] **or ASIC design** for the intended application which needs more commitment, energy and financial support although there is no guarantee that the system will function properly and effectively. A novel and functioning **ASIC design**[3.10] example might be an integrated approach developed to an artificial nose based on ASICs and conducting polymers as an intelligent system.

3.9 Conclusion

Intelligent sensors are a rapidly expanding topic of research which will inevitably bring extraordinary changes in current medical, industrial, avionics, consumer products, space research, manufacturing, and many other real life problems. Therefore, at this stage what is needed urgently for intelligent sensor design is a deeper understanding of the basics of sensor systems and their application to technology. Unless the basics of traditional sensor systems are digested very well, new intelligent sensor design will be difficult. Specifically, the area of medicine might get much more benefit from intelligent sensors; probably much more from intelligent material based intelligent sensors, i.e. biosensors, although nowadays microcomputer based intelligent sensors are much more popular.

The principal sensing element is the heart of any intelligent sensor design and implementation. Unless it works properly, little can be done about the rest of the system. That is why this research tested the sensor array in a sequence with binary solutions, mixed solutions, artificial blood, and finally with whole human blood to make sure that all kinds of problems associated with the sensor system have been solved before integration with electronics.

This sensor system does not need any external excitation since the chemical energy produced converts into electrical energy directly, and is the main reason why zero-current potentiometry is preferred in ion-sensor integration with electronics.

Amplification of the output of the principal sensing elements is perpetually a fundamental requirement. This requirement has been achieved by using a proper pH meter during the tests of the sensor array with binary and mixed solutions whereas for the artificial and whole human blood analysis a picoscope has been used in conjuction with a very high input impedance two-channel electrometer. Signals are displayed by using either the digital displayers of the pH meter or the picoscope having two channels.

Chapter Four

Methods of Ion Sensing

Summary

This chapter gives a comprehensive review of the methods used for ion detection in different media. It introduces ion-controlled diodes [ICDs], ion-sensitive field effect transistors [ISFETs], ion-selective electrodes [ISEs] and optrodes and their sensing mechanisms, and mentions briefly attempts to miniaturize the reference electrodes for ion detection.

4.1 Introduction

There are various methods used for ion detection either in solutions or in body fluids, for example in human blood. However, the main concern behind the experimental work with solutions has always been the possibility of recommmending new alternative methods, either partial or full, for real life constituents, in analysing the constituents of human blood.

Human blood contains many constituents having significantly different characteristics requiring complex or simple analysis in collaboration with high-tech instruments.

It is well known that biologists and/or physicians are mostly interested in the concentration or activity of a certain ion and its variations under conditions operative in a living organism. Therefore, the most common task is the determination of various ions or even non-ionic substances in *extracellular biological fluids*, e.g. blood, plasma, cerebrospinal fluid, urine, gastric juices, bile, either in vitro or in vivo, *and in intracellular liquids*.

All biological fluids are very complex electrolytes, containing considerable amounts of proteins and other organic substances.

The main ions are potassium, sodium, chloride, phosphate; **with important roles played by calcium,** magnesium and bicarbonate ions[4.1]. It is evident that, from the biological and medical point of view, the most important inorganic levels to be determined are the H^+, Na^+, K^+, Ca^{2+}, Cl^-, and pCO_2 and pO_2, depending on the application intended.

4.2 Ion-detection methods

4.2.1 Ion-controlled diodes and their sensing mechanism

The structure of this device is shown schematically in **Fig. 4.1**.

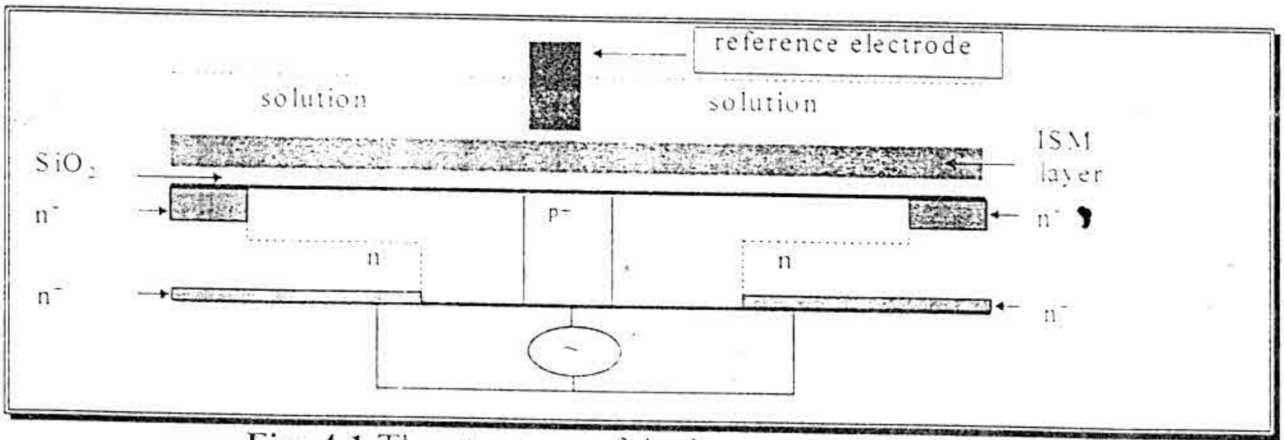


Fig. 4.1 The structure of the ion-controlled diode [ICD]

A p^+-type area is surrounded by two n-type areas thus forming a pn junction. On top of the structure is a double layer consisting of a thin SiO_2 layer and a layer that forms an ion-selective membrane [ISM]. As the pn diode is properly covered by the SiO_2 layer it shows the normal I-V characteristics. A depletion region is associated with the pn junction when the diode is zero or reversed biased. **The differential capacitance of this depletion region** can be measured. The structure is immersed in a solution of which the ion concentration is to be measured. Above the structure a reference electrode is positioned. **When a negative voltage is applied, electrons are depleted from**

the silicon surface under the SiO_2 layer. At sufficiently negative bias the surface is inverted and the inverted layer connects to the p^+-region which results in an increase in the depletion layer and its capacitance. When the device is placed in a solution containing ions and the ISM layer is such that the ions can penetrate this layer, the ions arrive at the oxide/ISM layer interface. This affects the capacitance which can be measured. By carefully choosing the ISM layer, chemical sensors for the measurement of, for instance, hydrogen ion activity (*pH*) or potassium (K^+) ion concentrations can be obtained. The advantage of ICDs is that the connecting wires can be mounted on the side of the device that is not in contact with the solution to be measured[4.2].

4.2.2 Ion-sensitive field-effect transistors [ISFET] and their sensing mechanism

The most extensively investigated *MOSFET-based chemical sensor* is the so-called ion-sensitive field-effect transistor or *ISFET*. The device was invented by P. Bergveld 1972 and was capable of measuring the *pH* of an electrolyte[4.2].

An *ISFET* consists of a *MOSFET* where the oxide layer is replaced by a combination of an oxide layer and a solution containing ions. In more sophisticated devices, called *CHEMFETs*, the oxide layer is covered with an ion-permeable layer, thus broadening the application field of *ISFETs*. *The gate of an ISFET is positioned at a certain distance from the silicon substrate and is more often called the reference electrode*. Application of a potential to the gate will induce a current to flow through the *p-Si* area. By measuring this current it will be possible to detect the activity of ion(s) in the solution under the test.

The usual method of measuring *pH* is based on the measurement of the potential difference between an ion-selective and a reference electrode. The most important part of the ion-selective electrode is an ion-sensitive membrane. **When the membrane is in contact with the ionic solution, ions diffuse through the membrane causing a potential difference to occur across the membrane.**

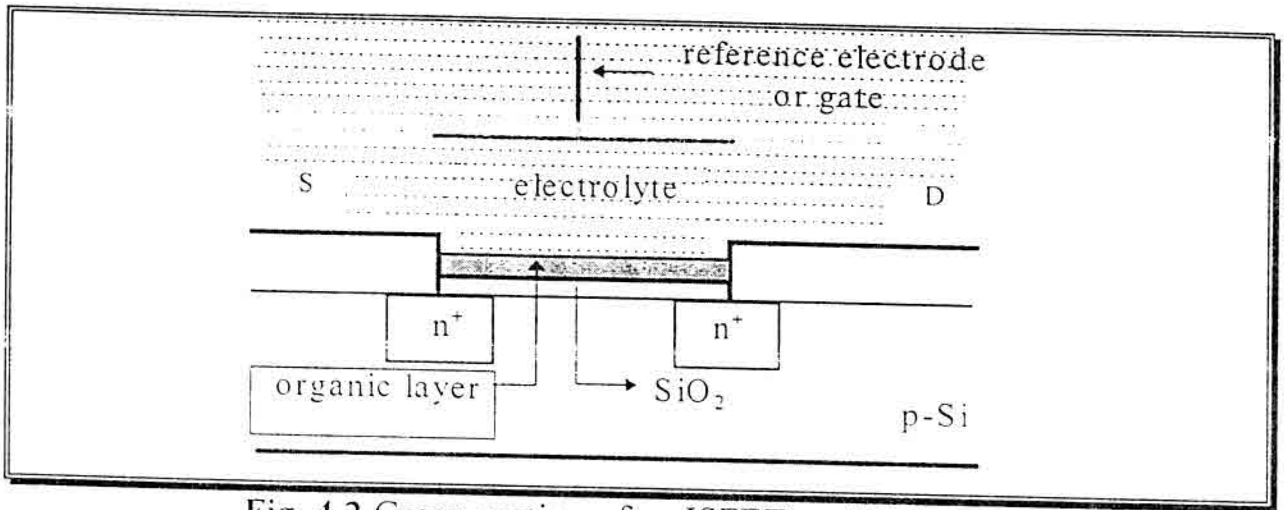


Fig. 4.2 Cross-section of an ISFET or CHEMFET

The working mechanism of *ISFETs* has always been a debatable subject among researchers but finally agreement has been reached on the "*site binding model*" in electrochemistry as a model explaining almost all the features of *ISFETs* today. This model is based on the *assumption* that the *pH* response of an *ISFET* arises from surface reactions between the electrolyte and the oxide and *not* from hydrogen diffusion through the oxide. Dependent on the ion concentration of the electrolyte and the oxide used in the *ISFET*, two cases can be identified for the electrolyte-oxide interface. At "*unblocked*" interfaces interfacial potentials are generated by the process of ion exchange between the electrolyte and the oxide. At "*blocked*" interfaces the charge distribution at the interface is governed solely by electrostatics. **In principle, only unblocked interfaces show ideal Nernstian response**, but *blocked interfaces* as observed in *ISFETs* can, under certain conditions, also show a response that comes close to a Nernstian response.

ISFETs are mainly *pH-sensitive* devices. When the insulator is covered with a layer that allows some ion exchange to take place, the device can be made sensitive to other ions. Such devices are known as *CHEMFETs*. **However, the use of conventional reference electrodes very much reduces the attractiveness of the *ISFET* concept**[4.2].

Bipolar (normal) transistors are current-driven semiconductors, ie, they amplify a current. They have a low input impedance, and are *not* suitable for the input stage of an electrode voltmeter. *FETs* are voltage-driven semiconductors; amplification is related to the voltage applied to the device which produces an electrostatic field. *FETs* do not consume input current, operating only on voltage. They have, therefore, a high input impedance, and are widely used for the first stage of an electrode voltmeter.

The elimination of intermediate processes is taken one step further than with the coated wire electrode by connecting the first stage of the millivoltmeter (a field-effect transistor) directly with the ion-selective membrane since **the ion selective component of *ISFETs* is similar to membrane electrodes**. The advantage of this arrangement is that the high impedance of the electrode is transformed directly to the low impedance output of the transistor, eliminating the need for heavy shielding.

The attraction of this approach is that with current micro-electronic technology, it should be possible to construct a cluster of electrodes on one chip, for possible *in vivo* applications. *ISFETs* have been developed for K^+, Na^+, Ca^{2+}, H^+, and NH_4^+ and have been used *in vivo*[4.3]. Unfortunately development of this sensor has been retarded by problems of electrical insulation of the chip from the solution. The present *ISFETs* have a limited active life time of about 10-20 days. Mass production of stable *ISFETs* would provide inexpensive assays for many analytes[4.3].

Although ***ISFETs*** **are not very effective as the modern version of** *ISEs,* the range of them is expanding and now includes enzyme and immunochemical sensors. **In view of possible miniaturization,** *ISFETs* have interesting perspectives in the field of biology[4.4]. These devices contain an ion-selective membrane placed on the insulator layer, for example, *SiO_2 or a mixture of SiO_2 and Si_3N_4 etc.*, of the *MOSFET.* The potential difference between a reference electrode immersed in the analyzed solution and the contact to the silicon wafer is equal, providing a constant term, to the electrostatic potential difference (the Volta potential) between the surface of the insulator in contact with the ion-selective membrane and the opposite surface containing the silicon chip. This potential difference depends, in the Nernstian manner, on the logarithm of the activity of the ions which are in the equilibrium with the membrane. **Thus this device functions exactly as an** ***ISE*** **with rather high impedance**[4.5].

Solid-state sensor techniques include microelectronic sensors, thick- and thin-film sensors and microengineered devices[4.6]. All are based on fabrication techniques familiar in the microelectronics industry and refined in that field for high quality, high volume and very low cost. They are also all characterized by small physical size and rugged, monolithic construction.

Chemically sensitive field effect transistors (CHEMFETs) can be configured to exploit ion-selective elements (ISFETs), gas-sensitive membranes (GASFETs), immobilized enzyme-mediated reagents (ENFETs) and other mechanisms. This flexibility has led to the laboratory development of a very wide range of sensors with potential applications in air, water, and ground monitoring, including sensors for pH, nitrates, ammonia and metal ions. However, the only example to have reached the market in a significant way is the pH sensitive ISFET. Despite very heavy investment, it has proven difficult to achieve long term stability in more complex CHEMFETs, as would be essential for more widespread penetration of the environmental monitoring market. Cross-sensitivity has also been a considerable problem. However, the market prospects for ISFETs in particular, as replacements for ion-selective glass electrodes in water-based applications, are very high and there is still significant pressure to develop practical devices.

Thick-film and thin-film fabrication techniques, principally screen printing of reagents and electrodes onto plastic, glass or ceramic substrates, are increasingly being used for electrochemical sensor elements[4.6]. Commercially available examples include disposable sensors for lead and copper in drinking water based on anode-stripping voltammetry and vibrating-electrode sensors for residual chlorine and ammonia. A collaborative project is under way to develop a multi-sensor array fabricated using thick film techiques for water quality monitoring, including sensors for temperature, conductivity, pH and dissolved oxygen, with reference electrodes, all on a single substrate[4.6]. Chlorine and heavy metal sensors will be added in a later phase of the project[4.6]. Thick-film and thin-film sensors are also being researched for exhaust emission monitoring and engine management in automobile engines here at Sussex.

The principle attraction of thick-film technology in particular is the very low investment required to set up a production facility, compared for example to a silicon-based sensor fabrication line. Whilst the flexibility of silicon sensors is not available from thick-film sensors, this economic advantage will ensure a significant future market opportunity.

Microengineered silicon sensors have mainly appeared as physical sensors of temperature, strain or acceleration for example. However, there is a growing number of developments, mostly at very early stages, aiming to

reproduce the functionality of laboratory analytical instruments on a microscale. Examples include a gas chromatograph on a chip, which has been commercially available for some years, flow-injection analysis systems and various forms of mass spectrometer integrated in silicon. **The techniques are well established to form flow channels, reaction chambers, valves, pumps, heaters and other elements of conventional analytical instruments on a millimetre scale and with features on a micron scale.** Moreover, the silicon substrate can, in principle, also support the integration of sensor elements such as CHEMFETs or optical sensors and of signal-processing electronics. The long term prospect is therefore for devices with the specific analytical capabilities of laboratory instruments but small enough and cheap enough to operate in the field. The investment required to set up manufacturing facilities for microengineering will be high but, as with microelectronics fabrication, many different sensor designs for diverse markets could be made in a single facility.

It was expected that FETs would have an advantage over conventional potentiometric electrodes given their planar construction, ease of large scale production, **and the relative simplicity with which a multisensor device could be constructed.** The small dimensions, fast response time, and direct application after storage were also seen as advantages over established technology. Although at least one small self-contained instrument based upon ISFET technology was developed and evaluated[4.7-4.8]. **FETs have enjoyed little commercial success in medical applications to date.** However, these devices are begining to compete in other markets for selective ion detection such as fermentation and bioreactors. There are still serious problems with encapsulation and stability[4.9].

4.2.3 Ion-selective electrodes [ISEs] and their sensing mechanism

Among different methods of ion-sensing techniques, the use of ISEs is advantageous since many biological processes depend on ion activities and not the overall concentration.

The healthcare professional needs information about the physical and chemical/biochemical status of the patient. This information is used to diagnose disease or to monitor the progress of treatment of the disease. Traditionally physical measurements such as temperature and blood pressure have been performed by the physician at the patient's side, while chemical and biochemical measurements of analytes such as serum sodium and cholesterol have been carried out in a centralized laboratory. Both of these approaches have the disadvantage that they give readings taken at one point in time. If changes in the patient's condition are to be monitored the procedures must be repeated with the attendant inconvenience to the patient and physician. Another disadvantage of using the centralized laboratory, with its current technology, is the time taken for the physician to receive the results.

Sensor technology opens up the possibility of continuous real-time or near real-time monitoring of a patient so that changes can be detected quickly in life threatening conditions or the effect of therapy can be seen immediately. So, beyond the year 2000 it is expected that the most rapid areas of innovation and growth will be in new methods of diagnosis and patient monitoring which will be closer to the patient and less invasive. The technologies considered include electrochemical sensors, optical sensors, biosensors, and neural activity sensors.

The fact is that the majority of the electrochemical sensors used in healthcare are ion-selective potentiometric electrodes used for the measurement of specific ions such as potassium, sodium, or hydrogen, or for the detection and measurement of specific gases such as carbon dioxide or ammonia.

A potentiometric electrode is used in conjunction with an ion-selective membrane to produce an ion-selective electrode (ISE). ISEs for the single measurement of sodium, potassium and lithium were developed in the 1920s and a glass electrode for the selective measurement of hydrogen ions, and therefore pH, has been commercially available since the mid 1930s. The technology was developed to include calcium-selective electrodes, which were commercialized in about 1970.

The uptake of ISEs in the clinical laboratory was quite small, probably due to the availability of flame photometric technology for sodium and potassium analysis and to the fact that **ISEs measure ion activity** and not the total concentration of the ion. The activity of an ion is an indication of the amount of free unbound ion present in the sample. The proportion of an ion which is in the free state is affected by the dilution of the sample and by the other constituents of the sample such as proteins and lipids. These constituents tend to bind ions. Such factors mean that the results obtained by measuring ion activity using an ISE do not directly correlate to the analytical results obtained by flame photometry. This led to difficulties in standardizing and interpreting the results.

If the sample is diluted most of the matrix effects are removed and the result obtained using an ISE is comparable to flame photometry. For this reason clinical samples are frequently diluted before analysis using ISEs. Alternatively, a "factor" is applied to the analytical reading to convert it to a "total" ion concentration.

It appears that the extra information obtained by assaying for an ion activity as opposed to an ion concentration has not been found to give clinically useful information. **The exception to this is calcium**. Calcium ion activity is a more meaningful assay than total calcium, therefore calcium assays are carried out on undiluted samples.

ISEs are frequently used in critical care where there is a requirement for rapid test results. One study has shown that the turnaround time for receiving analytical results can be decreased from 25 minutes to less than three minutes by having an analytical device in the operating suite rather than using the services of a laboratory even when this is adjacent to the operating room. The complexity and urgency of many diseases require a timely analytical results, especially for K^{+}, Na^{+}, Ca^{2+}, pH, O_2, CO_2, and for the management of the patient before, during and after surgery.

Ion-selective electrodes [ISEs] are devices that permit the activity of a given ion in a solution, usually aqueous, to be determined potentiometrically despite the presence of other ions.

The measured parameter is voltage in millivolts (mV). The mathematical equation governing this phenomena is called the Nernst Equation and is given in (4.1) below:

$$= E \pm E_0 = \pm(RT/nF)\cdot \ln a_i \qquad (4.1)$$

where a_i is the activity of the ion i, F is the Faraday constant, n is the number of electrons involved in the potential-determining reaction, R is the gas constant, and T is the absolute temperature.

All measuring devices in potentiometry are called electrodes (ion-sensors), the term sensor is used for the active part of the electrode only. Potentiometric measurement has to be carried out under zero-current condition which necessitates very high input impedance electronic systems (electrometers), effectively very high input impedance instrumentation amplifiers, in the order of $>10^{12}$ Ω. The main task expected from a potentiometric measuring electrode is mainly to respond quickly and with high selectivity to changes in the concentration of the sensed ion in the sample, in this case human blood. In potentiometry miniaturised reference systems are also needed. Good reference systems/electrodes must show a potential not depending on the properties of different samples.

Most of the reference systems used are based on electrodes of the second kind following the scheme $M / MX / X^-$, where M is a metal that forms sparingly soluble salts MX. By selecting the activity (or concentration) a_{x-} the potential of the system is fixed. The mechanism of such electrodes is that the electrode M detects the activity of M^{n+} which arises from the solubility of MX in relation to the solubility product of MX. This again is ruled by the a_{x-} activity. Most of the reference systems in practice make use of the combination $Ag/AgCl/Cl^-$ with potassium chloride as the reference electrolyte. KCl as reference electrolyte is used in high concentrations for most applications. a good compromise owing to the same mobility of the K^+ and Cl^- ions, "equitransference"[4.10].

4.3 Optrodes for ions

4.3.1 Optrode for potassium

Reliable and sensitive ion sensors are needed for various applications including clinical ion sensing. Quite a number of schemes for optically sensing alkali and earth alkali ions have been reported in the past few years[4.11]. While some are based on the use of chromoionophores or crown ethers, the most promising sensing schemes at present appear to rely on the use of *neutral ion carriers*. Three different methods based on the use of ion carriers, such as valinomycin, have been reported. One is based on the use of potential-sensitive indicators, the other on the use of proton-carrier dyes, and the third on a widely unexplored mechanism through the use of lipophilized acridinium dyes whose fluorescence obviously is highly polarity-sensitive.

Ion-exchange or coextraction sensing schemes have provided a most promising way for sensing a number of ions. Here, the carrier-mediated transport of a cation into a PVC membrane is coupled to the release of a proton from a lipophilic proton carrier contained in the sensing membrane. This deprotonation causes a change in the optical properties of the proton carrier to occur.

An optrode for potassium ion has been designed that exploits the inner filter effect (*IFE*) of fluorescence. The sensing scheme makes use of neutral ion carriers as described by Simon et al. [4.11], but transduction is based on the use of two different dyes, viz., an absorber and a fluorescer. The absorber acts as the proton carrier, while the other is a stable and *pH-independent* fluorophore bound to minute particles contained in the sensor membrane. Fluorescence varies as a result of the varying absorption of the dye which in turn is modulated by the potassium concentration through the *IFE*. The sensor material is extremely sensitive to potassium to which it fully reversibly responds over the *1* μ*M* to *10 mM* concentration range with a useful dynamic range from *1* μ*M* to *1 mM*. The sensing approach presented is generic in that it can be applied to almost any species for which respective carriers are known. Thus, by replacing valinomycin by carriers for ammonium and calcium ions, respective ion sensors are obtained without requiring any changes in the optical system [4.11].

The membranes were prepared from a batch of 2.4 mg of PVC, 0.4 mg of PTCB, 1.8 mg of valinomycin, 30 mg of the aqueous suspension of the FluoSphere particles, 0.4 mg of KFU 111, and 6.0 mg of NPOE, all of which have been dissolved in 1.5 mL of freshly distilled tetrahydrofuran[4.11].

Optical measurement of chemical species through an optical fiber is one of the most advanced sensing techniques. Various fiber-optic sensors have been developed, especially for measurements of pH[4.12], oxygen[4.13], and metal ions[4.14]. Some suitable indicators specific to the chemical species of interest have been used as transducing materials in these studies. However, there is difficulty in construction of a fiber-optic sensor for alkali-metal ions, since few chromogenic dyes interact with these ions.

The first sensor sensitive to an alkali-earth ion was reported by Zhujun et al. [4.15]. The sensing mechanism is based on fluorescence quenching by a copper ion; a weak quencher, Na^{+}, competitively binds to a fluorescent dye with a strong quencher, Cu^{2+}. Wolfbeis and co-workers have developed an optical K^{+} sensor, in which the membrane potential between an aqueous sample solution and a Langmuir-Blodgett layer is measured by using a potential-sensitive fluorescent dye[4.16]. The sensor has a logarithmic response to the analyte concentration and has a wide dynamic range. Alder et al. have constructed a different type of K^{+} sensor using a chromo-ionophore consisting of a crown ether and a dye molecule[4.17]. For these sensors, the preparation of the optically sensitive membrane is rather difficult, since the structure of the membrane is highly sophisticated and should be carefully controlled. Furthermore, it is necessary to synthesize a new chromogenic dye sensitive to a specific alkali-metal ion.

For the determination of alkali-metal ions, an ion-selective electrode with a plasticized poly(vinyl chloride) (PVC) membrane is currently used[4.18]. This plasticized PVC membrane consists of poly(vinyl chloride), a neutral ionophore, a hydrophobic ion exchanger, and a plasticizer. Optical sensors using plasticized PVC membranes have also been developed. However, an indicator that directly interacts with a specific cation must be synthesized to construct a fiber-optic sensor. To solve this difficulty, Ishibashi, N. et al.[4.19] report a general approach to constructing a fiber-optic sensor using the plasticized PVC membrane. The fluorescence intensity of the hydrophobic probe, dodecyl acridine orange (dodecyl-AO^+), is known to be dependent on the polarity around the probe molecule. Dodecyl-AO^+ is incorporated in the membrane containing valinoymcin, and it moves to an aqueous phase by ion-exchange with the potassium ion in a sample solution. If so, the fluorescence intensity of dodecyl-AO^+ decreases with increase of the polarity of the microenvironment around the probe molecule. Dodecyl-AO^+, used as a chromogenic indicator, does not directly interact with the potassium ion. Thus this technique can be generally applied to fiber-optic sensors responding to other cations by changing the plasticized PVC membrane with a different neutral ionophore.

4.3.2 Optrodes for calcium

The development of sensors with an optical transduction of the chemical recognition process (optical sensors; optrodes) has become a highly relevant and rapidly expanding area in analytical chemistry. These optical sensing devices are based on absorption, reflection, fluorescence, or chemiluminescence measurements, and they commonly make use of chemical compounds that drastically change their optical properties as the result of the actual recognition process. With only few exceptions, immobilized indicator dyes, especially fluorigenic substances, have so far been applied as the active materials. Very often, optical fibers are employed for focusing the incident light onto the corresponding sensing area of the probe and for guiding it back to the detector, but this is not required for fundamental studies of the presented type of membranes.

Recently, many reports on the design features, the morphological aspects, and the theoretical description of a new class of optrodes which are based on conventional, electrically neutral ionophores have been published[4.14-4-18]. These highly selective compounds have been widely applied in membranes of a large variety of ion-selective electrodes. For the preparation of the optical sensing layers, such neutral carriers for cations or anions can be combined in the same bulk membrane phase with a second sort of highly selective neutral ionophores, so-called chromoionophores which dramatically change their absorption spectrum in the UV-vis region upon complexation of the corresponding ionic species. The well-proved poly(vinyl chloride) (PVC) membrane technology can basically be adopted for these new optode systems, which is in clear contrast to some earlier approaches.

Formally, two functional principles are possible for the present optical sensing devices: (i) a carrier-induced coextraction of cations and anions from the sample into the membrane phase, or (ii) a carrier-induced exchange of cations or of anions between sample and membrane. In the first case the absorbance response depends under ideal conditions on the product of the respective ion activities in the sample solution, while in the second case the response is controlled by the corresponding ratio of ion activities.

The group reports on the development of a novel optode membrane belonging to the group of neutral-ionophore-based cation exchangers[4.19]. This system has been realized by combining a conventional Ca^{2-} -selective carrier (ETH 1001) and a newly prepared H^{+} -selective chromoionophore (ETH 5294) together with lipophilic anionic sites in the same plasticized PVC membrane phase. Membrane-coated glass instead of optical fibers has been used for aforementioned studies.

4.3.3 Sensor/membrane preparation for optodes

Optode membranes have been prepared from a batch of 20 mg of ETH 1001, 5.5 mg of ETH 5294, 11.2 mg of the additive $NaTm(CF_3)_2PB$ to create lipophilic anionic site, 143 mg of the plasticizer DOS, and 70 mg of PVC.

The membrane components were dissolved in 2 ml of freshly distilled THF. A 0.2 ml portion of this solution was injected onto a rotating, dust-free glass plate of 35 mm diameter which was located in a THF-saturated atmosphere. A hand-made spinning device with a closed aluminium/Plexiglas cell was used rotating with a frequency of 600 rpm. This allows the preparation of glass-supported membranes having controlled and highly reproducible thicknesses in the range between 0.5 and 7 μm. After a spinning time of only about 4 s, the glass support with the optical sensing membrane was removed and left open for some minutes for further drying[4.20].

The membrane composition of the calcium ion selective electrode used consisted of 3.4 wt % ETH 1001, 2.0 wt % KTpClPB, 31.2 wt % poly(vinyl chloride), and 63.4 wt % DOS[4.20].

4.4 Miniaturized reference electrode

Compared with ISEs, the ion-selective field effect transistor (ISFET) is small and physically robust and can be produced by microelectronic methods, with a future prospect of low-cost bulk production. However, until now, ISFETs were used mostly in combination with conventional reference macroelectrodes. This reduces considerably the benefits gained from the miniaturization of the sensor. Attempts to miniaturize reference macroelectrodes of the **second kind** [4.21-4.22-4.23] resulted in a serious reduction of the electrode lifetime due to the reduced electrolyte quantity. Therefore, reference electrodes without a reference electrolyte appear to be advantageous. Decreasing the quantity of hydroxy groups at the gate surface of an ISFET leads to an insensitivity of the ISFET toward the *pH* value[4.22]. To achieve this goal, insensitive polymer membranes have been used as the gate surface[4.24-4.22]. However, there are practical limits of these reference field effect transistors [REFETs], which is the difficulty in defining the interface between the insensitive membrane and sample[4.25].

A REFET based on a fluoride-selective ISFET with CaF_2 as the *internal reference electrolyte* and a polymer membrane which reduces its diffusion has also been described[4.26]. The sensor potential is influenced by fluoride and calcium ion concentrations in the sample and by varying diffusion potentials at the interface of the internal electrolyte and sample because of the low electrolyte concentration within a saturated CaF_2 solution. A further REFET with reference electrolyte has been described by Comte and Janata[4.27]. A stable potential is obtained using buffered agarose on top of a *pH-selective ISFET*, with a glass capillary as liquid junction. Nevertheless, a significant influence of the *pH* on the REFET potential due to the capilllary tip potential remains. To combine the benefits of a longer lifetime with an excellent potential stability, another reference electrode based on a perchlorate-selective field effect transistor has been successfully developed[4.28].

In this reference electrode, a perchlorate-sensitive sensor is in contact with a saturated solution of $KClO_4$ *and* $CaSO_4$ over solid $KClO_4$ *and* $CaSO_4$. The perchlorate activity in this solution is determined by the solubility of $KClO_4$ *(0.148 mol/l at 25 °C)*[4.28], and the corresponding constant perchlorate activity leads to a constant electrode potential. The perchlorate-sensitive sensor and the solution are located in a cavity, with a liquid junction to the analyte solution.

A prolonged lifetime compared with those of *Ag/AgCl reference electrodes* is achieved by adjusting the perchlorate activity, considering the solubility of $KClO_4$, *which is much lower than that of KCl.* Furthermore, interferences are low due to the high selectivity of the perchlorate-sensitive membrane. In addition, no influence of redox couples is expected. So, the results show that the perchlorate reference electrode is very well suited for *integration on an ISFET chip*[4.28] because of its feasibility of being miniaturized, its good reference potential stability, and its prolonged lifetime.

4.5 ISFETs for Na^+ and Cl^-

Differential measurement with ion-selective electrodes has been used widely. The chief advantage of this type of measurement is that it eliminates the uncertainty of possible changes of the liquid junction of the reference electrode and that it provides some measure of compensation of temperature variations and improved rejection of common noise. The price that has to be paid for these advantages is that the information about the activity of individual ions is not explicitly available from these measurements. From the instrumentation point of view both electrodes require a high-input impedance electrometer, which is not a common feature of most ion-selective electrode meters.

With a mixture of advantages and disadvantages such as these it is not surprising that this approach will be suitable only for some specialized applications. Ion-sensitive field effect transistors (ISFET) are ideal amplifiers for this approach because several high-input impedance channels can be readily available on the same chip. Furthermore, they naturally form a compact differential ion-measuring probe, which may be suitable for biomedical applications in which the experimental space or the volume of the available sample may be small. The group has developed such a probe especially for in situ measurement of the activity of sodium chloride in sweat[4.29]. This probe is a dual ion sensitive field effect transistor prepared by applying sodium and chloride selective membranes to the adjacent gates of the same transistor chip. Without a stable reference electrode this probe can measure the activity of sodium chloride as a difference of signals from the two transistors. This probe has lower drift and better temperature stability (~0.1 mV/°C, between 25 and 40 °C) than individual ISFETs. It has been tested in the range of 10^{-4} - 1 M concentration of NaCl.

4.6 ISEs using an ionophore covalently attached to carboxylated poly(vinyl chloride)

ISEs based on ionophore-impregnated polymer membranes, typically plasticized poly(vinyl chloride) (PVC), are now commonly employed in a variety of analyses. The operational lifetime of these ISEs is affected by the solubility of the ionophore in the plasticizer and can be limited by the leaching of the ionophore and the plasticizer from the polymer matrix. Usually, this leaching also worsens the detection limits of the electrode and results in gradual deterioration of the response.

Covalently grafting the ionophore to a polymeric backbone has been suggested as a possible solution to the problem of leaching[4.30]. The chemical immobilization of an ionophore on a polymer matrix involved the use of phosphorylated VAGH to develop an electrode for Ca^{2+} where a VAGH is a copolymer composed of vinyl chloride, vinyl acetate, and vinyl alcohol[4.31].

4.7 Conclusions

There are different techniques used to detect ions, either cations or anions, in solutions or in all other types of body fluids. Amongst these techniques, ion-selective electrodes have always been important and played a crucial role in clinical analysis. Their mathematical, chemical, and physical background are well established. Ion-controlled diodes, ion-sensitive field effect transistors, and optrodes are actually a direct result of the ion-selective electrodes principles practised in a different way. This clearly means that without proper, deeper, and better understanding of ion-selective electrodes, none of the others could well be implemented for practical issues.

The technology hospitals use is based on ion-selective electrodes although ion-controlled diodes and ion-sensitive field effect transistors are still in their research phase for commercial success which is other evidence of the importance of ion-selective electrodes for medical and clinical analysis.

For these reasons this research used ion-selective electrodes as the core part of the intended multi-sensing intelligent instrumentation system. In such systems, electrochemical sensors are favoured. They convert directly the chemical signal (kind and concentration of electrochemically active species, eg, ions in the analyte) into an electronic signal[2.13, 2.24]. In the case of ion-selective electrodes (ISEs) and ion-sensitive field effect transistors (ISFETs), an electrochemical potential established at the solution/sensor interface is controlled by the ion concentration of the solution. A thin ion-sensitive membrane (ISM) which is placed at this interface has a determining influence on the basic characteristics of these potentiometric sensors. Any distortion of this sensitive interface will disturb the sensor function, thus demanding self-control and self-calibration which leads to the requirement for an intelligent instrumentation system.

Chapter Five

Disposable Ag/AgCl/Cl⁻ Reference Electrode based on Coated Silver-Stripe (CSS)

Summary

This chapter introduces a new type of a disposable miniaturized all solid-state reference electrode made by incorporating the internal solution required in poly(vinylalcohol), PVA, and based on coated silver-stripe (CSS) and intends to give in detail the method used to prepare the subject reference electrode of a second kind which acts as a half-cell of a sensor system measuring K^{+}, Na^{-} and Ca^{+2} ion activity in binary solutions, multi-solutions (mixed electroyte), artificial and whole human blood.

Engineers who have been studying in biomedical engineering or medicine-related research areas find it very difficult to cope with some of the basic principles in this field and the chapter goes some way to alleviate this problem as well, at least for those dealing with basic principles of electrochemistry.

The technique described is a cost-effective and time saving method for biomedical measurement.

5.1 Introduction

A solid-state reference electrode is defined as any electrode or material in direct contact with a test solution that maintains a constant or predictable interfacial potential .difference despite changing type and concentration of chemical species in the test electrolyte[5.1] whereas a conventional $Ag/AgCl/Cl^{-}$ reference electrode consists of a solid silver chloride on silver wire and in contact with a solution of a soluble chloride[5.2]. This new application method alleviates the requirement for the solution by incorporating it in *PVA*.

The reference electrode is the most complicated and crucial part of a measuring system especially in the case of biological fluids. When application problems do arise with the system, they mostly involve the reference electrode since there are several major factors affecting the reference potential established by either silver/silver chloride, copper/copper sulphate or calomel reference electrodes which are mainly temperature, light, contaminants, and solute concentration[5.3].

An electrochemical measuring system always consists of four parts: a sensing electrode, an electrometer that translates the signal into a readable format, a reference electrode, and the sample being measured or tested. Each part of the system plays a critical role in the measurement process.

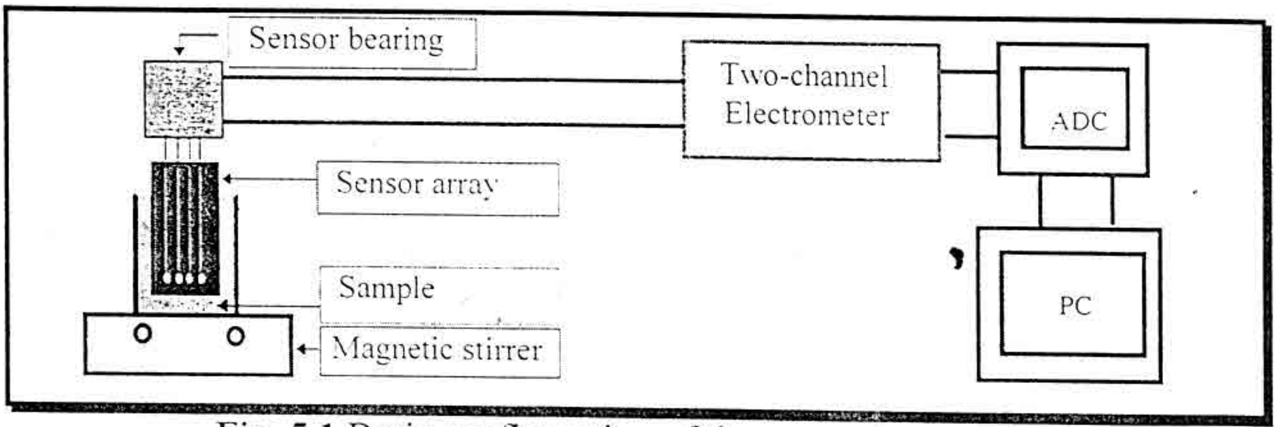


Fig. 5.1 Basic configuration of the measuring system

The sensing electrode is technically a transducer and its potential (E) as given in the formula (5.1) is a function of the activity of the free ion and a voltage of value E_0 which is supposed to be the rest potential of the system. In other words, E_0 is the voltage of the system when the ionic-activity is one.

$$E = E_o + 0.00591 \cdot \log a_i \qquad \text{(Volts)} \qquad (5.1)$$

where a_i is the activity of the ion i ,

and E_o is the standard electrode potential.

The reference electrode is also a transducer but its voltage does not vary with the activity of free ion or other ions cross-talking, but is only a function of the voltage value E_0 and the concentration of the ion involved in the electrode reaction, Cl^- in case of KCl and $NaCl$ but SO_4^{2-} in case of $CaSO_4$, which will usually be constant.

It is accepted that a sensing electrode is a source of a varying voltage whereas a reference electrode is a constant voltage source in measurement and instrumentation systems.

The reference electrode used by the author in this research consists of three principal parts leading to a reproducible and constant voltage: a vapour deposited 99.9% pure silver layer of $8.3\ \mu m$ thickness, a very thin layer of silver chloride over the silver layer or a silver conductive layer coated with silver chloride, and finally a very thin layer of KCl solution because the Cl^- ion is needed in order to establish the potential of the $Ag / AgCl$ electrode since the chemical reaction is: $Ag^+Cl^- + e \leftrightarrow Ag + Cl^-$. One important point is that silver chloride is more soluble in very high concentrations of chloride, [because of the formation of silver complexes like $AgCl_2^-$] than it is in low concentrations.

In actual fact, $AgCl$ is taken to be nearly insoluble and light sensitive. Light can cause the reduction of some silver chloride to free silver with the formation of some additional chloride ions and the white $AgCl$ turns to a purple colour.

5.2 Preparation of a miniaturized all solid-state $Ag / AgCl / Cl^-$ reference electrode based on coated silver-stripe for whole human blood analysis

5.2.1 A deposit of pure (99.9%) metallic silver is made using any appropriate technique onto an appropriate substrate. Silver was deposited onto a glass substrate using <u>V</u>apor <u>D</u>eposition <u>T</u>echnique (VDT) using the facilities of the laboratories of Physics Department of the University of Sussex.

The substrate used need not necessarily be glass; one might use some other material such as ceramic or polycarbonate.

5.2.2 The thickness of the silver layer deposited was about $8.3\ \mu m$ which is a thickness appropriate for the intended application. However, different applications might need different thickness values. In the application described four electrodes were used with $8.3mg$ silver on each glass slide. The dimensions of the glass slide are given in Chapter One in **Fig.1.1**. To make sure the silver sticks to the glass slide properly a rough surface was created by using sandpaper and stickness test was performed after it.

5.2.3 The preparation of a solution of $FeCl_3$ in HCl: $0.8g$ solid $FeCl_3$, $0.8ml$ concentrated HCl were added to a $20ml$ plastic tube and deionised water added to give a mixture of $20ml$. The color of this solution is yellow. The reason HCl was used is to create the environment which helps the chemical reaction converting $FeCl_3$ to $FeCl_2$ to happen.

5.2.4 Using a $10\ \mu l$ pipette, a $10\ \mu l$ of $FeCl_3 + HCl + H_2O$ mixture solution was poured on to the reference electrode area for a very short time, e.g. for 2-5 seconds. A chemical reaction takes place on the substrate, converting silver metal deposited into silver chloride salt. The silver layer changes color, becoming brown or brownish: $FeCl_3 + Ag \rightarrow FeCl_2 + AgCl$.

The important point is to make sure that the silver deposition will not come off from the substrate surface by waiting too long, e.g. for 2-5 minutes, because most of the Ag has been converted to non-coherent (leaching easily) $AgCl$.

5.2.5 Since silver chloride is light sensitive, it is necessary to ensure that during and after the chemical reaction, the silver chloride not exposed to light.

5.2.6 $5\ \mu l$ of $KCl + Ethanol + Water(Distilled) + Poly(vinylalcohol)$ solution was put onto the $Ag / AgCl$ electrode area and left until dry. It is important to ensure that all the surface area of the reference electrode, **Fig.5.2**, is totally covered with the solution and there are no air bubbles left in the $KCl + Ethanol + Water(Distilled) + Poly(vinylalcohol)$ layer since air bubbles and/or holes are fatal for the sensing and reference electrodes. A check was made by examining the electrode using a low power microscope.

5.2.7 Having dried the reference electrode, in time it will get non-conductive. Hence, it is very important at this stage to find out how to keep the reference electrode reasonably wet, assuring low resistivity. One way of doing this is to have a plastic container with a lid and a platform and keeping the container wet by putting a wet tissue in it. Due to air circulation it will remain reasonably wet with a resistance value between 100Ω-$2k\Omega$ which is an acceptable value.

5.2.8 The overall layer structure of the reference electrode (CSS_RE) and of the three ion-sensors ($K^{+}\_CSS\_IS$, $Na^{+}\_CSS\_IS$, and $Ca^{2+}\_CSS\_IS$) , all on the same substrate (4 on 1), are as follow respectively:

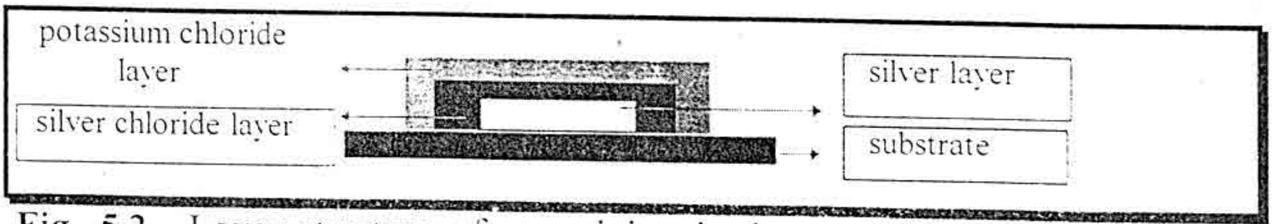


Fig. 5.2 Layer structure of a miniaturized coated silver-stripe reference electrode incorporating the internal solution

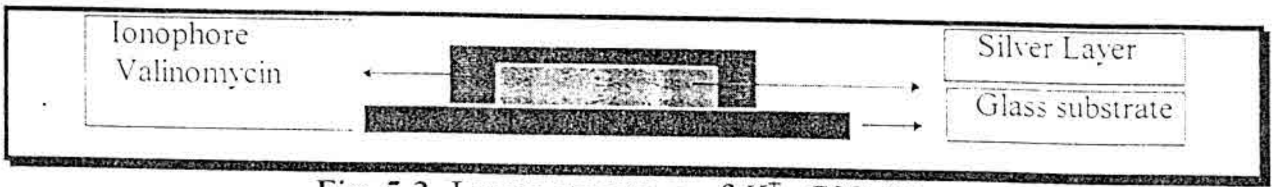


Fig. 5.3 Layer structure of $K^{+}\_CSS\_IS$

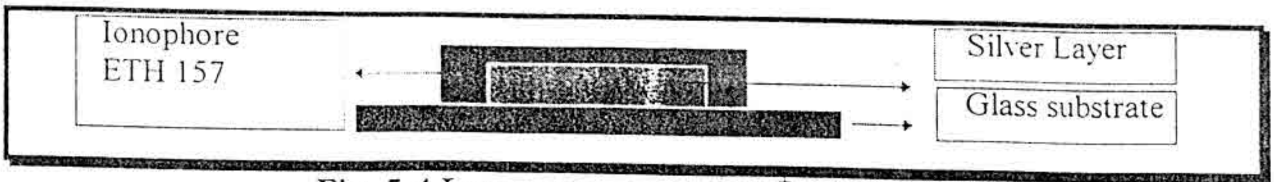


Fig. 5.4 Layer structure of $Na^{+}\_CSS\_IS$

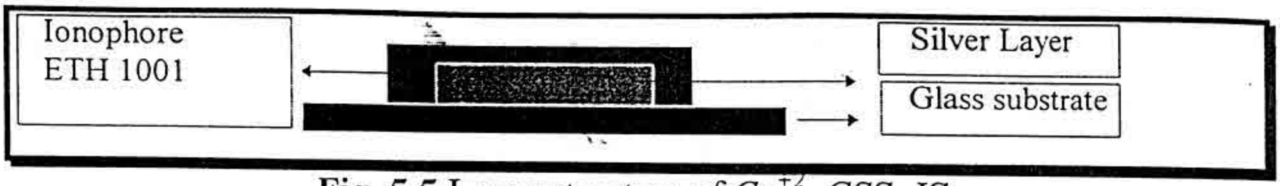


Fig. 5.5 Layer structure of Ca^{+2}*_CSS_IS*

5.2.9 A test of *CSS_RE* versus conventional double junction silver-silver chloride reference electrode with *6 mol/l KCl* internal solution is as follows, having preconditioned the *CSS_RE in 1 mmol/l KCl* solution for *one minute.* The readings of three KCl solutions are in mV with respect to conventional reference electrode. Three tests (t1, t2, and t3) are carried out with 2, 4, and 6 mmol/l KCl solutions each test per concentration lasting for 60 seconds.

	KCl solutions			
Tests	2 mmol/l	4 mmol/l	6 mmol/l	Time (second)
t1	24	22	24	60
t2	26	22	24	60
t3	24	25	24	60

Table 5.1 The response of *CSS_RE* versus traditional *Ag/AgCl RE*

As the tests indicate, the response of *CSS_RE* versus the conventional *Ag/AgCl* reference electrode is reasonably reproducible which supports that the *CSS_RE* might be an appropriate electrode for measuring ion-activity in various kind of solutions and consequently in whole human blood. This claim has been experimentally proved as the test results in Chapter Six and Chapter Seven show.

Different compositions have been tested to obtain the chemical mixture with the most repeatability since the reference electrode is a constant voltage source for different concentrations. The experimental set-up for these measurements is given in **Fig.5.1**. The following table gives a concise summary of tested options.

No	HMW Poly(vinyl)alcohol (g)	Distilled Water (μl)	Ethanol (μl)	KCl concentration
1	0.1002	800	1000	200 μl of 2 mol/l of KCl
2	0.1502	800	1000	200 μl of 2 mol/l of KCl
3	0.2005	2000	2000	250 μl of 2.5 mol/l of KCl
4	0.2505	2000	2000	250 μl of 2.5 mol/l of KCl
5	0.3005	2000	2000	250 μl of 2.5 mol/l of KCl
6	0.3505	2000	2000	250 μl of 2.5 mol/l of KCl
7	**0.4008**	**3000**	**2000**	**500 μl of 3 mol/l of KCl**
8	0.4508	3000	2000	500 μl of 3 mol/l of KCl
9	0.5008	3000	2000	500 μl of 3 mol/l of KCl
10	0.5508	3000	2000	500 μl of 3 mol/l of KCl
11	**0.6042**	**3000**	**4000**	**1000 μl of 6 mol/l of KCl**
12	0.6542	3000	4000	1000 μl of 6 mol/l of KCl
13	0.7042	3000	4000	1000 μl of 6 mol/l of KCl
14	0.7542	3000	4000	1000 μl of 6 mol/l of KCl
15	0.8024	3500	4000	1500 μl of 6 mol/l of KCl
16	0.8524	3500	4000	1500 μl of 6 mol/l of KCl
17	0.9024	3500	4000	1500 μl of 6 mol/l of KCl
18	0.9524	3500	4000	1500 μl of 6 mol/l of KCl
19	1.000	3500	4000	2000 μl of 6 mol/l of KCl

Table 5.2 Optimum mixture of miniaturized CSS_RE

Although different compositions have been tested, it has been experimentally shown that composition number 7 is the best mixture for the intended application due to its reproducibility within ±2 millivolts whereas the reproducibility of the composition number **11** is within ±5 millivolts. That is why this research used the composition number **7** as the standard mixture and its voltage response is measured with respect to a conventional silver-silver chloride double-junction reference electrode, as shown in **Table 5.1** above. Except solutions number **7** and **11** the remaining seventeen solutions were **not** reproducing a constant voltage value less than ±**2** mV and ±**5** mV which is highly important in order to be used as the chemical composition of the reference electrode of the sensor array.

5.2.10 The reference electrode (*CSS_RE*) was tested to measure K^+ ion-activity in *KCl* solutions with *2 mmol/l*, *4 mmol/l*, and *6 mmol/l* concentrations. The results are tabulated in **Table 5.3.**

	tests (mV)			time
Solutions	t1	t2	t3	(second)
2 mmol/l	195	197	196	60
4 mmol/l	207	205	210	60
6 mmol/l	227	227	227	60

Table 5.3 Test results of K^+*_CSS_IS* versus *CSS_RE*

The *CSS_RE* is prepared for single use or at most for two uses since with more than two uses it might lose its characteristics, due to leaching effect of PVA into the solutions or vice versa, in such a way that it cannot measure and identify the test solutions properly.

The detailed configuration of the sensing and reference electrodes, in this case for K^+, connected to a high impedance electrometer to measure the potential difference between the two half cells of the measurement system is shown in **Fig. 5.6**:

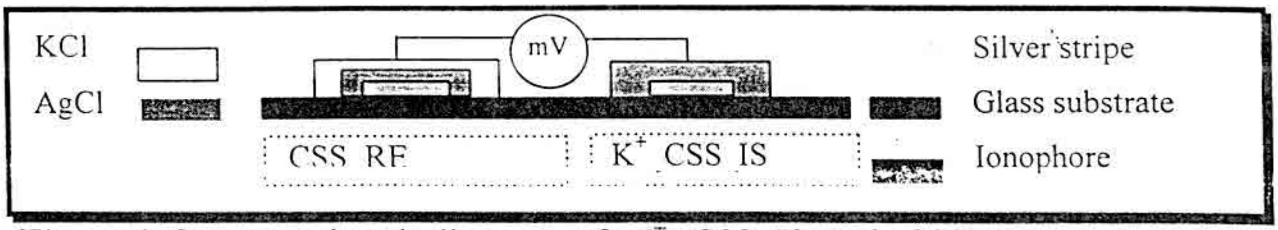


Fig. 5.6 Cross-sectional diagram of *K^+_CSS_IS* and *CSS_RE* on the same substrate

5.2.11 The chemical composition of the coated silver-stripe ion-selective sensor (*K^+_CSS_ISS*)[5.4] and coated silver-stripe reference electrod (*CSS_RE)* [5.4] are given in **Table 5.4** and **Table 5.5**.

Ion	Ionophore (wt.%)	Plasticizers (wt. %)	PVC (wt. %)	Salt (wt. %)
K^+	*1 %* *Valinomycin*	*65.5 %* *ETH 469*	*33*	*0.5 %* *K[B(p-ClPh)4]*

Table 5.4 The chemical composition of *K^+_CSS_IS*

PVA (hmw) (wt. %)	Distilled water (µl)	Ethanol (µl)	3 mol/l KCl (µl)
0.4008	*3000*	*2000*	*500*

Table 5.5 The chemical composition of *CSS_RE* for Potassium

Fig. 5.7 shows the effectiveness of the technique described for reliable measurement at differing levels of activity provided the sensor is not used for more than three or four measurements in rapid succession.

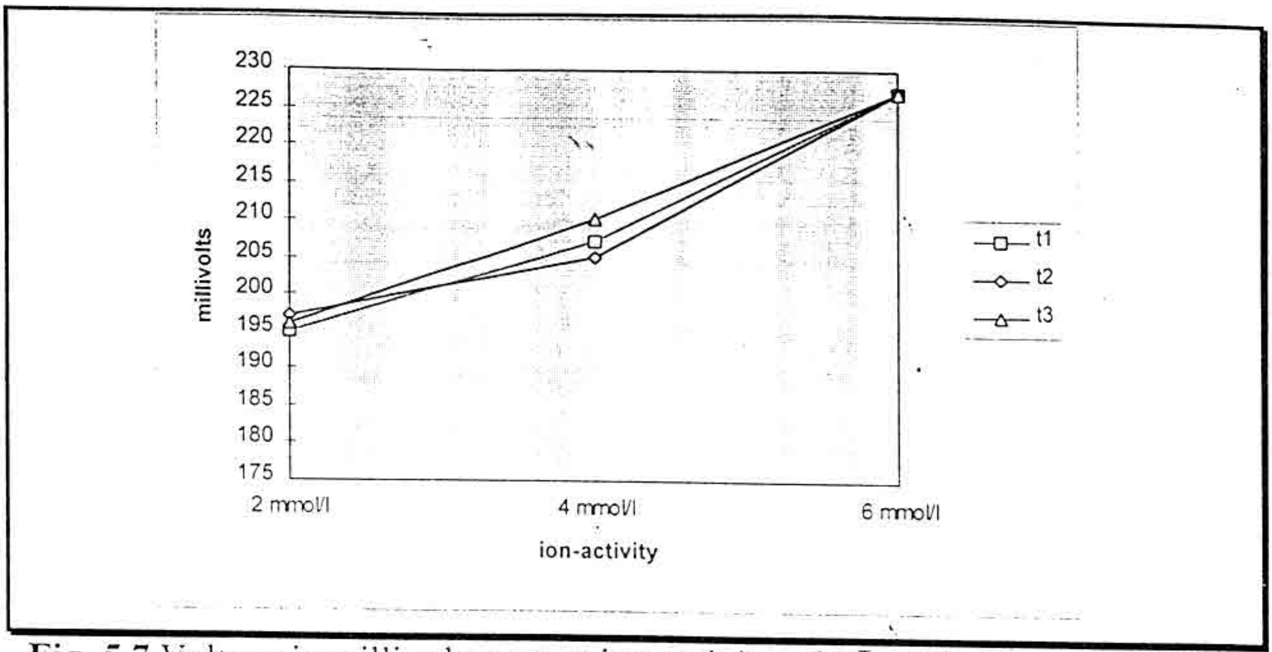


Fig. 5.7 Voltage in millivolts versus ion-activity of K^{+}_*CSS_IS* and *CSS_RE*

5.2.12 The reference electrode (*CSS_RE*) was tested to measure Na^{+} ion-activity in $NaCl$ solutions with *50 mmol/l*, *100 mmol/l*, and *200 mmol/l* concentrations. The results are tabulated in **Table 5.6.**

	Tests (mV)				Time
Solutions	t1	t2	t3	t4	(second)
50 mmol/l	94	95	97	94	60
100 mmol/l	115	117	116	115	60
200 mmol/l	136	138	135	137	60

Table 5.6 Test results of Na^{+}_*CSS_IS* versus *CSS_RE*

Again the *CSS_RE* is prepared for single use or at most for two uses since for more than two uses it might lose its characteristics in such a way that it cannot measure and identify the test solutions properly.

The detailed configuration of the sensing and reference electrodes, in this case for Na^{+}, connected to a high impedance electrometer to measure the potential difference between the two half cells of the measurement system is shown in **Fig. 5.8**:

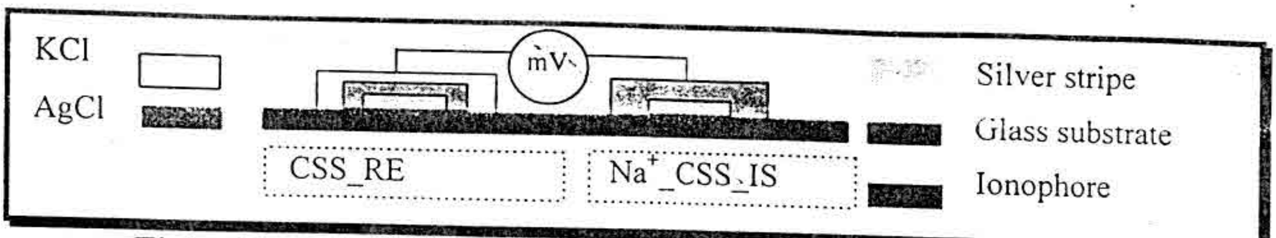


Fig. 5.8 Cross-sectional diagram of *Na^+_CSS_IS* and *CSS_RE*

5.2.13 The chemical composition of the coated silver-stripe ion-selective sensor (*Na^+_CSS_ISS*)[5.4] and coated silver-stripe reference electrode[5.4] (*CSS_RE*) are given in **Table 5.7** and **Table 5.8** respectively.

Ion	Ionophore (wt.%)	Plasticizers (wt. %)	PVC (wt. %)
Na^+	*1 %* *ETH 157*	*66 %* *BBPA*	*33*

Table 5.7 The chemical composition of *Na^+_CSS_IS*

PVA (hmw) (wt. %)	Distilled water (µl)	Ethanol (µl)	3 mol/l KCl (µl)
0.4008	*3000*	*2000*	*500*

Table 5.8 The chemical composition of *CSS_RE* for Sodium

Fig. 5.9 shows the effectiveness of the technique described for reliable measurement at differing levels of activity provided the sensor is not used for more than three or four measurements in rapid succession.

The reason different concentrations have been used is as potassium, sodium, and calcium each has its own unique concentration in human blood. The solutions are in fact trying to imitate the actual concentrations. If the sensor-array composition is not going to work with the solutions, either binary or mixed, it is highly likely that it will not function as expected in real human blood as well which means that with the current chemical composition at hand it is not possible to sense the ions intended to be sensed.

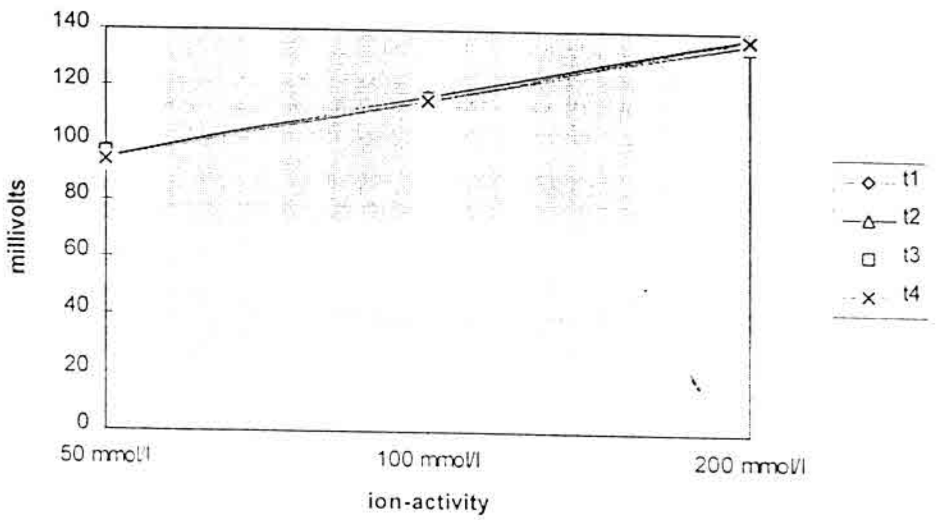


Fig.5.9 Voltage in millivolts versus concentration of Na^{-} _CSS_IS and CSS_RE

5.2.14 The reference electrode (*CSS_RE*) was tested to measure Ca^{2-} ion-activity in $CaSO_4$ solutions with *5 mmol/l, 10 mmol/l,* and *20 mmol/l* concentrations. The results are tabulated in **Table 5.9**.

	Tests [mV]			Time
Solutions	t1	t2	t3	(second)
5 mmol/l	209	207	237	60
10 mmol/l	218	216	248	60
20 mmol/l	226	224	256	60

Table 5.9 Test results of Ca^{-+} _CSS_IS versus CSS_RE

Once again *CSS_RE* is prepared for single use or at most for two uses since for more than two uses it might lose its characteristics in such a way that it cannot measure and identify the test solutions properly.

The detailed configuration of the sensing and reference electrodes, in this case for Ca^{+2}, connected to a high impedance electrometer to measure the potential difference between the two half cells of the measurement system is shown in **Fig. 5.10**:

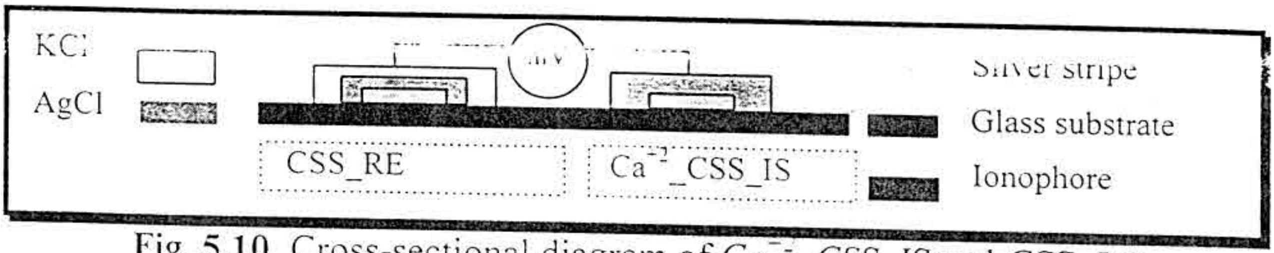


Fig. 5.10 Cross-sectional diagram of *Ca^{+2}_CSS_IS* and *CSS_RE*

5.2.15 The chemical composition of the coated silver-stripe ion-selective sensor (*Ca^{2+}_CSS_ISS*)[5.4] and coated silver-stripe reference electrode[5.4] (*CSS_RE*) are given in **Table 5.10** and **Table 5.11** respectively.

Ion	Ionophore (wt.%)	Plasticizers (wt. %)	PVC (wt. %)	Salt (wt. %)
Ca^{++}	*3.3 %* *ETH 1001*	*64.7 %* *ETH 469*	*30.9*	*2.1 %* *K[B(p-ClPh)4]*

Table 5.10 The chemical composition of *Ca^{++}_CSS_IS*

PVA (hmw) (wt. %)	Distilled water (µl)	Ethanol (µl)	3 mol/l KCl (µl)
0.4008	*3000*	*2000*	*500*

Table 5.11 The chemical composition of *CSS_RE* for Calcium

Fig. 5.11 shows the effectiveness of the technique described for reliable measurement at differing levels of activity provided the sensor is not used for more than three or four measurements in rapid succession.

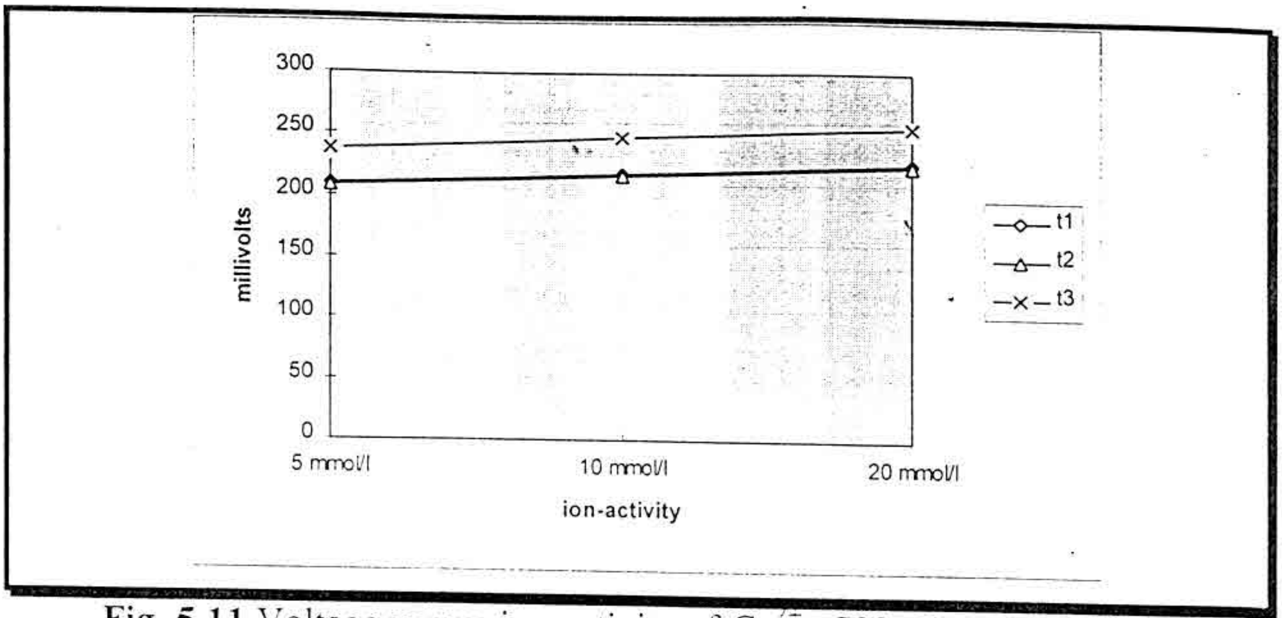


Fig. 5.11 Voltage versus ion-activity of Ca^{2+}_*CSS_IS* and *CSS_RE*

5.3 Conclusions

The *CSS_RE* is prepared for single use or at most for two uses. With more than two uses, it might lose its characteristics, due to leaching of PVA into the solutions or vice versa, in such a way that it cannot measure and identify the test solutions properly. The reason is that the water is diffusing into the reference electrode membrane in the meantime and at the same time PVA is leaching as well. Different ratios of *high molecular weight (hmw) poly(vinylalcohol), (PVA),* have been tested although for the purpose of this work it has been experimentally realized that the composition used is the most optimum one. This work tends to develop some disposable miniaturized ion-sensors for simultaneous multi-sensing of K^+, Na^+, *and* Ca^{2+} ion concentration or ion activity in the human blood.

In comparison with conventional reference electrodes, the life-time of the reference electrode developed by this research is quite short because of the diffusion of *KCl* into the test solution or vice versa but it has the advantages of small size, disposability, cheapness, easy usage and preparation, and is easily mass produced yet gives reproducible results. Finally it gives better possibility of integration with a microelectronic design of chemical sensors.

Until recently, the ISFETs, miniaturized ISEs and CWEs were used mostly in combination with conventional reference macroelectrodes. Hence, this reduces considerably the benefits gained from the miniaturization of the sensors or sensor-arrays. Attempts to miniaturize reference macroelectrodes of the second kind resulted in a serious reduction of the electrode lifetime due to the reduced electrolyte quantity[5.5]. Therefore, reference electrodes without a reference electrolyte appear to be advantageous. Due to this fact this research incorporates a small amount of electrolytes in *PVC* leading to negligible electrolyte and disposable reference electrode of a second kind. The advantage it provides is questionable but the research point is to show that the idea of a reference electrode without an electrolyte is important specifically for disposable sensor arrays.

The main reason for using silver vapour deposition instead of traditional silver wire is that with this method it is expected to be easier to have an array of sensors interfaced with microelectronics based systems.

Each shell of the electrode system has been checked under the microscope with a two-level magnification system to observe whether there exists any air bubble, hole or any impurity in it or not since air bubbles are especially very detrimental to the effective functioning of the electrodes. It has been observed that the more diluted the solution the more homogenous it gets. One of the reason the electrodes get air bubbles might be due to the use of pipettes, so great attention was paid to this fact during electrode preparation. Any discontinuity within the electrodes directly effects the proper functioning of the electrodes. A second important factor is that the chemicals of the ion sensors and reference electrodes must adhere very well to the silver stripe deposited onto the glass substrate. This fact is one of the reasons behind the usage of high molecular weight poly(vinyl chloride). Since in *poly(vinyl alcohol)* bacteria can not survive that is another preferable reason for using *PVA* in such systems.

One of the reasons for drift with the measurement system is the small quantity of the sample solutions used and the long waiting time for a stable reading; meanwhile due to evaporation of the sample a constant drift from the system might be observed. The main reason *3 mol/l KCl* solution has been used is to minimise the junction potential effect, if indeed there is any such effect.

Chapter Six

The Measurements of the Sensor Array of K^+, Na^+, and Ca^{2+} with Binary and Mixed Solutions

Summary

This chapter introduces the sensing mechanisms of each ion sensor, the preparation of the stock and test solutions, membrane composition of the ion sensors, and the test results of the ionophores based sensor array with binary and mixed solutions in single- or multi-sensing modes respectively. Finally it gives the calibration curves of each ion sensor separately.

6.1 Introduction

Increasing interest in the use of miniaturized ion-selective electrodes (M-ISEs) where the degree of miniaturization depends entirely on the application, as chemical sensors has been observed in recent years[6.1]. Potentiometry allows a simple and fast determination of the activity of inorganic ions to be measured and it is not as expensive as other analytical methods such as flame photometry which is often used in clinical laboratories and atomic absorption. However, there exist some disadvantages in the practical and readly use of conventional ISEs in clinical laboratories since they are expensive, bulky, and they have fairly large dimensions, complex structures and *their reproducibility is diminished after the membrane has been in contact with body fluids.* Therefore, some simple and possibly disposable electrodes permitting ion-activity measurement are required.

Among the smallest and simplest electrodes are those of the "*first kind*", namely fine metallic wires such as *Ag* and *Cu* that respond to changes in $a_{Ag}{}^{+}$ *and* $a_{Cu}{}^{2+}$, respectively. However, their availablity is only for a few cations. *A wider variety of ions give potentiometric responses to electrodes of the "second kind" in which a metal wire is coated with a poorly soluble salt of that metal, as with the Ag/AgCl electrode which responds to changes in* $a_{Cl}{}^{+}$. Although the salt layer is usually deposited by anodizing the metal electrode in a suitable electrolyte, *recently it has been shown*[6.2] *possible to incorporate the poorly soluble salt in a polymeric matrix*, affixed to the metal wire, provided that the salt particles are in continuous contact from the outer surface of the *polymer film* to the metal surface underneath.

Eastman Kodak company have developed *film-electrodes*[6.1] as a new generation of low-cost chemical sensors for clinical applications. These electrodes are composed of multilayer elements containing *flat reference electrodes* in contact with hydrophobic ion-selective membranes. They are based on the principle of *zero-point potentiometry;* the reference and measuring systems are identical. Controlled manufacture of these *multilayer film electrodes* requires very sophisticated and expensive methods, so it was decided to investigate the construction of similar but simpler electrodes with good properties.

This research is looking for ion-sensors having multi-layers based on basic chemicals without going through sophisticated and expensive multilayer film electrodes techniques but capable of measuring ion-activity as a disposable sensor-array.

To do this, the present work deals with the construction of K^{+}, Na^{+}, and Ca^{2+} ion-sensors in a new way which is a new version of the coated-wire ion-selective electrodes [6.3]. A thin silver-stripe (approximately *8.3 μm* thick) is vapour deposited on glass and directly contacted with various ion-selective membranes incorporated in PVC, valinomycin, ETH 157, and ETH 1001, corresponding to the cations to be measured. The realized multisensor sensor-array is a simple cost-effective system with disposable reference electrode showing reasonable properties. Actually at the beginning what *Freiser, H. and his research group* wanted were to show that a molecular dispersion, or solution, of a salt or complex in a polymer matrix such as polyvinyl chloride, epoxy resin, or methyl methacrylate painted on a platinum wire should function as an electrode responsive to one of the ions of the salt. Then the whole range of so-called liquid membrane electrodes could be produced in inexpensive, compact form. Finally they proved the very promising idea with coated wire Ca^{2+} ion-selective electrode[6.3]. *To measure the potential response of the coated wire Ca^{2+} ion-selective electrode they used a saturated conventional calomel electrode as the reference electrode.*

The chemical composition of coated wire Ca^{2+} ion-selective electrode is given by *Freiser, H. et al.,* in [6.3] as below: " Coat a *0.018-in. platinum wire with a 6:1 mixture of 5% polyvinyl chloride dissolved in cyclohexanone and 0.1M calcium didecylphosphate in dioctylphosphonate*, then dip the wire in the mixture several times and allow to set overnight in air. Cover the remainder of the wire by wrapping it tightly with a paraffin film to prevent direct contact of the metal surface with the test solution." By the year 1974, *Freiser, H., Cattrall, R. W., and Tribuzio, S.* [6.3] had proved that coated wire ion-selective electrodes could successfully be applied to a variety of cationic and anionic species involving ion association and chelate systems. This had encouraged the team to investigate the feasibility of this approach in the so-called neutral carrier electrode system. They tried the first potassium ion-selective electrode based on valinomycin and its application to the analysis of potassium in whole sheep blood and in sea water[6.3]. The chemical composition they had preferred to use is: " *Various* *coating mixtures of PVC, a plasticizer (either di(2-ethyl hexyl)-2-ethylhexylphosphonate (DOOP) or di-n-decylphthalate (DDP) and valinomycin prepared by weighing out the materials and dissolving the mixture in the minimum amount of tetrahydrofuran.*" *The reference electrode they used was a double junction calomel electrode with 10%* NH_4NO_3 *in the outer compartment.*

The most relevant fields of application of neutral-carrier-based ion-selective electrodes are clinical chemistry and bioelectrochemistry[6.4]. In both fields the response time of the sensors is of utmost importance when the aim is to achieve a large sample throughput or to follow fast biological processes. On the other hand, there are very compelling requirements concerning the selectivity, sensitivity, potential stability, reproducibility, and lifetime of the sensors. To find the best compromise, one should know the exact influence of the different components and of their properties on the response time of the sensor and this is actually the job that must be left to chemists. This has been done. To find the best or optimum chemical combination needed for sensor design will take some tough research and there is a highly unpredictable financial side of it.

The ion-selective membranes used in such macroelectrodes usually consist of about 1 wt % ionophore, 60-65 wt % plasticizer, 30-35 wt % PVC, and a strictly limited amount of lipophilic salt additives like sodium tetraphenylborate (NaTPB) or potassium tetrakis(p-chlorophenyl)borate (KTpClBP). Only the appropriate selection of each of these constituents finally guarantees a highly selective and sensitive electrode that exhibits a stable and reproducible potential response[6.4]. On the other hand, the specifications required for special applications such as in clinical chemistry can be fulfilled only by a very careful optimization of the membrane composition, which often involves compromises between the competitive influences of components to be made. To study the influence of the lipophilicity of the ligand, the lipophilicity of the plasticizer, the presence of lipophilic salt additives in the membrane, and modifications of the membrane matrix on the response time of selected cation-selective neutral-carrier-based PVC membrane electrodes is very important and interesting since only sensors based on relatively lipophilic membrane components have practical relevance for clinical routine analysis. High lipophilicity is even more crucial when minielectrodes, fabricated with modern planar technologies, are concerned[6.4].

Hence, the sensor array under consideration for this specific research is neither a microelectrode (μm sized)) nor macroelectrode (conventional) sensor array, in actual fact, it is somewhere in between (20 mm^2). The research intention was and is to find a intermediate way which will help to integrate readily the sensor array with microelectronics involving very high input impedance circuit design (CMOS design).

However, it is difficult to investigate the dependence of, for example response time, on a single parameter because by varying the lipophilicity of the ligand and/or of the plasticizer, not only the extraction properties but also several other decisive parameters of the membrane, (e.g. viscosity, ion permeabilities, etc.) are changed. As a lipophilic anionic-site additive, *potassium tetrakis (p-chlorophenyl)borate* was used. The incorporation of additional anionic sites (*KTpClPB*) into conventional neutral-carrier-based PVC membranes generally leads to fast responding electrodes and the dynamic behavior is often found to become almost independent of the properties of the ionophore applied[6.4]. The electrodes with *KTpClPB* additive become so fast that the overall transients are primarily controlled by the time of switching and mixing.

Membrane thickness is determined by the solutions of *20* μl poured onto the silver-stripe deposited on glass substrate. The active membrane area was around *20* mm^2 depending on the liquid distribution and viscosity. The lipophilicities of the plasticizers and of the ionophores, log P, are determined by thin-layer chromatography[6.4]. Membrane electrodes with *poly(vinyl chloride) (PVC)* matrices incorporate a number of different types of selective binding sites, such as neutral carrier or ion exchange molecules. *The most successful example is the K^+ electrode based on valinomycin.* This antibiotic molecule is a doughnut-shaped complex with an electron-rich pocket in the center into which K^+ ions are selectively extracted, causing the desired charge separation.

To provide mechanical strength, the binding sites are typically incorporated into the semi-rigid, but liquid-state, plasticized PVC membrane[6.5]. The commercial successes for single sensing only in binary solutions to date include neutral carrier-based electrodes for K^+, Na^+, Ca^{2+}, NH_4^+, H^+, and an ion-exchanger-based electrode for Cl^-. The PVC-based electrodes are simple, inexpensive, long-lived, and easy to maintain, but they do have a sensitivity to interfering lipophilic ions. Charged surfactants, preservatives, or interferences with lipophilic character will be extracted into the lipophilic membrane phase and either enhance the charge separation or depolarize the membrane. So it must be remembered that lipophilic ions may cause errors with this type of sensing membrane[6.5].

6.2 Sensing mechanism of ion sensors

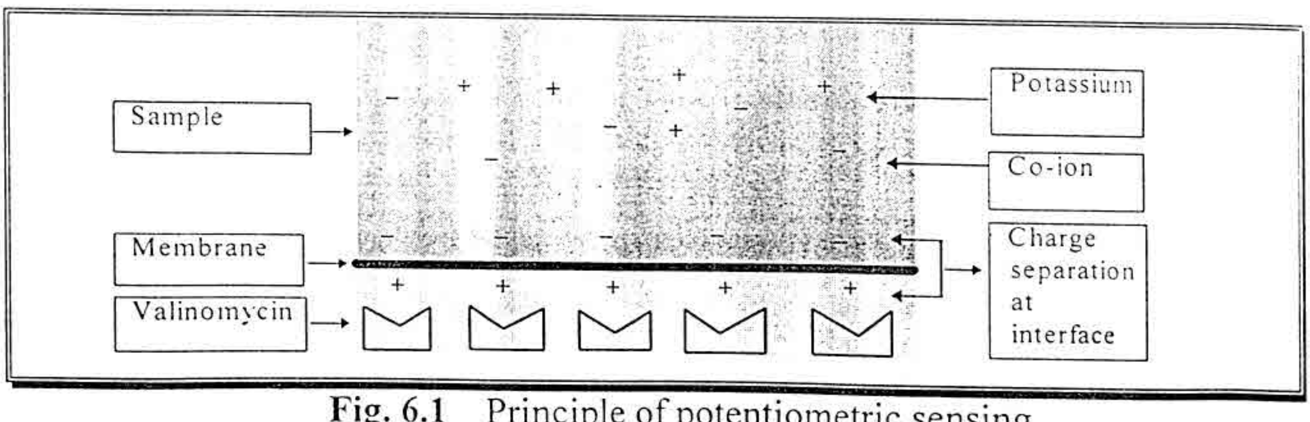


Fig. 6.1 Principle of potentiometric sensing

The ISE membrane extracts target ions, leaving the co-ions behind. The resulting charge separation established across the sample-membrane interface generates a potential that is related to the concentration of the target ions in the sample[6.5].

Potentiometric transducers are based on the principle of the accumulation of a membrane potential as a result of the selective binding of ions to a sensing membrane[6.7]. The change in potential is measured, there being a logarithmic relationship between potential and concentration.

The development of ion-selective electrodes (ISE), in which ions present in the sample become selectively bound to the surface of the sensor and set up a charge separation between the sample and sensor surface, has proved successful. The potential generated is measured against a reference electrode maintained at zero current flow, and is proportional to the activity of ions present in the solution.

The principle of potentiometric sensing, illustrated in **Fig.6.1** with the potassium ISE, in which the antibiotic valinomycin selectively binds potassium ions within the membrane, leaving behind the co-ions in the sample. The resulting charge separation, which is established across the sample/membrane interface, generates a potential that is related to the concentration of potassium ions in the sample[6.7].

All electrochemical sensors fall into one of two categories: potentiometric or amperometric. In the potentiometric type, a membrane or sensing surface acts as a source of EMF, generating a potential proportional to the logarithm of analyte concentration (activity). This potential is measured relative to an inert reference electrode also in contact with the sample. Because most membranes are poor conductors, potentiometric measurements are made under conditions of essentially zero current flow. Current flow tends to discharge the battery and disturb the equilibrium at the sample-membrane interface.

The other side of the coin is the amperometric sensor. Here a voltage is applied between the working and reference electrodes. This imposed potential encourages electron transfer (redox) reactions to occur, causing a current flow that is proportional to the concentration of the analyte.

Potentiometric devices can be used for direct and indirect determinations; there are advantages and disadvantages to each. In direct testing, the sensor is placed in contact with the whole blood, plasma, serum or any sample available. *As a result, direct potentiometry lends itself to an emergency type of application*[6.5]. The disadvantage, however, is that measurements in undiluted samples are sensitive to activity coefficient and residual liquid junction potential errors. In the case of K^- and Na^+ determinations, for example, the assay values obtained by direct potentiometry do not exactly match those obtained with a diluted technique. This is a source of confusion in the clinical arena, and it requires that the clinician and physician know and understand the subtle differences between the two techniques.

With indirect potentiometry the sample is diluted prior to measurement. A major feature is that samples can be assayed in the highly automated batch analyzers found in the main hospital laboratory. Also, it is not necessary to worry much about junction potentials and activity coefficients because their effects are essentially swamped out by the diluent. However, it is required that more awareness is made of the importance of total solids content of the sample. For example, if a clinical sample has an abnormally high lipid or protein content, the assay results for K^+ or Na^- will be lower than expected due to the displacement effect of the elevated solids content.

Thus, there is a trade-off in the use of direct versus indirect measurements. With direct determinations there must be awareness of junction potentials and activity coefficients, and with indirect analyses a realization that the solids content will have an impact. Indirect potentiometric measurements are popular because they match the historical reference methods, e.g., flame photometry, that have been used for years. In the real world of the clinical lab, where people are especially busy, new technology (direct potentiometry) that upsets the status quo is accepted slowly. Thus, a method that gives historical values is attractive[6.5].

6.3 Chemical composition of ion sensors

Solvent(plasticizer)/polymeric membranes function as ion-selective transducers only when an appropriate ionophore is incorporated within the polymeric film or level. The ionophore serves as a reversible and reusable binding reagent that selectively extracts the analyte ion into the organic membrane phase, thereby creating a charge separation or phase boundary potential at the membrane sample/interface. Selectivity is largely determined by the relative affinity of cations for the carrier (ionophores), and is dependent on the use of a proper solvent. The solvent is the vehicle which allows the neutral carrier mobility within the PVC matrix and both influences and allows the selectivity process to occur. These solvents, or plasticizers, perform their function best when they display high lipophilicity and viscosity[6.6]. Very often, the addition of lipophilic additives accentuates/intensifies the effect of the solvent alone in an inert matrix such as poly(vinyl chloride), PVC, or silicone rubber.

Since coated silver-wire electrodes are mostly and preferentially used in research and development projects, this research uses a vapour deposition technique to deposit on to glass substrate a thin silver layer as the conduction medium of the ion sensors as well as the miniaturized reference electrode.

A *8.3 μm* thick, *70 mm* long, and *1 mm* wide with *circular crosssectional* area of *20* mm^2 four electrodes are deposited onto the glass substrate. A pH meter is used to measure the output voltage of the sensor system in millivolts. The input impedance of the meter is $10^{12}\ \Omega$. The larger the input impedance of the measuring system the better the measurements because the larger the input impedance the smaller is the current passing through the electrodes leading to less drift and higher polarization of the ion-sensors. For example, with a $10^{15}\ \Omega$ input impedance the electrodes will have 1000 times less current passing through.

Three coated silver-stripe ion-sensors (*CSS-ISs*) for K^+, Na^+, *and* Ca^{2+} ion-activity measurement of total human blood and one coated silver-stripe disposable reference electrode incorporating the internal solution (*CSS-RE*) have been prepared and tested with different binary solutions and mixed solutions of each cation under investigation. This emf, a function of the ionic activities within the cell, can be used analytically to measure ionic concentrations in titrations, process streams, biological fluids and a multitude of other situations. Hence this is why potentiometry is the most widely used analytical technique. An ion sensor often yields a reasonable response over a wide analyte concentration range of 10^{-} *to 1* mol/dm^3 and it can be employed in both aqueous and non-aqueous environments. The apparatus required to carry out the analyses is both simple and inexpensive and ion sensors often remain effective for long period of time. The ion-sensor array can be coupled directly into an analogue-to-digital converter for data acquisition by a computer since analytical methods involving galvanic cells can therefore be readily automated and computerized.

6.4 The preparation of stock solution [3mol/l KCl]

The molecular weight of *KCl* is *74.56*g. So, three molecular weight is equal to $3\times74.56 = 223.68$g and by adding this quantity into one litre of deionised water, then *3 mol/l* KCl solution is obtained hence this solution is called *KCl stock solution.*

6.5 The preparation of test solutions

Using this stock solution, it is easy to prepare *1 mmol/l, 2 mmol/l, 3 mmol/l, 4 mmol/l, 5 mmol/l and 6 mmol/l KCl* solutions for ion-sensors testing. For example, to get *3 mmol/l KCl* solution it is needed to add *1000 µl KCl* stock solution into *1 l* of water. Others can be prepared accordingly, the table below shows the µl quantities required to obtain recommended mmol/l solutions.

1 mmol/l	2 mmol/l	3 mmol/l	4 mmol/l	5 mmol/l	6 mmol/l
333 µl	666 µl	1000 µl	1333 µl	1666 µl	2000 µl

Table 6.1 The preparation of test solutions of *KCl*

Sometimes 2, 4, and 6 mmol/l solutions and sometimes 1, 3, and 6 mmol/l solutions have been used for testing purposes. The following are potassium ion-sensor responses to its own different solutions with various concentrations and with different sensor-array preconditioning times [SAPT] of 3, 5, 6, and 7 minutes.

6.6 Tests results of K^+-ion sensor with different SAPTs

In actual fact, there are **many parameters effecting the measurement of such nature, among them are some**: the structure of the reference electrode, the ion-sensor structure in terms of ionophore, plasticizer, salt, and PVC, and chemical interference, selectivity of the membrane, space occupying effects of protein and lipid in the sample, variation in ionic strength and activity coefficient, rate of response of the membrane (change in concentration, thickness, dimension, and ion-exchange rate, change in activity, temperature, and stirring rate), temperature, interfering ions and junction potentials. Of these factors, the activity coefficient is the most important and is influenced by ionic strength, particularly if direct measurements are required. These comments equally apply to sodium and calcium measurements as well.

1^{st} $K^{\pm}$-Ion Sensor response:

	[SAPT] is 3 minutes			
	Tests (mV)			Time
Solutions	t1	t2	t3	(second
2 mmol/l	195	197	196	60
4 mmol/l	207	205	210	60
6 mmol/l	227	227	227	60

Table 6.2 K^{-}-Ion-sensor response with [SAPT] 3 minutes

2^{nd} $K^{\pm}$-Ion-Sensor response:

	SAPT is 5 minutes			
	Tests (mV)			Time
Solutions	t1	t2	t3	(second)
2 mmol/l	149	147	146	60
4 mmol/l	164	162	165	60
6 mmol/l	182	180	183	60

	SAPT is 5 minutes			
	Tests (mV)			Time
Solutions	t1	t2	t3	(second)
2 mmol/l	192	190	190	60
4 mmol/l	212	214	216	60
6 mmol/l	226	228	227	60

	SAPT is 5 minutes			
	Tests (mV)			Time
Solutions	t1	t2	t3	(second)
2 mmol/l	180	156	142	60
4 mmol/l	212	161	162	60
6 mmol/l	234	178	175	60

This ion-sensor was left to dry for 24 hours before being tested.

	SAPT is 5 minutes			
	Tests (mV)			Time
Solutions	t1	t2	t3	(second)
2 mmol/l	179	178	186	60
4 mmol/l	195	198	211	60
6 mmol/l	212	208	222	60

Table 6.3 K^+-Ion-sensor response with [SAPT] 5 minutes

3^{rd} $K^{\pm}$-Ion-Sensor response:

	SAPT is 6 minutes			
	Tests (mV)			Time
Solutions	t1	t2	t3	(second)
2 mmol/l	159	161	157	60
4 mmol/l	173	172	175	60
6 mmol/l	185	187	190	60

	SAPT is 6 minutes			
	Tests (mV)			Time
Solutions	t1	t2	t3	(second)
2 mmol/l	162	164	163	60
4 mmol/l	182	180	181	60
6 mmol/l	194	190	191	60

Table 6.4 K^+-Ion-sensor response with [SAPT] 6 minutes

4^{th} $K^{\pm}$-Ion-Sensor response:

	SAPT is 7 minutes			
	Tests (mV)			Time
Solutions	t1	t2	t3	(second)
2 mmol/l	120	122	121	60
4 mmol/l	135	137	138	60
6 mmol/l	156	154	153	60

Table 6.5 K^+-Ion-sensor response with [SAPT] 7 minutes

5^{th} $K^{\pm}$-Ion-Sensor response:

	SAPT is 6 minutes		
	Tests (mV)		Time
Solutions	t1	t2	(second)
2 mmol/l	224	222	60
4 mmol/l	229	227	60
6 mmol/l	233	233	60

Table 6.6 K^+-Ion-sensor response with [SAPT] 6 minutes

The duration of all the measurements is only 60 seconds. Only three successive tests have been carried out for each group having different SAPT times. With all SAPT times of 3, 5, 6, and 7 minutes reasonable tests results have been obtained. However, the shorter the SAPT time, the better the *CSS_RE* lifetime leading to a preference for choosing 3 minutes as the SAPT time.

The effect of temperature on the measurement (potential) is negligibly small for it is around *1 or 2 mV per 5 °C*. This is of importance when automatically compensating for the effects of temperature on the potential E to be applied in case of intelligent sensor-array design. To do this, another sensor measures the temperature of the sample, and the signal from that sensor is fed to a temperature-compensating circuit. In actual fact, the temperature stability depends mainly on the chemistry of the system for reference electrodes as well as ion-sensors.Test results show that the ion-sensor array is capable of differentating various test solutions with the proviso that the system is ready to use with real samples.

6.7 The characteristics of potassium coated silver-stripe ion sensor

Potassium Coated Silver-Stripe Ion Sensor (K^{+}-CSS-IS)	
Technology	*Thin-film (8.3 μm)*
Deposition Technique	*Vapour Deposition (VD)*
Sensor Type	*Electrochemical*
Sensor Area	*20 mm^2*
Sensor Chemical Structure or mixture	*Ionophore+Plasticizer+ PVC +Salt*
Sensor Layers	*2*
Sensor Reference Electrode	*CSS-RE All solid-state $Ag/AgCl/Cl^-$*
Measurement Method	*Direct Potentiometry*
Test Solutions	*2, 4, and 6 mmol/l or* *1, 3, and 6 mmol/l*
Test Solutions Technique	*Separate Solutions Method (SSM)*

Response Time	*5-10 sec.*
pH range	*3-4*
Sensor Preconditioning Time (SPT)	*1,3,5,6, or 7 minutes*
Sensor Preconditioning Solution	*1 mmol/l of KCl*
Sensor Response Type	*Nernstian*

Table 6.7 The Characteristics of Potassium Coated Silver-Stripe Ion Sensor

6.8 Calibration chart of the potassium ion Sensor

	Log K^+ [mol/l]				
	-5	**-4**	**-3**	**-2**	**-1**
Millivolts	220	280	320	370	450

Table 6.8 Calibration characteristic of the potassium ion sensor

6.9 Preparation of stock solution for sodium ion sensor

Stock solution for NaCl test solutions is *1 mol/l* which is equal to one molar. The formula used for molar calculations is given by the equation as $M = \frac{n}{V}\left(\frac{mol}{L}\right)$.

The molecular weight of NaCl is 58.44g. So, 58.44g/l = 1 mol/l =1000 mmol/l. By adding 58.44g of NaCl to one litre of distilled water *1 mol/l stock solution* is obtained.

6.10 The preparation of test solutions for sodium ion sensor

The formula used for solutions with different concentration is as follows, $M_1 \cdot V_1 = M_2 \cdot V_2$. The clinically acceptable value of Na^+ in human blood is 145 mmol/l. More simply and in a practical way, the tests solutions have been prepared as follows:

100 mmol/l =0.1 mol/l

Add 5 ml of 1 mol/l stock solution to 45 ml of distilled water to get 50 ml solution with 0.1 mol/l concentration.

$\left(\frac{5}{50}\right)1 = 0.1$ mol/l which is equal to 100 mmol/l test solution.

110 mmol/l=0.11 mol/l

Add 5.5 ml of 1 mol/l stock solution to 44.5 ml of distilled water to get 50 ml solution with 0.11 mol/l concentration.

$\left(\frac{5.5}{50}\right)1 = 0.11$ mol/l which is 110 mmol/l test solution.

120 mmol/l=0.12 mol/l

Add 6 ml of 1 mol/l stock solution to 44 ml of distilled water to get 50 ml solution with 0.12 mol/l concentration. $\left(\frac{6}{50}\right)1 = 0.12$ mol/l which is equal to 120 mmol/l test solution.

130 mmol/l=0.13 mol/l

Add 6.5 ml of 1 mol/l stock solution to 43.5 ml of distilled water to get 50 ml solution with 0.13 mol/l concentration. $\left(\frac{6.5}{50}\right)1 = 0.13$ mol/l which is equal to 130 mmol/l test solution.

140 mmol/l=0.14 mol/l

Add 7 ml of 1 mol/l stock solution to 43 ml of distilled water to get 50 ml solution with 0.14 mol/l concentration. $\left(\frac{7}{50}\right)1 = 0.14$ mol/l which is equal to 140 mmol/l test solution.

150 mmol/l=0.15 mol/l

Add 7.5 ml of 1 mol/l stock solution to 42.5 ml of distilled water to get 50 ml solution with 0.15 mol/l concentration. $\left(\frac{7.5}{50}\right)1 = 0.15$ mol/l which is equal to 150 mmol/l test solution.

160 mmol/l=0.16 mol/l

Add 8 ml of 1 mol/l stock solution to 42 ml of distilled water to get 50 ml solution with 0.16 mol/l concentration.

$\left(\frac{8}{50}\right) \cdot 1 = 0.16$ mol/l which is equal to 160 mmol/l test solution.

6.11 Tests results of Na^+-Ion-sensor with different SAPTs

1st $Na^{\pm}$-Ion-Sensor Response:

	SAPT is 3 minutes				
	Tests (mV)				Time
Solutions	t1	t2	t3	t4	(second)
50 mmol/l	120	123	125	125	60
100 mmol/l	136	138	147	147	60
200 mmol/l	156	161	164	162	60

Table 6.9 Na^--Ion -Sensor Response with SAPT 3 minutes

2nd $Na^{\pm}$-Ion-Sensor Response:

	SAPT is 5 minutes				
	Tests (mV)				Time
Solutions	t1	t2	t3	t4	(second)
50 mmol/l	120	121	124	122	60
100 mmol/l	137	138	135	136	60
200 mmol/l	150	154	152	151	60

	SAPT is 5 minutes				
	Tests (mV)				Time
Solutions	t1	t2	t3	t4	(second)
50 mmol/l	94	95	97	94	60
100 mmol/l	115	117	116	115	60
200 mmol/l	136	138	135	137	60

Table 6.10 Na^+-Ion-Sensor Response with SAPT 5 minutes

3rd Na^+-Ion-Sensor Response:

	SAPT is 6 minutes				
	Tests (mV)				Time
Solutions	t1	t2	t3	t4	(second)
50 mmol/l	70	71	73	72	60
100 mmol/l	85	87	86	88	60
200 mmol/l	104	106	105	103	60

Table 6.11 Na^+-Ion-Sensor Response with SAPT 6 minutes

4th Na^+-Ion-Sensor Response:

	SAPT is 7 minutes				
	Tests (mV)				Time
Solutions	t1	t2	t3	t4	(second)
50 mmol/l	78	77	78	78	60
100 mmol/l	95	96	94	97	60
200 mmol/l	114	116	115	113	60

Table 6.12 Na^+-Ion-Sensor Response with SAPT 7 minutes

5^{th} $Na^{\pm}$-Ion-Sensor Response:

	SAPT is 3 minutes		
	Tests (mV)		Time
Solutions	t1	t2	(second)
50 mmol/l	83	82	60
100 mmol/l	107	103	60
200 mmol/l	137	128	60

Table 6.13 Na^{+}-Ion -Sensor Response with SAPT 3 minute

6.12 The characteristics of sodium coated silver-stripe ion sensor

Sodium Coated Silver-Stripe Ion Sensor (Na^{+}-CSS-IS)	
Technology	*Thin-film (8.3 μm)*
Deposition Technique	*Vapour Deposition (VD)*
Sensor Type	*Electrochemical*
Sensor Area	*20 mm^2*
Sensor Chemical Structure or mixture	*Ionophore+Plasticizer+ PVC +Salt*
Sensor Layers	*2*
Sensor Reference Electrode	*CSS-RE All solid-state $Ag/AgCl/Cl^{-}$*
Measurement Method	*Direct Potentiometry*
Test Solutions	*100, 120, and 140 mmol/l or* *50, 100, and 200 mmol/l*
Test Solutions Technique	*Separate Solutions Method (SSM)*
Response Time	*5-10 sec.*
pH range	*3-4*
Sensor Preconditioning Time (SPT)	*1,3,5,6, or 7 minutes*
Sensor Preconditioning Solution	*50 mmol/l of NaCl*
Sensor Response Type	*Nernstian*

Table 6.14 The characteristics of sodium coated silver-stripe ion sensor

6.13 Calibration chart of the sodium-ion sensor

	Log Na^+ [mol/l]		
	-3	**-2**	**-1**
Millivolts	80	130	220

Table 6.15 Calibration characteristic of the sodium-ion sensor

6.14 The preparation of stock solution for calcium-ion sensor

The molecular weight of *$CaSO_4$* is equal to *136.14g* which is one mol, so *136.14g* in one mole deionized water makes *1 mol/l* solution. Therefore, 0.1 mol/l solution needs *13.614g/l $CaSO_4$* which is equal to *100 mmol/l* which means *1.3614g/100 ml stock solution*. So, stock solution is *1.3614g/100 ml = 100 mmol/l*.

6.15 The preparation of tests solution for calcium-ion sensor

To prepare from the stock solution, 20 mmol/l, 10 mmol/l, and 5 mmol/l solutions respectively, it is needed to calculate and prepare as follows:

<u>For 20 mmol/l solution:</u>

2 units of stock solution into 8 units of deionized water since

$$\left(2/(2+8) \right) \cdot 100 = 20 \text{ units.}$$

<u>For 10 mmol/l solution:</u>

1 unit of stock solution into 9 units of deionized water since

$$\left(1/(1+9) \right) \cdot 100 = 10 \text{ units.}$$

For 5 mmol/l solution:

1 unit of stock solution into 19 units of deionized water since

$$\left(\frac{1}{(1+19)}\right)\cdot 100 = 5 \text{ units.}$$

6.16 Tests results of Ca^{2+}-Ion sensor with different SAPTs

1st Ca^{+2}-Ion Sensor Response

	SAPT time is 3 minutes			
	Tests [mV]			Time
Solutions	t1	t2	t3	second
5 mmol/l	- 186	- 207	- 237	60
10 mmol/l	- 201	- 219	- 248	60
20 mmol/l.	- 215	- 234	- 256	60

Table 6.16 Ca^{+2}-Ion-Sensor Response with SAPT 3 minutes

2nd Ca^{+2}-Ion-Sensor Response

	SAPT time is 5 minutes					
	Tests [mV]					Time
Solutions	t1	t2	t3	t4	t5	second
5 mmol/l	- 222	- 222	- 223	- 223	- 221	60
10 mmol/l	- 229	- 230	- 231	- 232	- 233	60
20 mmol/l	- 237	- 239	- 240	- 242	- 243	60

Table 6.17 Ca^{+2}-Ion-Sensor Response with SAPT 5 minutes

3^{rd} Ca^{+2}-Ion-Sensor Response

	SAPT time is 3 minutes		
	tests [mV]		
solutions	**t1**	**t2**	Time (seconds)
5 mmol/l	-209	-207	60
10 mmol/l	-218	-216	60
20 mmol/l	-226	-224	60

Table 6.18 Ca^{2+}-Ion-sensor response with SAPT 3 minutes

6.17 The characteristics of calcium coated silver-stripe ion sensor

Calcium Coated Silver-Stripe Ion-Sensor (Ca^{2+}- CSS-IS)	
Technology	*Thin-film (8.3 μm)*
Deposition Technique	*Vapour Deposition (VD)*
Sensor Type	*Electrochemical*
Sensor Area	*20 mm^2*
Sensor Chemical Structure or mixtur	*Ionophore+Plasticizer+ PVC +Salt*
Sensor Layers	*2*
Sensor Reference Electrode	*CSS-RE All solid-state $Ag/AgCl/Cl^-$*
Measurement Method	*Direct Potentiometry*
Test Solutions	*1, 1.5, and 2 mmol/l*
Test Solutions Technique	*Separate Solutions Method (SSM)*
Response Time	*5 sec.*
pH range	*3-4*
Sensor Preconditioning Time (SPT)	*1,3,5,6,8 minutes*
Sensor Preconditioning Solution	*1 mmol/l of $CaSO_4$*
Sensor Response Type	*Nernstian*

Table 6.19 The characteristics of calcium coated silver-stripe ion-sensor

6.18 Calibration chart of the calcium-ion sensor

	Log Ca^{2+} [mol/l]			
	-4	**-3**	**-2**	**-1**
Millivolts	150	200	250	not tested

Table 6.20 Calibration characteristic of the calcium-ion sensor

6.19 The preparation of the multi-electrolytes (or mixed) solutions

In order to prepare mixed electrolytes, it is necessary to get the stock solutions ready, so for the three electrolytes three stock solutions with concentrations of *140 mmol/l (NaCl), 5 mmol/l (KCl),* and *2 mmol/l ($CaSO_4$)* were prepared. To prepare mixed electrolytes, three stock solutions were made as follows:

700 mmol/l NaCl ....(1)

25 mmol/l KCl ....(2)

5 mmol/l $CaSO_4$ ....(3)

To prepare 700 mmol/l NaCl stock solution

700 mmol/l = 0.7 mol/l.

Molecular weight of *NaCl* is equal to *58.44g*. So, *58.44g of NaCl* in *1 l of H_2O* is equal to *58.44g/l or 1 mol/l.* Therefore, *0.7 mol/l* = *(58.44 × 0.7) g* per litre which is equal to *40.908g/l*. This means that to get *700 mmol/l* stock solution, it is necessary to dissolve *40.908g of NaCl* in one litre of deionized water.

To prepare 25 mmol/l KCl stock solution

25 mmol/l = 0.025 mol/l.

Molecular weight of *KCl* is equal to *74.6g*. So, *74.6g of KCl* in *1 l of H_2O* is equal to *74.6g/l or 1 mol/l*. Therefore, *0.025 mol/l = (74.6 × 0.025)g* per litre which is equal to *1.8650g/l*. This means that to get *25 mmol/l stock solution*, it is necessary to dissolve *1.8650g of KCl* in one litre of deionized water.

To prepare 5 mmol/l $CaSO_4$ stock solution

5 mmol/l = 0.005 mol/l. Molecular weight of $CaSO_4$ is equal to *136.14g*. So, *136.14g of $CaSO_4$* in *1 l of H_2O* is equal to *136.14g/l or 1 mol/l*. Therefore, *0.005 mol/l=(136.14 × 0.005) g/l* which is equal to *0.6807g/l*. This means that to *get 5 mmol/l stock solution*, it is necessary to dissolve *0.6807 gr of $CaSO_4$* in one litre of deionized water.

1st Group Solutions

Keep Na^+ and K^- ion-concentrations constant, change Ca^{2+} ion-concentration accordingly. 140 mmol/l NaCl, 5 mmol/l KCl, and **1**, **1.5**, and **2 mmol/l $CaSO_4$**. To do that:

Solutions	1	2	3
Volume of stock NaCl	10 ml	10 ml	10 ml
Volume of stock KCl	10 ml	10 ml	10 ml
Volume of stock $CaSO_4$	**10** ml	**15** ml	**20** ml
Volume of H_2O	20 ml	15 ml	10 ml
Total of	50 ml	50 ml	50 ml.

2nd Group Solutions

Keep Na^+ and Ca^{2-} ion-concentrations constant, change K^+ ion-concentration accordingly. 140 mmol/l NaCl, **1**, **3**, and **5 mmol/l KCl**, and 2 mmol/l $CaSO_4$. To do that:

Solutions	1	2	3
Volume of stock NaCl	10 ml	10 ml	10 ml
Volume of stock KCl	**2** ml	**6** ml	**10** ml
Volume of stock $CaSO_4$	10 ml	10 ml	10 ml
Volume of H_2O	28 ml	24 ml	20 ml
Total of	50 ml	50 ml	50 ml.

3rd Group Solutions

Keep K^+ and Ca^{2+} ion-concentrations constant, change Na^+ ion-concentration accordingly. **100**, **120**, and **140 mmol/l NaCl**, 5 mmol/l KCl, and 2 mmol/l $CaSO_4$. To do that:

Solutions	**1**	**2**	**3**
Volume of stock NaCl	**8** ml	**9** ml	**10** ml
Volume of stock KCl	10 ml	10 ml	10 ml
Volume of stock $CaSO_4$	10 ml	10 ml	10 ml
Volume of H_2O	22 ml	21 ml	20 ml
Total of	50 ml	50 ml	50 ml.

6.20 Multi-sensing of ion-sensors with mixed electrolytes

6.20.1 Simultaneous measurement of K^+-CSS-IS and Na^+-CSS-IS

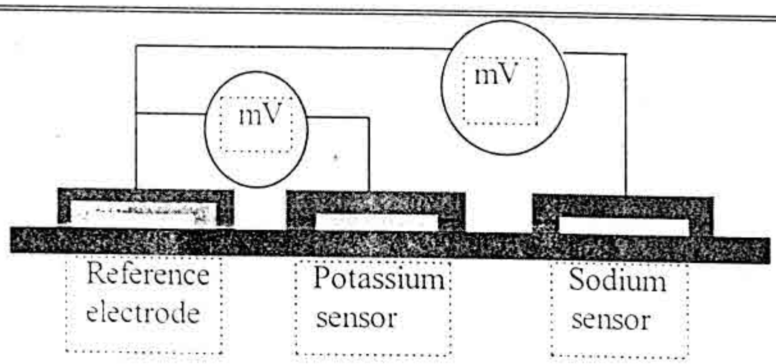


Fig.6.2 System for simultaneous measurement of K^+-CSS-IS and Na^+-CSS-IS with respect to miniaturized silver-silver chloride reference electrode

As the **Fig.6.2** shows, three electrodes are prepared on a single glass slide each having *20 mm²* electrode area. To fabricate the electrodes, first vapor deposition must be done and then special chemicals with specific quantities have been used. (see Chapter Five for details).

1st Simultaneous measurement of potassium- and sodium-ion sensors in mV

	NaCl solutions			KCl solutions			
sensors	50 mmol/l	100 mmol/l	200 mmol/l	sènsors	2 mmol/l	4 mmol/l	6 mmol/l
K^+	102	101	102	**K^+**	**210**	**223**	**245**
Na^+	**65**	**81**	**100**	**Na^+**	40	42	41

Table 6.21 Simultaneous measurement of potassium- and sodium-ion sensors

The simultanous measurement of K^+ with Na^+ and vice versa shows that the reference electrode and the ion sensors are functioning properly in NaCl and KCl solutions. The reference electrode response is nearly constant, within the range of a variation of $\pm 1\ mV$, while the Na^+-Ion Sensor is effectively responding to the media with various concentrations.

When the test medium is changed to *KCl* solutions with differing concentrations, the response of the ion sensors changes accordingly as expected. That is the K^+-Ion Sensor starts to respond while the Na^+-Ion Sensor responds almost constantly within the range of variation of $\pm 1\ mV$ and $\pm 2\ mV$.

2nd Simultaneous measurement of potassium- and sodium-ion sensors in mV

	NaCl solutions			KCl solutions			
sensors	50 mmol/l	100 mmol/l	200 mmol/l	sensors	2 mmol/l	4 mmol/l	6 mmol/l
K^+	98	100	102	**K^+**	**205**	**220**	**245**
Na^+	**75**	**88**	**105**	**Na^+**	40	42	44

Table 6.22 Simultaneous measurement of potassium- and sodium-ion sensors

The simultaneous measurement of K^+ with Na^+ and vice versa shows that the reference electrode and the ion sensors are functioning properly in NaCl and KCl solutions.

The reference electrode response is nearly constant, within the range of a variation of ±2 *mV* for NaCl test solutions and ±2 *mV* and ±4 *mV* for KCl solutions, while Na^+-Ion Sensor is effectively responding to the medium with various concentrations.

When the test medium is changed to KCl solutions with differing concentrations, the response of the ion sensors changes accordingly as expected. The K^+-Ion Sensor produces a variable output whilst the Na^+-Ion Sensor responds almost constantly within the range of variation of ±2 *mV*.

6.20.2 Simultaneous measurement of Na^+-CSS-IS and Ca^{2+}-CSS-IS

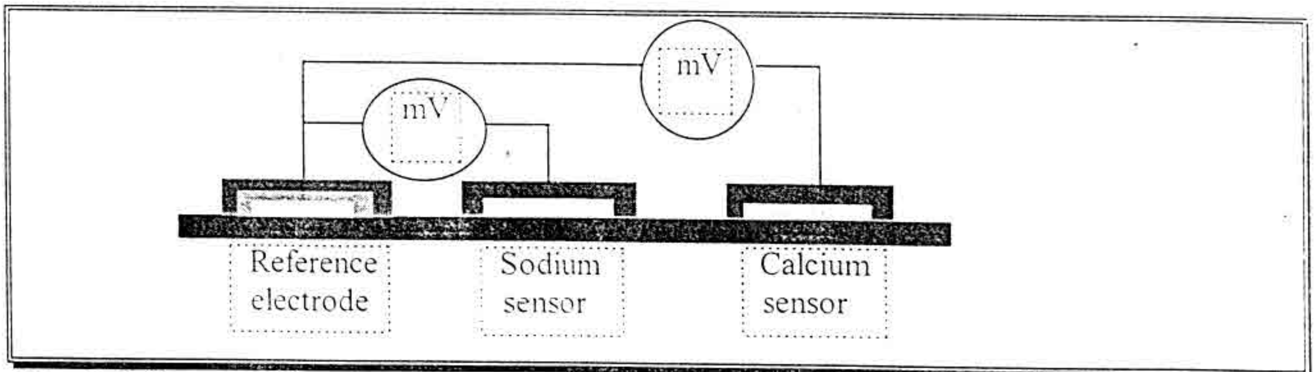


Fig.6.3 System for simultaneous measurement of Na^+-CSS-IS and Ca^{2+}-CSS-IS with respect to miniaturized silver-silver chloride reference electrode

<u>1st Simultaneous measurement of sodium- and calcium-ion sensors in mV</u>

	NaCl solutions				CaSO4 solutions		
sensors	50 mmol/l	100 mmol/l	200 mmol/l	sensors	5 mmol/l	10 mmol/l	20 mmol/l
Na^+	67	**82**	**105**	**Na^+**	41	43	40
Ca^{2+}	143	142	142	**Ca^{2+}**	**210**	**224**	**231**

Table 6.23 Simultaneous measurement of sodium- and calcium-ion sensors

The simultanous measurement of Na^+ with Ca^{2+} and vice versa shows that the reference electrode and the ion sensors are functioning properly in NaCl and $CaSO_4$ solutions. The reference electrode response is nearly constant, within the range of a variation of *−1 mV* and *zero* for NaCl test solutions and *−2 mV* and *−3 mV* for $CaSO_4$ solutions, while the Na^+-Ion-Sensor is effectively responding to the medium with various concentrations.

When the test medium is changed to $CaSO_4$ solutions with differing concentrations, the response of the ion sensors changes accordingly as expected. That is the Ca^{2+}-Ion Sensor starts to respond while Na^+-Ion Sensor responds almost constantly within the range of variation of *−2 mV* and *−3 mV*.

The change in variation of the Ca^{2+}-Ion Sensor to test solutions is *+14 mV* and *+7 mV* while the Na^+-Ion-Sensor is more reasonable with *+15 mV* and *+23 mV*. With *20 mmol/l* $CaSO_4$ solution the response expected is to be reasonably higher than with *10 mmol/l* solutions. The reason why the response is not as expected as high might be due to the maximum dissolveable limit of $CaSO_4$ in water which is *20 mmol/l*. The maximum limit might not be dissolved completely in the test solution.

<u>2nd Simultaneous measurement of sodium- and calcium-ion sensors in mV</u>

	NaCl solutions				CaSO4 solutions		
sensors	50 mmol/l	100 mmol/l	200 mmol/l	sensors	5 mmol/l	10 mmol/l	20 mmol/l
Na^+	70	82	**108**	Na^+	43	41	43
Ca^{2+}	140	142	140	Ca^{2+}	**210**	**220**	**231**

Table 6.24 Simultaneous measurement of sodium- and calcium-ion sensors

The simultanous measurement of Na^+ with Ca^{2+} and vice versa shows that the reference electrode and the ion sensors are functioning properly in *NaCl* and

$CaSO_4$ solutions. The reference electrode response is nearly constant, within the range of a variation of ±2 *mV* for *NaCl* test solutions and ±2 *mV* for $CaSO_4$ solutions, while Na^+-Ion Sensor is effectively responding to the medium with various concentrations.

When the test medium is changed to $CaSO_4$ solutions with differing concentrations, the response of the ion sensors changes accordingly as expected. That is the Ca^{2+}-Ion Sensor starts to respond while Na^+-Ion Sensor responds almost constantly within the range of variation of ±2 *mV*. The change in variation of the Ca^{2+}-Ion Sensor to test solutions is +*10 mV* and +*21 mV* while that of the Na^--Ion Sensor is +*12 mV* and +*26 mV*.

6.20.3 Simultaneous measurement of K^+-CSS-IS and Ca^{2+}-CSS-IS

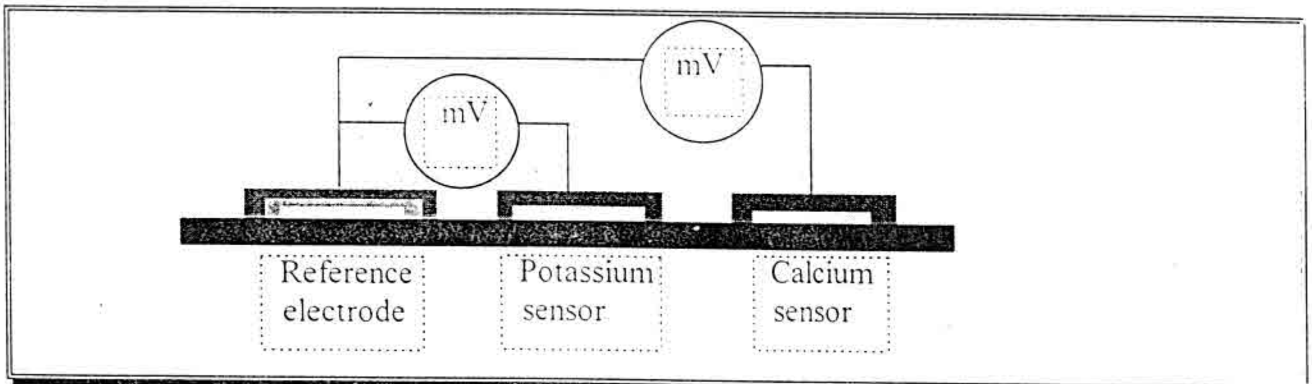


Fig. 6.4 System for simultaneous measurement of K^+-CSS-IS and Ca^{2+}-CSS-IS with respect to miniaturized silver-silver chloride reference electrode

<u>1st Simultaneous measurement of potassium- and calcium-ion sensors in mV</u>

	KCl solutions			CaSO4 solutions			
sensors	2 mmol/l	4 mmol/l	6 mmol/l	sensors	5 mmol/l	10 mmol/l	20 mmol/l
K^+	**210**	**225**	**254**	K^+	102	102	101
Ca^{2+}	143	144	142	Ca^{2+}	**208**	**220**	**236**

Table 6.25 Simultaneous measurement of potassium- and calcium-ion sensors

The simultanous measurement of K^+ with Ca^{2+} and vice versa shows that the reference electrode and the ion sensors are functioning properly in *KCl* and $CaSO_4$ solutions. The reference electrode response is nearly constant, within the range of a variation of *−1 mV* and *−2 mV* for *KCl* test solutions whereas *zero* and *-1 mV* for $CaSO_4$ solutions, while K^+-Ion Sensor is effectively responding to the medium with various concentrations.

When the test medium is changed to $CaSO_4$ solutions with differing concentrations, the response of the ion sensors changes accordingly as expected. That is the Ca^{2+}-Ion Sensor starts to respond while K^+-Ion Sensor responds almost constantly within the range of variation of *zero* and *−1 mV* of reference electrode response.

The change in variation of the Ca^{2+}-Ion Sensor to tests solutions is *+12 mV* and *+16 mV* while that of the K^+-Ion Sensor is *+15 mV* and *+29 mV*.

2nd Simultaneous measurement of potassium- and calcium-ion sensors in mV

	KCl solutions			CaSO4 solutions			
sensors	2 mmol/l	4 mmol/l	6 mmol/l	sensors	5 mmol/l	10 mmol/l	20 mmol/l
K^+	**212**	229	254	K^+	101	101	101
Ca^{2+}	144	143	145	Ca^{2+}	**202**	**220**	**232**

Table 6.26 Simultaneous measurement of potassium- and calcium-ion sensors

The simultaneous measurement of K^+ with Ca^{2+} and vice versa shows that the reference electrode and the ion sensors are functioning properly in *KCl* and $CaSO_4$ solutions. The reference electrode response is nearly constant, with zero variation $CaSO_4$ solutions and *−1 mV* and *+2 mV* for *KCl* test solutions, while

K^+-Ion Sensor is effectively responding to the medium with various concentrations.

When the test medium is changed to $CaSO_4$ solutions with differing concentrations, the response of the ion sensors changes accordingly as expected. That is the Ca^{2+}-Ion Sensor starts to respond while K^--Ion Sensor responds almost constantly within the range of variation of zero and $-1\ mV$ and $+2\ mV$ of reference electrode response. The change in variation of the Ca^{2+}-Ion Sensor to test solutions is $+18\ mV$ and $+12\ mV$ while that of the K^--Ion Sensor with $+17\ mV$ and $+25\ mV$.

The system is a simultaneous measuring set-up of two parameters at a time as the experimental set-up was only for two variable measurement. Therefore it is not measuring potassium, sodium, and calcium at the same time.

6.21 Conclusions

This research has developed and tested various ion sensors having a multi-layer sensor-array configuration based on specific chemicals without resorting to sophisticated and expensive multilayer film electrode techniques but neverthless capable of measuring ion activity.

The sensor array considered in this specific research is neither a microelectrode (μm sized) nor macroelectrode (conventional) sensor-array. In actual fact, it is a disposable sensor array. The research intention was and is to find an intermediate way which will help to integrate readily the sensor array with microelectronics involving very high input impedance circuit design (CMOS design).

Each shell of the electrode system has been checked under a microscope with a two-level magnification system to observe whether there exists any air bubble or hole or any impurity present or not since air bubbles are especially very detrimental for effective functioning of the electrodes.

Any discontinuity within the electrodes directly affects the proper functioning of the electrodes.

Potentiometry allows a simple and fast determination of the activity of inorganic ions to be measured by using M-ISEs as chemical sensors. Direct potentiometry lends itself to an emergency type of application.

The *reproducibility of ISEs is diminished after the membrane has been in contact with body fluids.* Therefore, some simple and possibly disposable electrodes permitting ion-activity measurement is required. Hence the research has investigated disposable miniaturized special chemical based ion sensors as a possible cost-effective alternative to conventional methods with comparable performance and with the advantage of multiple sensing.

The reference electrode of first kind unfortunately responds to a few cations whilst the reference electrode of the second kind *which is a metal wire coated with a poorly soluble salt of that metal, responds to a wider variety of ions. Thus the choice of the reference electrode is a disposable miniaturized special chemical based $Ag/AgCl/Cl^-$ electrode of the second kind.* Although the salt layer is usually deposited by anodizing the metal electrode in a suitable electrolyte, *it has been shown*[6.2] that it is *possible to incorporate the poorly soluble salt in a polymeric matrix. This is an alternative way of miniaturizating the subject sensor array.* The main point is to incorporate the correct amount of appropriate chemicals in a polymetric matrix. The salt must be a poorly soluble salt of the ion.

Only the appropriate selection of each of these constituents finally guarantees a highly selective and sensitive electrode that exhibits a stable and reproducible potential response. The M-ISEs and M-RE composition is given above.

High lipophilicity is even more crucial when minielectrodes fabricated with modern planar technologies are concerned. Integration of the sensor array with microelectronics involving M-ISEs as chemical sensors needs a very high input impedance circuit design, namely CMOS design. A thin silver layer on a glass substrate as the conduction medium of the ion-sensors is quite effective and leads to direct coupling into an ADC for data acquisition by a PC and traditional analytical methods involving galvanic cells can therefore be readily automated and computerized.

CW-ISEs are not integratable with microelectronic circuit design which is a major reason that led to vapour deposition of pure silver onto the glass substrate.

The most relevant fields of application of neutral-carrier-based ion-selective electrodes are clinical chemistry and bioelectrochemistry[6.4]. Hence this explains the reason behind the decision to use neutral-carrier based M-ISEs, since in both fields the response time of the sensors is of utmost importance when the aim is to achieve a large sample throughput or to follow fast biological processes.

To provide mechanical strength, plasticized PVC membranes[6.5] have been used as a reference. However, the PVC-based membranes/electrodes have a sensitivity to interfering lipophilic ions.

The duration for all measurements is only 60 seconds. Only three successive tests have been carried out for each group having different SAPT (**S**ensor **A**rray **P**reconditioning **T**ime) times. With all SAPT times of 3, 5, 6. and 7 minutes reasonable tests results have been obtained. However, the shorter the SAPT time, the better the *CSS_RE* lifetime leading to 3 or one minute as the preferred SAPT time. The test results clearly show that the ion-sensor array is capable of differentating various test solutions provided that the system is ready for use with real samples[6.8].

Chapter Seven

The Sensor-Array Measurements with Artificial Blood and Whole Human Blood

Summary

This chapter introduces a very concise but comprehensive review of human blood as well as the test results of the sensor-array with artificial and whole human blood respectively.

7.1 Introduction

Blood provides nutrients, oxygen, chemical instructions, and a mechanism for waste removal from each of the roughly 75 trillion individual cells in the human body. The blood also transports specialised cells that defend peripheral tissues from the infection and disease. These services are absolutely essential, and a region completely deprived of circulation may die in a matter of minutes. So, the function of blood is absolutely crucial.

The functions of blood include the following[7.1]:

[a] **Blood transports dissolved gases,** bringing oxygen from the lungs to the tissues and carrying carbon dioxide from the tissues to the lungs.

[b] **Blood distributes nutrients** absorbed at the digestive tract or released from storage in adipose tissue or the liver.

[c] **Blood transports metabolic wastes** from peripheral tissues to sites of excretion, such as the kidneys.

[d] **Blood delivers enzymes and hormones** to specific target tissues.

[e] Blood regulates the pH and electrolyte composition of interstitial fluids throughout the body. An extensive array of buffers enables the blood stream to deal with the acids generated by active tissues, such as the lactic acid produced by skeletal muscles.

[f] Blood restricts fluid losses through damaged vessels or at other injury sites. The clotting reaction seals the breaks in the vessel walls, preventing changes in blood volume that could seriously affect blood pressure and cardiovascular function.

[g] Blood defends the body against toxins and pathogens. It transports *white blood cells*, specialised cells that migrate into peripheral tissues to fight infections or remove debris, and delivers *antibodies*, special proteins that attack invading organisms or foreign compounds. The blood also receives toxins produced by infection, physical damage, or metabolic activity and delivers them to the liver and kidneys, where they can be inactivated or excreted.

[h] Blood helps regulate body temperature by absorbing and redistributing heat. Blood is nearly 50 % water which has an extraordinarily high heat capacity. In the body, active skeletal muscles and other tissues generate heat, and the blood stream carries it away. When body temperature is already high, that heat is lost across the surface of the skin. When body temperature is too low, warm blood is directed to the most temperature-sensitive organs.

7.2 The composition of blood

Blood is normally confined to the circulatory system, and it has a characteristic and unique composition as follows[7.1]:

Plasma is the ground substance of blood and has a density only slightly greater than that of water. It contains dissolved proteins rather than the network of insoluble fibers found in loose connective tissue or cartilage.

Formed elements are blood cells and cell fragments that are suspended in the plasma. These elements are present in great abundance and are highly specialised. Red blood cells transport oxygen and carbon dioxide. The less numerous white blood cells are components of the immune system. Platelets are small packets of cytoplasm that contain enzymes and factors important to the blood clotting process.

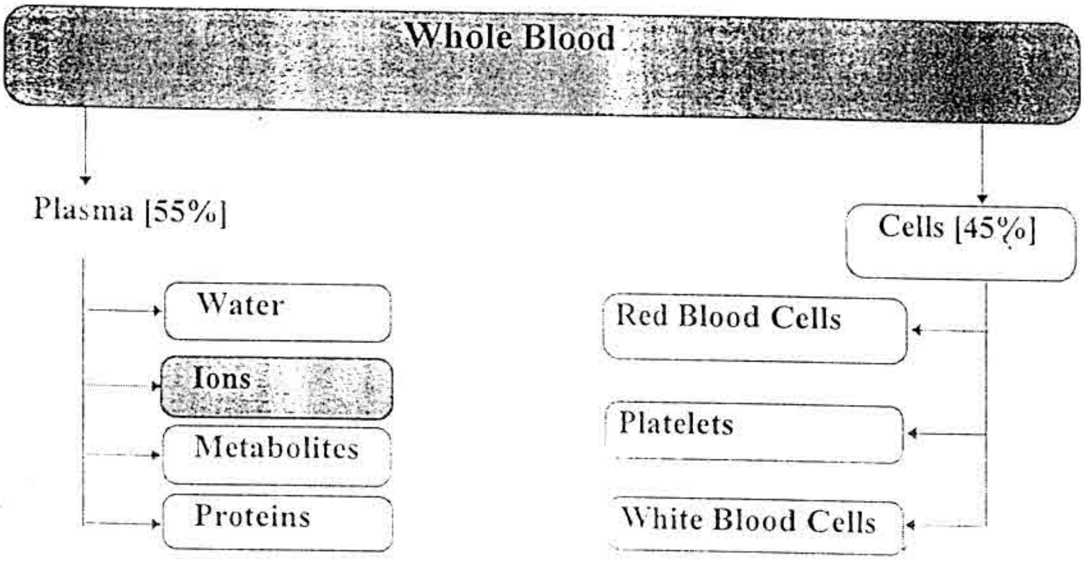


Fig. 7.1 Whole human blood structures

Together the plasma and formed elements constitute whole blood. These components may be separated, or fractionated, for analytical or clinical purposes.

The cation- and anion-ions in whole human blood are K^+, Na^-, Ca^{2-}, Mg^{2+} and Cl^-, HCO_3^-, HPO_4^{2-}, and SO_4^{2-} respectively. Since this research is looking for ion measurements in whole human blood, it is necessary to give the subject electrolytes and their normal ranges in blood below:

Electrolytes	Ranges			
K^+	3.6	-	5.5	mmol/l
Na^+	135	-	155	mmol/l
Ca^{2+}	1.14	-	1.31	mmol/l

Table 7.1 The ions (or electrolytes) and their normal ranges in blood

To give an idea about the accuracy required in clinical practice, in conjuction with **Table 7.1,** the following data taken from the department of Clinical Pathology of Royal Sussex County Hospital at Brighton in United Kingdom from real samples using indirect potentiometry might help:

Na^+ (mmol/l)	K^+ (mmol/l)	Ca^{2+} (mmol/l)	Comments
139	3.6	3.6	Normal
140	4.5	4.5	Normal
136	4.7	4.7	Low Na^-
140	5.1	5.1	High K^- and Ca^{2+}
137	3	3	Low K^- and Ca^{2+}
134	3.1	3.1	Low Na^-, K^+ and Ca^{2+}
135	3.1	3.1	Low Na^-, K^- and Ca^{2+}
137	4.7	4.7	Normal
147	3.6	3.6	High Na^-

Table 7.2 Real whole human blood measurements with indirect potentiometry

Readings given in **Table 7.2** are a small selection from 650 real blood samples from different age groups and sexes. From the table it can be seen that values taken as normal are (137,139 and 140), (3.6.4.5 and 4.7) and (3.6, 4.5 and 4.7) for Na^+, K^+, and Ca^{2-}, respectively. The table shows that ±2% accuracy is required for potassium, for example, although the interpretation of the reading might change according to patient conditions.

Clinicians interpret the data according to age, sex, and even geographical backgrounds. The most important point is to be able to differentiate clearly between different ion concentrations, ion activities or samples.

In clinical practice there is an emphasis on the importance of reproducibility rather than accuracy and that is real clinical practice. However, a reproducibility of 1% or 2% at a level of 2 mmol/l to 2.5 mmol/l of concentration is required.

7.3 Tests with artificial blood

7.3.1 K^+-IS and Na^+-IS tests with artificial blood

		Tests [mV]				
		t1	t2	t3	t4	tt (min.)
PV1	K^+	360	333	320	350	5
	Na^+	340	330	280	340	5
PV2	K^+	409	450	440	450	5
	Na^+	410	415	380	420	5

Table 7.3 K^+ and Na^+ sensor-array measurements in mV with artificial blood samples PV1 and PV2

The chemical composition of PV1 and PV2 artificial blood samples is 2.6 mmol/l K^+, 119 mmol/l Na^+, and 2.02 mmol/l Ca^{2+} for the former and 5.3 mmol/l K^+, 141 mmol/l Na^+, and 2.94 mmol/l Ca^{2+} for the latter, respectively.

The ion-sensor array response is quite reasonable since for all measurements the system is able to differentiate between the two samples tested for both of the ions. For t1, t2, t3, and t4 of K^+-IS the measurement difference in mV is 49 mV, 117 mV, 120 mV, and 100 mV respectively which clearly indicates that the sensor array is responding mostly in a Nernstian way.

Whereas for t1, t2, t3, and t4 of Na^+-IS the measured differences in mV are 70 mV, 85 mV, 100 mV, and 80 mV respectively which clearly illustrates that the ion-sensor array is responding in a Nernstian way. The justification of the Nernstian response is given in the calibration graph of each ion-sensor in Chapter Six.

Although the system is clearly making a distinction between the samples there is one point to be emphasised here which is the reproducibility of the system. One reason might be the structure of the miniaturised reference electrode since it is for single use. Another reason might be chemical composition of the ion-sensor array which may not be the exact composition for artificial blood testing for electrolytes. These comments apply equally to sections **7.3.2** and **7.3.3** as well.

7.3.2 Na^+-IS and Ca^{2+}-IS tests with artificial blood

		Tests [mV]				
		t1	t2	t3	t4	tt (min.)
PV1	Na^+	280	280	284	304	5
	Ca^{2+}	110	126	116	124	5
PV2	Na^+	348	392	391	345	5
	Ca^{2+}	138	164	149	168	5

Table 7.4 Na^+ and Ca^{2+} sensor-array measurements in mV with artificial blood samples PV1 and PV2

The chemical composition of PV1 and PV2 artificial blood samples is 2.6 mmol/l K^+, 119 mmol/l $\mathbf{Na^+}$, and 2.02 mmol/l $\mathbf{Ca^{2+}}$ for the former and 5.3 mmol/l K^+, 141 mmol/l $\mathbf{Na^+}$, and 2.94 mmol/l $\mathbf{Ca^{2+}}$ for the latter, respectively.

The ion sensor array response is quite reasonable since for all measurements the system is able to differentiate between the two samples tested for both of the ions. For t1, t2, t3, and t4 of Na^+-IS the measurement differences in mV are 68 mV, 112 mV, 107 mV, and 41 mV respectively which clearly illustrates that the sensor array is responding mostly in a Nernstian way.

Whereas for t1, t2, t3, and t4 of Ca^{2+}-IS the measured differences in mV are 28 mV, 38 mV, 33 mV, and 44 mV respectively which clearly shows that the ion-sensor array is responding in a Nernstian way.

7.3.3 K^+-IS and Ca^{2+}-IS tests with artificial blood

		Tests [mV]				
		t1	t2	t3	t4	tt (min.)
PV1	K^+	370	353	340	367	5
	Ca^{2+}	185	187	160	158	5
PV2	K^+	419	430	440	450	5
	Ca^{2+}	205	210	205	200	5

Table 7.5 K^+ and Ca^{2+} sensor-array measurements in mV with artificial blood samples PV1 and PV2

The chemical composition of PV1 and PV2 artificial blood samples is 2.6 mmol/l $\mathbf{K^+}$, 119 mmol/l Na^+, and 2.02 mmol/l $\mathbf{Ca^{2+}}$ for the former and 5.3 mmol/l $\mathbf{K^+}$, 141 mmol/l Na^+, and 2.94 mmol/l $\mathbf{Ca^{2+}}$ for the latter, respectively.

The ion-sensor-array response is quite reasonable since for all measurements the system is able to differentiate between the two samples tested for both of the ions. For t1, t2, t3, and t4 of K^+-IS the measurement differences in mV are 49 mV, 77 mV, 100 mV, and 83 mV respectively which clearly shows that the sensor array is responding mostly in a Nernstian way.

Whereas for t1, t2, t3, and t4 of Ca^{2+}-IS the measurement differences in mV are 20 mV, 23 mV, 45 mV, and 42 mV respectively which similarly illustrates that the ion-sensor array is responding in a Nernstian way.

An alternative explanation for the reproducibility of the system might be the interference due to the ions being measured and the Na^+ concentration in the samples.

7.4 Tests with whole human blood

7.4.1 K^+-IS and Na^+-IS tests with whole human blood

		Tests [mV]				
		t1	t2	t3	t4	tt [min]
Whole Human Blood [WHB]	K^+	330	316	345	341	5
	Na^+	156	143	162	185	5

Table 7.6 K^- and Na^+ sensor-array measurements in mV with whole human blood

Four ion sensors have been prepared and tested for K^+ and Na^+ simultaneous measurement. The reproducibility of the K^+-ion sensor and the Na^+-ion sensor is thought to be reasonable due to the disposable reference electrode used. These values are read during five-minute time intervals between ±5 mV drift for each test measurement. The ion activity measurements of each ion sensor are given in millivolts.

The whole sensor array is used as a disposable sensor array to get rid of negative effect of proteins on measurements in the whole human blood. As direct potentiometric measurements read normally 40% less than flame photometry and other techniques used in clinical practice for whole human blood, the results are fairly encouraging.

In actual fact, there are **many parameters affecting the measurement of such nature, among them are**[7.2]: the structure of the reference electrode, the ion-sensor structure in terms of ionophore, plasticizer, salt, and PVC, and chemical interference, selectivity of the membrane, space-occupying effects of protein and lipid in the sample, variation in ionic strength and activity coefficient, rate of response of the membrane (change in concentration, thickness, dimension, and ion-exchange rate, change in activity, temperature, and stirring rate), temperature, interfering ions and junction potentials.

Of these factors, the activity coefficient is the most important and is influenced by ionic strength, particularly if direct measurements are required.

The membrane of the ion sensors has a limited life time since valinomycin, ETH 157, or ETH 1001 is slowly leached out into the aqueous phase[7.2].

7.4.2 Na^{+}-IS and Ca^{2+}-IS tests with whole human blood

		Tests [mV]				
		t1	t2	t3	t4	tt [min]
Whole Human Blood [WHB]	Na^{+}	250	245	238	242	5
	Ca^{2+}	157	186	162	167	5

Table 7.7 Na^{+} and Ca^{2+} sensor-array measurements in mV with whole human blood

Four ion sensors have been prepared and tested for Na^{+} and Ca^{2+} simultaneous measurement. The reproducibility of the Na^{+}-ion sensors and the Ca^{2+}-ion sensors is fairly reasonable because the reference electrode used is disposable. These values are read during a five-minute interval between ±7 mV drift for each test measurement. The ion-activity measurements of each ion sensor are given in millivolts.

Although the ion sensors are not disposable, the whole sensor array is used as a disposable sensor array to get rid of the negative effect of different constituents in blood, especially of proteins, on measurements in the whole human blood. As already stated, direct potentiometric measurements read normally 40% less than flame photometry and other techniques used in clinical practice for whole human blood, the results are again fairly encouraging.

In actual fact, there are many parameters affecting the measurement of such nature and these were mentioned in subsection **7.4.1** above.

7.4.3 K^+-IS and Ca^{2+}-IS tests with whole human blood

		Tests [mV]				
		t1	t2	t3	t4	tt [min]
Whole Human Blood [WHB]	K^+	340	345	316	332	5
	Ca^{2+}	180	156	155	157	5

Table 7.8 K^+ and Ca^{2+} sensor-array measurements in mV with whole human blood

Four ion sensors have been prepared and tested for K^+ and Ca^{2+} simultaneous measurement. The reproducibility of the K^+-ion sensors and the Ca^{2+}-ion sensors is quite reasonable for disposable reference electrode of the sensor array. These values are read over five-minute period between ±6 mV drift for each test measurement. The ion activity measurements of each ion sensor are given in millivolts.

5. Conclusions

Multi-sensing of potassium, sodium, and calcium in whole human blood by using neutral charge carriers has gone through four phases which are testing the sensor-array system with (i) binary solutions, (ii) mixed solutions, (iii) artificial blood[7.3], and (iv) whole human blood[7.3], respectively.

50 ml of solutions, either binary or mixed, have been used as the test sample for the first two phases whereas 20 μl of blood, either artificial or real whole human blood, has been used as the test sample for the last two phases. During the course of the research, the sensor array has been able to differentiate between different concentrations properly. However from time to time the reproducibility has remained a problem with the chemical composition used which implies that different chemical compositions must be tried in order to find

the optimum chemical composition with most reproducibility for such a multi-sensing measurement.

Although optimum chemical compositions have been studied for potassium, sodium, and calcium in solutions for single sensing[7.4-7.5], it is not possible to claim that the same ratios are perfectly applicable for multi-sensing systems using chemicals as sensing material.

The most critical point in such sensor arrays is the capability of the sensors to differentiate between various samples which requires a proper chemical composition as well as a very high input impedance electrometer. The main disadvantage of the sensor array was the disposable reference electrode as it affects the reproducibility of the measurements.

It also alters the cell emf when its ionic strength in the sample solution changes. Consequently it is difficult to know whether the changes in the measured emf originated from the reference electrode or from the remaining cell.

Unless a reproducible constant voltage source was realized no other measurement could have been done with the sensor array. To that aim, nineteen solutions have been tested to find the most reproducible chemical composition. That is why Chapter Five gives comprehensively the composition and experimental details of the disposable reference electrode which is the constant voltage source of the measurement system. The response of the reference electrode has been tested over a long period of time with a conventional silver/silver-chloride reference electrode to ensure that the reproducibility has been accomplished. The reproducibility achieved was 22±2 mV, 25±1 mV, and 22±4 mV for various tests. This reproducibility is considered reasonable in comparision with the mV levels of the test samples. For details see Chapter Five and Chapter Six.

After a working and reproducible reference electrode was established the sensor array was tested with binary and mixed solutions respectively. This experimental phase had to be carried out before any attempt with real human blood. Unless the sensor array works reasonably well with binary and mixed solutions it is not possible to rely on the measurements done with real human blood. Until the sensor array was able to differentiate between various binary and mixed solutions, no measurement was recorded with real samples. For details of binary and mixed solutions see Chapter Six.

Chapter Seven gives the test results from artificial and real human blood. Each time four successive measurements have been carried out. The test time (tt) was increased to five minutes. The test sample quantity was reduced to 20 μl. The factors affecting the measurement of such nature are given in Chapter Seven subsection 7.4.1.

Table 7.6 shows that the reproducibility of potassium and sodium ion sensors with whole human blood, with the average values of 333 mV and 161 mV for potassium and sodium respectively, is 0%, 5.3%, 3.5% and 2.5% and 3.5%, 13%, 0.46% and 12.5%, respectively.

Table 7.7 shows that the reproducibility of sodium and calcium ion sensors with whole human blood, with the average values of 243 mV and 168 mV for sodium and calcium respectively, is 2%, 1%, 2.4% and 0.7% and 7%, 10%, 3.7% and 10.5%, respectively.

Table 7.8 shows that the reproducibility of potassium and calcium ion sensors with whole human blood, with the average values of 333 mV and 162 mV for potassium and calcium respectively, is 2%, 3.5%, 5.4% and 0.3% and 10%, 3.8%, 4.5% and 3.1%, respectively.

By comparing the average values of potassium, sodium, and calcium ion sensors readings of whole human blood, it will be easy to see the effects of each ion sensor on the others.

K^+ ion sensor average (mV)	Na^+ ion sensor average (mV)	Ca^{2+} ion sensor average (mV)
333	161.5	
	243	168
333		162

Table 7.9 Comparision of average values of readings from K^+, Na^+, and Ca^{2+} ion sensors

It is clear that the potassium- and calcium-ion sensors have good reproducibility whereas the sodium ion sensor is not as good as them in whole human blood. This shows the interference effect of potassium and calcium on sodium measurements. The opposite is not true and the influence of sodium on calcium and potassium is negligible.

Chapter Eight

Conclusions and Future Work

8.1 Conclusions

8.1.1 The first difficulty this research faced was having found no standard definition made for intelligent sensors. Therefore, the second chapter of this thesis discusses this problem and tries to give a definition applicable specifically to this research but generally to the medical area. [Chapter Two]

An intelligent biomedical sensor could have a data processing function [see. **8.2.2**], automatic calibration and/or automatic compensation function, being able to detect and eliminate abnormal values or exceptional values[see.**8.2.2**]. It might incorporate an algorithm [see. **8.2.3**], capable of being altered, and has a certain degree of memory function[see. **8.2.1**]. It is also desirable to have ability to adapt to changes in environmental conditions as well.

Therefore, it is possible to define an intelligent sensor for biomedical application as one which has one of the following properties:

i) has a data processing function,

ii) has automatic calibration ability or function,

iii) has automatic compensation ability or function,

iv) has ability to detect or eliminate abnormal values or exceptional values,

v) has a special algorithm capable of being altered,

vi) has a certain degree of memory function,

vii) has ability to adapt to changes in environmental conditions, and

viii) has ability to multi-sense different parameters.

8.1.2 This research too shows that miniaturization of the reference electrode, with *20* mm^2 electrode area, based on vapour deposition and incorporation of proper amount of internal solution, *KCl*, in the *poly(vinyl alcohol)* matrix is possible. [Chapter Five]

8.1.3 It shows that miniaturization of ion-sensors based on neutral charge carriers [ionophores] incorporated *in poly(vinyl chloride) matrix* is also possible. [Chapter Six]

8.1.4 As far as miniaturization and integration are concerned. potentiometry is the best method for ion-activity measurements by ion-selective electrodes since they convert directly the chemical signal (kind and concentration of electrochemically active species, eg, ions in the analyte) into an electronic signal which facilitates electrochemical sensor array or chip design considerably. [Chapter Two and Chapter Six]

8.1.5 By using binary as well as mixed solutions as the testing medium, it is possible to get reasonable readings from a multi-ion measuring system within the reproducible limits obeying the related theoretical background. [Chapter Six]

8.1.6 *Multi-layer ion-sensors and reference electrodes* are more fragile than thin-film based ion- and reference-electrode systems since the sensor array is being put into the sample for testing leading to the electrolytes diffusion into the sample or vice versa. It may also be due to the lack of electrolytes incorporated into the matrices of the system. [Chapter Six]

8.1.7 The preconditioning time of the ionophores based ion sensors and *poly(vinyl alcohol)* based reference electrode must not exceed *1-3* minutes, preferably one minute, otherwise the reference electrode losses its stability largely leading to erroneous results. [Chapter Five and Chapter Six]

8.1.8 The stability of the ion-sensors is largely dependant upon the quantity of the *plasticizer* used which either leads to a positive drift or a negative drift in collaboration with consecutive measurements done.

8.1.9 The optimum chemical composition of the disposable reference electrode has been found as follows [Chapter Five]:

No	HMW Poly(vinyl)alcohol (g)	Distilled Water(μl)	Ethanol (μl)	KCl concentration
7	0.4008	3000	2000	**500 μL of 3 mol/l of KCl**
11	0.6042	3000	4000	**1000 μL of 6 mol/l of KCl**

8.1.10 The chemical composition of potassium, sodium, and calcium ion-sensors is as follows and found responsive enough for the intended application:

i) Potassium ion-sensor membrane composition

Sensor	**Ionophore** (Wt. %)	**Plasticizer** (Wt. %)	PCV (Wt.%)	**Salt** (Wt. %)
K^+	Valinomycin 1 %	ETH 469 65.5 %	33 %	0.5 % K[B(p-ClPh)4]

Ionophore (Valinomycin)	1.4	mg
Plasticizer (ETH 469)	82 [91.7]	mg
PVC	46.2	mg
Salt	0.7	mg
THF	1500	µl

ii) Sodium ion-sensor membrane composition

Sensor	**Ionophore** (Wt. %)	**Plasticizer** (Wt. %)	**PCV** (Wt.%)	**Salt** (Wt. %)
Na^+	ETH 157 1 %	BBPA 66 %	33 %	-

Ionophore (ETH 157)	1.8	mg
Plasticizer (BBPA)	111 [118]	mg
PVC	60 [59.4]	mg
THF	500	µl

iii) Calcium ion-sensor membrane composition

Sensor	**Ionophore** (Wt. %)	**Plasticizer** (Wt. %)	**PCV** (Wt.%)	**Salt** (Wt. %)
Ca^{2+}	ETH 1001 3.3 %	ETH 469 64.7 %	30.9 %	2.1 % K[B(p-ClPh)4]

Ionophore (ETH 1001)	17.90	mg
Plasticizers (ETH 469)	351.32	mg
PVC	167.78	mg
Salt	11.40	mg
THF	4100	µl

8.1.11 The test sample quantity was 50 ml and 20 μl for testing the sensor array with solutions (either binary or mixed solutions) and blood (either artificial or whole human blood), respectively. Therefore, the quantity used is reasonably small for multi-sensing purpose. [Chapter-6 and Chapter-7]

8.2 Future Work

8.2.1 The sensor array developed will be interfaced/integrated with discrete component based electronic circuit design. If it then functions properly the next stage will be designing surface mount technology based circuit design for the sensor array. Miniaturization of the electronic circuit designed would still be a concern with final CMOS circuitry developed and implemented for the sensor array of K^+, Na^+, and Ca^{2+}.

8.2.2 The reference electrode is a disposable, single-shot electrode whereas the ion sensors are not, their life-time could even be months. Therefore, it may not be reasonable to throw away the sensor unit having tested once with the disposable reference electrode. Perhaps it could be used a second time, especially for an industrial application. This is the point where intelligence could be introduced into the system. By storing into a RAM the first two measurements taken from the disposable reference electrode as it destabilizes after the second consecutive measurement, it will be possible to use the ion sensors more than twice. The software written for the sensor array could take the values stored into the RAM as the reference electrode values [virtual reference electrode]. With a compensation factor introduced into the software system it would be possible to make the system intelligent since a decision-making process will be done properly. This approach has been opened up during a discussion in the IEE Colloquium[8.1].

8.2.3 There is a formula (8.1) which gives a mathematical relationship among free potassium, sodium, and calcium ions for vertebrates[8.2].

$$\frac{\left([Ca^{2+}]_{free} + 0.005\right)}{\left([K^{+}]_{free} + 0.034[Na^{+}]_{free}\right)} = \text{constant} \qquad (8.1)$$

By using this mathematical relationship it becomes possible to produce nine different mathematical constants which lead to a microprocessor based intelligent system. There are three parameters with eight [2^3=8] different abnormal cases and one normal case as in the following table:

K^+	Na^+	Ca^{2+}	Constants
0	0	0	c_0
0	0	1	c_1
0	1	0	c_2
0	1	1	c_3
1	0	0	c_4
1	0	1	c_5
1	1	0	c_6
1	1	1	c_7
n	n	n	c_n

Zero stands for low values of K^+, Na^+, and Ca^{2+} ions with respect to clinically normal values (which is shown by c_n) whereas one stands for higher values of them with respect to clinically normal values in whole human blood. So, if it is possible to find **nine** different mathematical relationships between potassium, sodium, and calcium for whole human blood, or serum or plasma, then it will be possible to create an intelligent system deciding whether the tested sample is normal or having an abnormality. By loading clinically acceptable values of subject ions into a PROM as the reference values then for each sample tested the system will compare the measured values with the reference values and display one of the constants in the Constants column. So, the system will be an intelligent since it makes a decision properly. This approach was also opened up during discussion in the *IEE Colloquium*[8.1]. For the value of **n** for potassium, sodium, and calcium separately see Chapter Seven **Table 7.1** (The Ions and their normal ranges in blood).

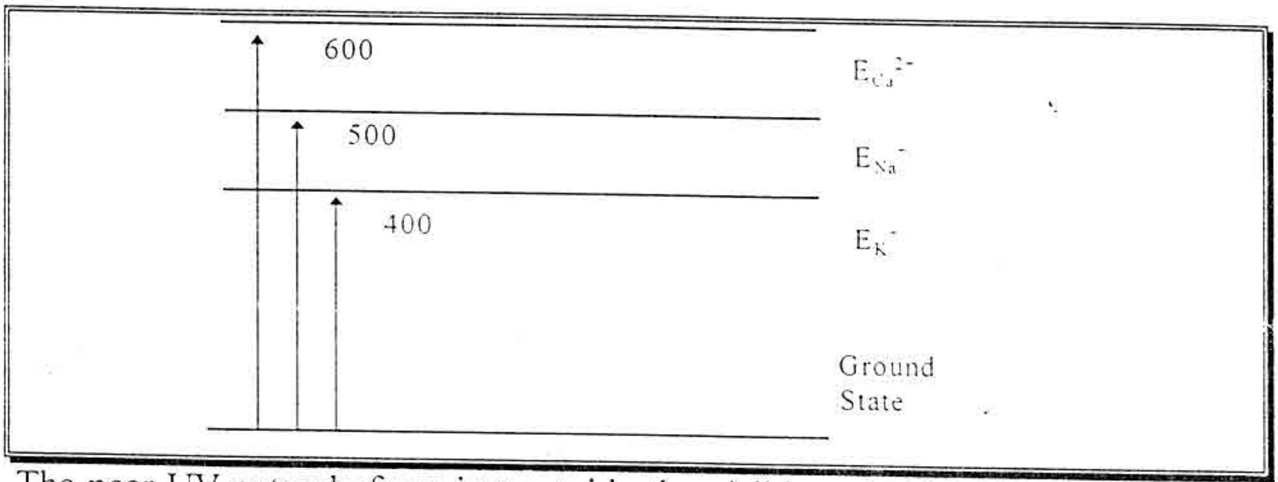
8.2.4 Software for a real-time application could be written detecting K^-, Na^+, and Ca^{2-} ions in real solutions depending upon three main parameters namely, the slope of the calibration curve, response times of the sensors, and voltage signal level of the ions under discussion. It is possible to differentiate between Ca^{2-} and K^- or Ca^{2+} and Na^+ by just looking for the slope of the calibration curves which are between 26-29 mV and 56-59 mV respectively, although it needs one more parameter, for example response times, for differentiating between K^+ and Na^+.

8.2.5 Children with cystic fibrosis have a marked and persistent increase in concentrations of Na^+ and Cl^- in sweat, as a result of a failure of reabsorption rather than increased secretion. Sweat samples are collected into a spiral microbore plastic tube which is attached to the skin. Sweat induction is accomplished by pilocarpine iontophoresis. Therefore, it is clear that there is a need for a direct reading device for detecting increased Na^+ and Cl^- concentrations in sweat as an aid to diagnosing cystic fibrosis. This should be possible with minibiosensors either potentiometric or conductivity based.

8.2.6 Optical and electroptical methods might also be used for sensing purposes, one way may be using the activation energy/ ionization energy of the K^+, Na^+, and Ca^{2+}.

K^+	400 kJ g-atom^{-1}	(4 eV)
Na^+	500 kJ g-atom^{-1}	(5 eV)
Ca^{2+}	600 kJ g-atom^{-1}	(6 eV).

By using the equation $\Delta E = h\gamma$, it might be possible to obtain sensors for the subject parameters.



The near UV extends from just outside the visible at 3 eV to the beginning of the vacuum UV at 6.7 eV, where air is no longer transparent and vacuum is required. This is the region where radiation begins to be energetic enough to be *ionizing*. The spectral region from 3 eV to 40 eV is to be emphasized since it is more likely to be of interest for electro-optics.

8.2.7 Ion sensors incorporating multi-ionophores (MIE) might be used to detect three parameters by creating different response times within the membrane mechanism of the electrode for each cation or by establishing poly-ion sensitive membranes.

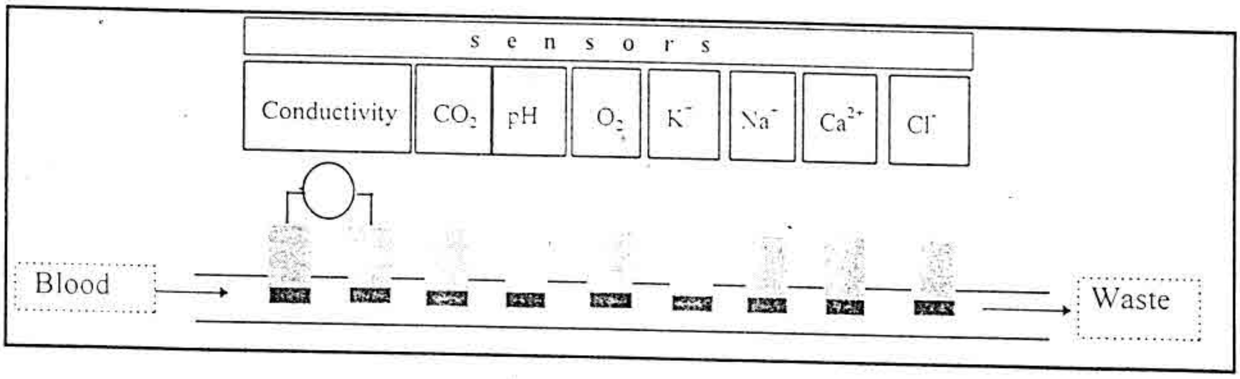
8.2.8 Since the ionic conductivities of the cations under consideration are reasonably different from each other, it might be possible to design sensors depending upon the conductivities. The ionic conductivities for K^+, Na^+, and Ca^{2+} are 73.5 $S/cm^2\ mol^{-1}$, 50.1 $S/cm^2\ mol^{-1}$, and 119 $S/cm^2\ mol^{-1}$ respectively.

8.2.9 The use of clever chemistry such as molecular recognition, in association with clever materials like sol-gel and other porous immobilisation media gives a high possibility of realizing new sensor systems. In principle, such materials can selectively detect and indicate cumulative exposures by visible colour changes that do not require complex instrumentation for their interpretation. *Typical examples are the chromo-ionophores, which combine selective recognition with specific colour changes indicating what species has been found among several possible analytes*. As an example, a charge-transfer chromophore bound to a crown ether recognition molecule might be given, in the chromophore, low energy electronic transitions, from the electron-donating *dimethylamino* end of the molecule to the *quinoid* electron-accepting end, leading to visible absorption. *Cations bound in the centre of the crown ether ring polarise the quinone oxygen, increasing the electron acceptor strength and hence the wavelength of the absorption maximum. The smaller and the more highly charged the metal ion, the greater this shift.*

8.2.10 ISFETs obey the same thermodynamic laws as CWEs. ISFETs may therefore be a better alternative for miniaturization of those three parameters. VLSI-based electrochemical sensor arrays are certainly an alternative way needing trying in the future for portable or bed side solutions. With silicon micromachining, it is possible to miniaturize ultimately the intelligent material or chemistry side of the sensors in such a way that it would be possible to use them during surgical operation to monitor the subject parameters. Intelligent materials (or special chemicals either natural or man-made) in collaboration with microelectronics will certainly lead to more promising sensor systems.

8.2.11 Since the mathematical relationship between cations is not yet a fixed one, it is a big problem in writing the software for an intelligent form of sensors requiring K^+, Na^+, and Ca^{2+} sensing capabilities. Further research should be done to find out the relationship between the parameters in different form of blood, for example, in serum, plasma and/or whole blood.

8.2.12 This multi-ion-sensing system could be extended for more than three parameters and probably the best alternative might be a typical miniaturized flow-through sensor array for multiparameter bedside testing. All these sensors might probably be realizable just by using intelligent materials accordingly.



8.2.13 Different ionophores should be tried to find out which one is the best option for intelligent sensors since there exits a lot of ionophores used for or probably might be used for, ion-sensing purposes either for single sensing or multi-sensing cases.

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