MODIFIED HYDROGEL MATRICES IN FIBRE OPTIC SENSORS

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SUMMARY

Common problems encountered in clinical sensing are those of non-biocompatibility, and slow response time of the device. The latter, also applying to chemical sensors, is possibly due to a lack of understanding of polymer support or membrane properties and hence failure to optimise membranes chosen for specific sensor applications.

Hydrogels can be described as polymers which swell in water. In addition to this, the presence of water in the polymer matrix offers some control of biocompatibility. They thus provide a medium through which rapid transport of a sensed species to an incorporated reagent could occur. This work considers the feasibility of such a system, leading to the design and construction of an optical sensor test bed. The development of suitable membrane systems and of suitable coating techniques in order to apply them to the fibre optics is described.

Initial results obtained from hydrogel coatings implied that the refractive index change in the polymer matrix, due to a change in water content with pH is the major factor contributing to the sensor response. However the presence of the colourimetric reagent was also altering the output signal obtained. An analysis of factors contributing to the overall response, such as colour change and membrane composition were made on both the test bed, via optical response, and on whole membranes via measurement of water content change.

The investigation of coatings with low equilibrium water contents, of less than 10%, was carried out and in fact a clearer signal response from the testbed was noted. Again these membranes were suprisingly responding via refractive index change, with the reagent playing a primary role in obtaining a sensible or non-random response, although not in a colourimetric fashion. A photographic study of these coatings revealed some clues as to the physical nature of these coatings and hence partially explained this phenomenon.

A study of the transport properties of the most successful membrane, on a coated wire electrode and also on the fibre optic test bed, in a series of test environments, indicated that the reagent was possibly acting as an ion exchanger and hence having a major influence on transport and therefore sensor characteristics.

KEYWORDS: Sensor, Biosensor, Fibre, Fibre optic, Hydrogel

To My Late Grandmother,
Phyllis Henry Davies.

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LIST OF ABBREVIATIONS

AcBPR	Acrylated bromopyrogallol red
AZBN	a-Azo bis isobutyronitrile
BPR	Bromopyrogallol red
CWE	Coated wire electrode
CWISE	Coated wire ion selective electrode.
EGDM	Ethylene glycol dimethacrylate
EWC	Equilibrium Water Content.
FET	Field effect transistor.
FITC	Fluoresceine Isothiocyanate
HEMA	Hydroxyethylmethacrylate
IGFET	Insulated gate field effect transistor.
ISE	Ion selective electrode
ISFET	Ion selective field effect transistor
MMA	Methylmethacrylate
MOSFET	Metal-Oxide-Semiconductor Field Effect Transistor.
NVC	N-vinyl carbazole
NNDMA	N,N-di methyl acrylamide
NVP	N -vinyl pyrollidone

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CHAPTER 1

INTRODUCTION AND LITERATURE SURVEY: THE OPERATING PRINCIPLES OF SENSORS AND THE RELATED FUNCTION OF INCORPORATED MEMBRANES

1.1 INTRODUCTION

The 1980's have seen a considerable increase in interest in clinically relevant chemical sensors. This interest is partially based upon the potential ability to miniaturise these devices and to provide rapid information relating to medical treatment, environmental contamination and chemical processes, thereby giving advantages over slower more traditional analytical techniques. There are however many problems in the design and performance of sensors that have yet to be resolved. For example, many presently available devices can only monitor a single variable, and are prone to interference from other species, whereas greater commercial viability is associated with the capability to measure several analytes with little or no cross-interference. Additionally, one of the major problems affecting the use of chemical sensors for *in vivo* monitoring is interaction and incompatibility with the body. This manifests itself in deposition on and encapsulation of the device by biological components and, more importantly, thrombus formation around the implanted device. It is in relation to these and similar problems that the design and selection of the membrane material has a part to play.

There are then several types of sensor used for many different potential applications. The most common may be subdivided as potentiometric and amperometric electrochemical devices on one hand and optical devices on the other. These differ both in the way in which a signal is transduced to give an electrical response and also in the form of the signal that the transducer is capable of detecting. They do however, have in common the need for a membrane interface between the transducer itself and the reservoir or source of the sensed species. This membrane may need to fulfill several roles. In addition to the physical protection of the device it is frequently involved in the

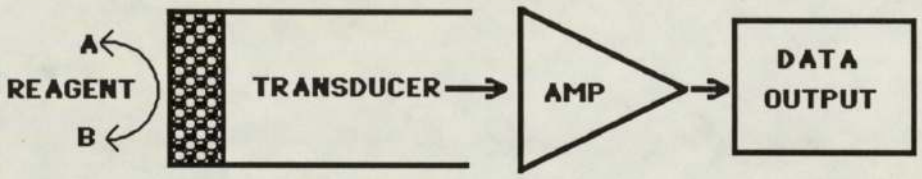
generation of an appropriate signal either through immobilisation of an active species such as an enzyme or the generation of a transmembrane potential by means of an incorporated charge carrier. The different aspects of membrane behaviour in relation to the different requirements of various sensor types are frequently poorly understood and imprecisely described in the sensor literature. There is rarely any evidence that membranes are designed for use with particular sensors. Indeed, they are most often selected from a limited range of materials whose most prominent feature is that they are available at the time of device construction. The work of this group, in this field, has been primarily concerned with the molecular design of polymers to meet the particular requirements of the sensor in relation to its proposed application (eg the environment in which it is to be used). This interest follows from, and is based on, work in other biomedical applications where membrane and surface properties of polymers are important. It is not normally the case that completely new materials as such are required since a vast range of different polymers have been synthesised and characterised in recent decades. The field of "speciality" or "effect" polymers consists in the design or selection of materials based upon a knowledge of the way in which polymer structure governs the properties of relevance to a particular application. As an initial step towards the rationalisation of the behaviour of the various types of polymer used in the literature therefore, it is appropriate to consider the behavioural requirements of different types of sensor, in terms of the range of properties that polymeric membranes can provide.

1.2 CLASSIFICATION OF SENSORS

In general terms a sensor can be described as a device capable of detecting a change in specific physical or chemical parameters in its environment and converting

the acquired information into an intelligible form. Biosensors could be described as either those sensors which are designed to analyse biologically important substances or, more specifically, as sensors which employ a biological reagent as the sensing species. Those sensors which fall into the more general definition but do not use a biological reagent, such as an enzyme or bacteria are described as clinical sensors, as opposed to chemical sensors, which are employed for non clinical purposes. The four components of a sensor can be represented as in FIG1.1

FIG 1.1 The Four Components Of A Sensor



At the reagent phase, there is an interaction between the sensed species and the reagent, forming some type of response. This could be directly or indirectly interpreted at the transducing phase, where the response is transformed into an electrical signal, which may require some amplification before it can be output, via a suitable means, in an intelligible form. The transducing phase, implemented in the sensor system, provides a means by which chemical sensors can be categorised. The four major types of transducers employed are POTENTIOMETRIC; AMPEROMETRIC; PIEZOELECTRIC and OPTICAL and these are described in detail.

1.3 POTENTIOMETRIC SENSORS

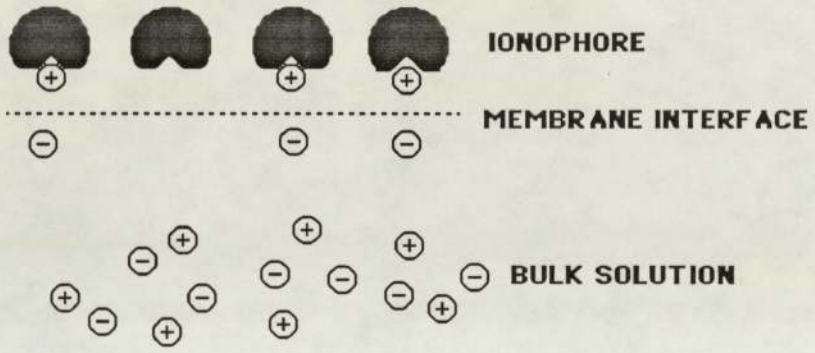
1.3.1 The Generation Of A Transmembrane Potential.

The operation of a potentiometric sensor depends on the generation of a potential difference across a membrane/solution interface, in response to changing concentration of analyte. The first type of ion selective electrodes, developed from the

glass pH electrodes[1,2], were produced in the sixties. Here ion-selectivity is determined by an ion exchanger or an ionophore encapsulated within a plasticised PVC membrane[3] as in FIG 1.2.

Since these membranes are widely used in many electrochemical sensors, the principle of generation across such an interface is described here.

FIG 1.2 The Generation Of A Potential Difference Across A Membrane/Solution Interface: The Transmembrane Potential.



Ionophores, such as valinomyacin, have electron rich pockets, which selectively extract those cations which will fit into the pockets, leaving the co-anions behind in the bulk solution and thus creating a charge separation at the membrane/solution interface. The trans-membrane potential, thus generated, is proportional to the log of the analyte activity and for ideal Nernstian behaviour is given by the equation[3]:-

$$E = \text{constant} + \frac{2.303\ RT}{ZF} \log a_{mz+}.$$

Where a_{mz+} is the activity of the cation; z is the charge number; T is the temperature; R and F are Boltzmann and Faraday's constants.

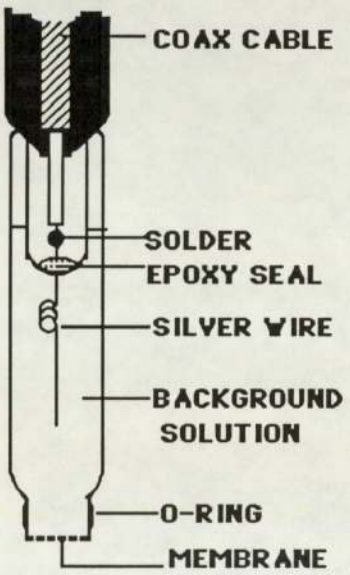
These PVC membranes are called solvent modified membranes, due to the high degree of plasticiser incorporated in them, which permits the diffusion of ionophores within the membrane matrix. A schematic diagram of a typical ion-selective electrode is

shown in FIG 1.3:

1.3.2 Ion Selective Electrodes

The best known example of an ISE is the potassium electrode, which uses the antibiotic valinomycin as its sensing component, incorporated into a plasticised PVC membrane. Other ISE's which work on similar operating principles including a calcium-sensitive electrode, have been described[4]. Recent research efforts have concentrated on expanding the range of species that can be determined with the solvent polymeric membranes to additional anions, to those which are commercially available and routinely used. i.e including electrodes for the determination of K^+, Na^+, Ca^{2+}, NH_4^+, $H^+$and CO_3^{2-}. Other uses of ion-selective electrodes which have been reported in the literature are for the detection and testing of pharmaceutical products, such as Cimetidine and Ranitidine, which are prescribed in the treatment of duodenal ulcers[5]. Considerable work has been carried out for the selective detection of cations but successful, selective, anion sensors are few and far between[6,7].

FIG 1.3 The Ion Selective Electrode



One of the great disadvantages of ISE's is that a reference solution is necessary, presenting problems such as added expense and bulkiness, combined with the inherent fragility of the glass encasement. Also, the response of the electrode is heavily dependent on interfering ions which are present within the bulk solution especially between sodium and potassium, since these both exist in the body in relatively large quantities, are singly charged and have approximately the same hydration radii[8]. In addition, the response of these ISE's is prone to drifting with age, due to the loss of the incorporated plasticisers and ionophores into the sample solution. One particular attempt to resolve this problem has been by the production of a film of Nafion which provides ionic clusters, randomly distributed across the backbone of polytetrafluoroethylene. Ions thus diffuse from cluster to cluster via interconnecting channels, through the polymer membrane[9]. A study of the specificity of paraffin membranes, solid polymeric membranes and Orion liquid ion-exchange-electrodes, for the measurement of calcium present in the body at activities 10^{-4} to 10^{-2}M in the presence of other species, such as Na^{+}($\sim 10^{-1}$M); K^{+}($\sim 10^{-2}$M); Mg^{2+}($\sim 10^{-3}$M); Cl^{-}($\sim 10^{-1}$M) has been given in the literature[10] as well as a table of relevant concentration ranges of clinically significant analytes[8].

1.3.3 Coated Wire Electrodes

The desire to reduce production costs, simplify and miniaturise the ISE led to the development of the coated wire electrode or CWE[11,12] where the principles are similar to the ISE but the selective membrane is coated directly onto the silver wire, therefore dispensing with the internal reference solution. Although there are no commercially available CWE's, their ease of construction means that they provide a useful testbed for ion-selective membranes. Applications for CWE's which are

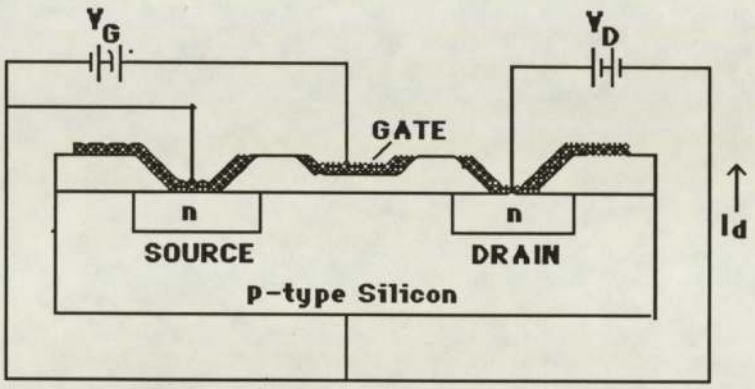
reported[12] are in the determination of NO_x, anionic detergents, phenobarbital, amino acids, potassium, iron and higher molecular weight quarternary amines.

1.3.4 Field Effect Transistors

A recent development in the potentiometric sensor field, which came to the fore in the early seventies, is the chemically selective field effect transistor (a chemFET)[2] or ISFET (Ion selective field effect transistor). This is a MOSFET (metal-oxide-semiconductor) device which has been modified to respond to the presence of ions or other species through charge separation at an interface, generating a potential which modulates the FET's source drain current. In order to illustrate the basic operation of the device, a schematic diagram of an insulated gate field effect transistor (IGFET) is drawn in FIG 1.4.

In the IGFET, the semiconductor has an insulating layer, formed at its surface between two highly doped regions of opposite polarity to the substrate. These form the source and drain. A conductor attached to the top of the insulating layer forms the gate and thus, the semiconductor substrate of p-type silicon and the metal gate of the IGFET constitute a parallel plate capacitor. Application of the gate voltage (V_G) induces the n-type inversion layer between the source and drain at the surface of the substrate, forming a conducting channel. The current thus flowing from the drain (Id) is determined by the gate voltage and the voltage across the drain. In ChemFETs and ISFETS, the metal gate layers of the conventional MOSFET devices are replaced by a chemically sensitive or ion selective layer in order to produce ISFETS or ChemFETS, thus making the gate potential and therefore the drain current, proportional to ion concentration[13,14].

FIG 1.4 An Insulated Gate Field Effect Transistor.



An ISFET device has been reported that can measure concentrations of H^+, K^+, Na^+, and Ca^{2+}, simultaneously during on-line blood monitoring[15,16]. FET sensors have also been developed for anion monitoring, which is a fairly sparse area as far as ISE's and CWE's are concerned. A device related to the ion selective field effect transistor (ISFET) is the suspended gate FET, the operation of which depends on changes of the work function and dipole orientation that result from the interaction of the sensing element with various gases. For example, palladium, which dissolves hydrogen, is used in the sensing element in a suspended gate FET for the detection of hydrogen. Another example of such a device is described[17], where polypyrrole is used as a reagent for an alcohol sensor.

A device related to the ISFET is a recently developed ammonia-sensitive, metal oxide semiconductor (MOS), that uses a catalytic metal such as iridium as part of the sensing element. Whereas ChemFET responses are monitored by measuring voltage induced changes in the conductivity of the channel region of the FET, the ammonia sensitive MOS device is based on the measurement of capacitance changes in this region. This has already proven useful, with the addition of a gas permeable membrane in

various biological and non-biological solutions such as whole blood, serum, rain water and river water.

A major advantage of the FET is its size, which creates opportunities for multiplexing and low production costs using microlithographical techniques. However, at the moment the problems incorporated in encapsulation prevent the production of ISFETs from being as low a cost as that for pH electrodes. ISFET's, as well as being prone to electrostatic damage, present great problems in their biocompatibility, since the locally induced field around the sensor can induce thrombosis. In addition, as with any electrode system, poisoning and fouling present added problems in the change of the electrode performance with time[18].

1.4 AMPEROMETRIC SENSORS

The difference between an amperometric sensor and a potentiometric sensor is that in the former, a potential is applied in order to induce an electrochemical redox reaction to occur and in the latter, the membrane acts as a battery, generating a potential difference, across an interface[1,2]. Amperometric sensors are devices which measure current changes and are therefore employed where the electrochemical reduction of reaction products is necessary for their detection. Amperometric sensors, reported in the literature are those for the determination of urea[19], glucose[20,21] or human chorionic gonadotropin[22], blood gases such as oxygen and carbon dioxide[23-27], vitamin B_{12} [28] and vitamin C [29].

The simplest type of sensor that may be used to illustrate these principles is based on the polarographic oxygen electrode. Several books and reviews have been published, in which the principles and detail of operation of these devices is described[30-32]. The majority of sensors which employ enzymes as reagents utilise

amperometric sensors in order to electrochemically reduce products, such as ammonia, oxygen and hydrogen peroxide.

1.5 ENZYMES AS REAGENTS IN ELECTROCHEMICAL SENSORS

Enzyme electrodes act according to the general scheme[33]:

Substrate + Cofactor_____> Product

Either the cofactor or product can thus be determined with an electrode, either amperometrically or potentiometrically. Thus, an enzyme immobilised on or near one of the electrodes can be used to produce or consume a species which alters the redox reaction rate and hence the observed current flow[33,1]. The most important and commercially available amperometric enzyme electrode is the glucose sensor. An example of this type of biosensor is the Yellow Springs Instrumentation (YSI) electrode: The detector system of the sensor consists of a small amount of the enzyme glucose oxidase, immobilised onto a cellulose acetate membrane upon platinum. The enzyme specific to glucose, converts it to gluconic acid plus hydrogen peroxide in the presence of oxygen. The production of hydrogen peroxide can be measured amperometrically by its reduction:-

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-$$

Thus by subtracting the current produced at the glucose electrode from that at the silver/silver chloride compensating electrode, the amount of hydrogen peroxide produced, and therefore glucose concentration can be determined[34]. Other sensors utilising glucose oxidase have been reported and an example of a coated wire electrode, where the enzyme is immobilised directly onto a palladium gold modified carbon electrode is given[35]. Similar sensors to this have been reported and electrodes utilising the same reaction but measuring the production of the acid by-product or decrease in

partial pressure of oxygen have also been suggested. However, the measurement of oxygen consumption is not recommended, since the background oxygen level must be controlled as it varies with such parameters as temperature and buffer constituents[35]. A comparison of these measuring principles for electrodes based on glucose oxidase is studied[36]. An alternative technique for measuring glucose concentration is by the use of the enzyme glucose dehydrogenase (GDH), which is not susceptible to oxygen interference, as is the case with glucose oxidase (GOD)[37]. Amperometric sensors have also been investigated, for the detection of galactose, glycolate and L-amino acids using the respective oxidases as catalytic enzymes. Galactose and lactose, are important substrates in fermentation and food production processes and the rapid assay of amino acids is critical in fermentation processes. Clinical applications include the monitoring of various metabolic disorders, such as diabetes[38].

Enzymes rather than ion exchangers have also been encapsulated in PVC and alternative membranes[39], in order to provide a naturally occurring highly specific reagent phase for many sensor systems[40]. The most commonly reported of these systems employs immobilised urease or glucose oxidase for the detection of urea and glucose respectively. In these devices an enzyme, specific for an organic molecule is immobilised in the vicinity of the ion-selective membrane. The binding of the organic molecule is sensed when an enzyme-catalysed reaction either produces or consumes an ion to which the ion-selective electrode is sensitive[20]. Other enzyme systems which have been utilised are whole or part plant and animal tissue[41], thus eradicating the need for expensive purification and extraction stages[42]. Although enzyme electrodes are highly selective, interference from intermediate metabolic products are frequent and their shelf lives are between 20-30 days with cold storage[7].

Similarly to enzyme electrodes, enzymes have been incorporated into the gate layers, in an attempt to produces Enzyme FETs or EnFETs for the detection of penicillin, glucose acetylcholine and urea[18,43-47]. In the detection of penicillin, the enzyme penicillinase is immobilised onto an albumin membrane at the FET gate. In the presence of penicillin, the following reaction occurs;

$$\text{Penicillin} \xrightarrow{\text{Penicillinase}} \text{Penicilloic acid}^- + H^+$$

and the hydrogen ion released from the penicilloic acid, reduces the pH at the surface of the ISFET[46,48].

Unfortunately, enzymes are not always stable at body temperature and these electrodes need to be able to monitor glucose levels for continuously long periods. This is evidently a common problem with enzyme systems and has led to the development of synthetic enzymes or synzymes, providing more stable reagents with longer life times[49].

1.6 PIEZOELECTRIC DEVICES

Piezoelectric devices[14] are sensitive to changes in mass density and viscosity of samples in contact with the surface of a vibrating crystal, since these parameters will alter the vibrating frequency. The most obvious application, therefore is for microweighing of substances deposited at its surface, such as ammonia, gases in aqueous solution, hydrogen chloride in air, hydrogen sulphide, explosives (TNT and MNT) and organophosphorous pesticides[50]. A successful application of a piezoelectric device has been for the determination of antihuman IgG and human chorionic gonadotropin, which is an indication of pregnancy. The largest hurdle in the development of these devices seems to be either the immobilisation and coating techniques, in order to increase the selectivity of the device. Piezoelectric devices which do not possess a selective coating can be sensitive to parts per billion but any mass change at the crystal surface,

regardless of the compound, will produce the same frequency shift[51]

1.7 OPTICAL SENSORS

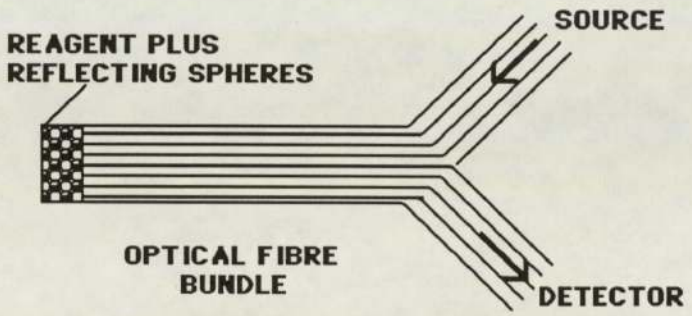
1.7.1 Introduction To Optical Fibres And Sensors.

An optical fibre is a thin strand of material, composed of an inner core and an outer cladding which is covered by an external coating to provide protection. Both the core and cladding can be constructed of either glass or plastic but the core can be quartz and is always of a higher refractive index than the cladding, causing incident light reflected at the core-cladding boundary to be continually reflected along the fibre, as it propogates through the core. The three types of refractive index profiles which are present in optical fibres are described[52] and these are single mode step-index, multimode step index and graded step index.

Optical sensor technology is one of the fastest growing fields in analytical chemistry today and so much so that a whole issue of Talanta[53-57] was dedicated to the subject of fibre optic chemical sensors in 1988. Optical sensors generally use a fibre optic as the transducing phase of the sensor and this can be introduced in the design of the sensor either intrinsically or extrinsically[58-60]. In the latter case, by definition the fibre is only used to carry light to and from the reaction cell[61,62] or waveguide[63-70], whereas intrinsic fibre optic sensors actively incorporate the fibre optic in one of three basic configurations[71].

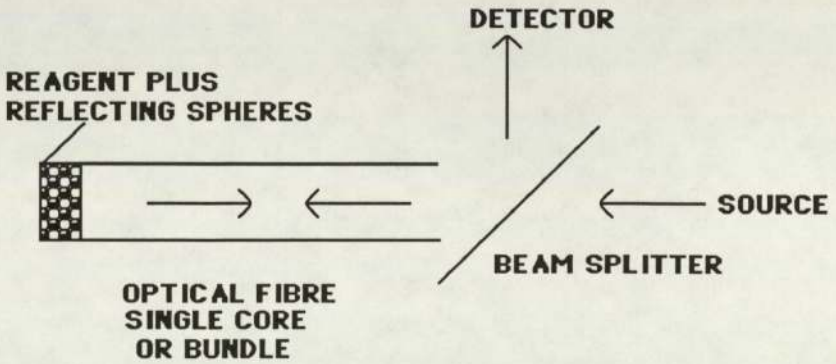
The first of the three sensor configurations, mentioned earlier, uses a bifurcated fibre bundle to pass light to and from the reagent phase situated at the tip of the fibre; FIG 1.5

FIG 1.5 Sensor Configuration Utilising A Bifurcated Fibre Bundle



In order to reflect the modified incident light back towards the detector, small spheres are usually incorporated at the tip of the fibre close to or with the reagent phase. The second, configuration requires only a single fibre coupled with a beam splitter in order to separate the incident and emergent radiation; FIG 1.6

FIG 1.6 Sensor Configuration Utilising A Single Fibre With Reagent At The Tip

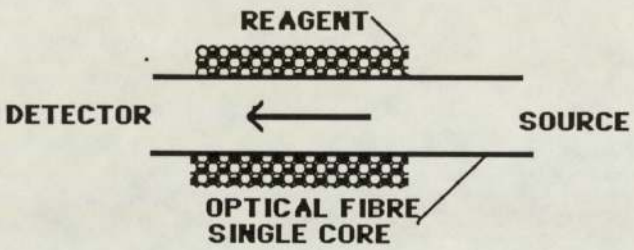


The incident or background light may need to be distinguished from the signal travelling along the fibre, towards the detector. In the case of a fluorescent reagent, this

could be achieved using a filter which would transmit the emission wavelength of the reagent and absorb the excitation wavelength.

The third uses the evanescent wave component or absorption of the incident light in the reagent phase situated concentrically along the fibre length; FIG 1.7

FIG1.7 Sensor Configuration Utilising The Evanescent Wave Component Of The Source Radiation



In this case, another parameter which can alter the intensity of the detected light is a change in refractive index of the reagent[71]. Since the signal is proportional to the amount of reagent which interacts with the incident light, the sensitivity in both colourimetric and fluorimetric sensors, can be increased by using this configuration rather than positioning the reagent at the small distal end of the fibre. Light is transmitted along the fibre by internal reflection and at each interaction at the surface of the fibre, a component of the incident light is transmitted into the surrounding medium, since light is not reflected at the point of contact between the two media but is parabolically reflected at some point in this rarer medium[72-74]. This component is known as the evanescent wave and is a standing wave, which penetrates the medium, decaying exponentially, to a depth of the order of a fraction of a wavelength of the incident light. This is a useful sensing technique to employ because only changes near the

surface of the fibre are detected and the properties of the bulk solution around the fibre are not. This is important when detecting change in samples which are coloured or turbid. Changes which can be detected are fluorescence and colour changes[70] by absorption and changes in the refractive index of the reflecting medium[75-77], as well as scattering of the incident light at the interface[78].

Fibre-optic sensing is generally based on the absorption, scattering or fluorescence of light in response to analyte concentration by the reagent phase, which is held within a membrane matrix, usually by covalently binding into ion exchange resins[54], including styrene divinylbenzene copolymers[79], polyacrylamide, polystyrene, porous glass beads[57,80,81] or directly onto the fibre itself[82-84]. The effect of immobilisation, onto ion exchange resins, on the sensitivity and fluorescent lifetime of certain fluorescent reagents is discussed[85].

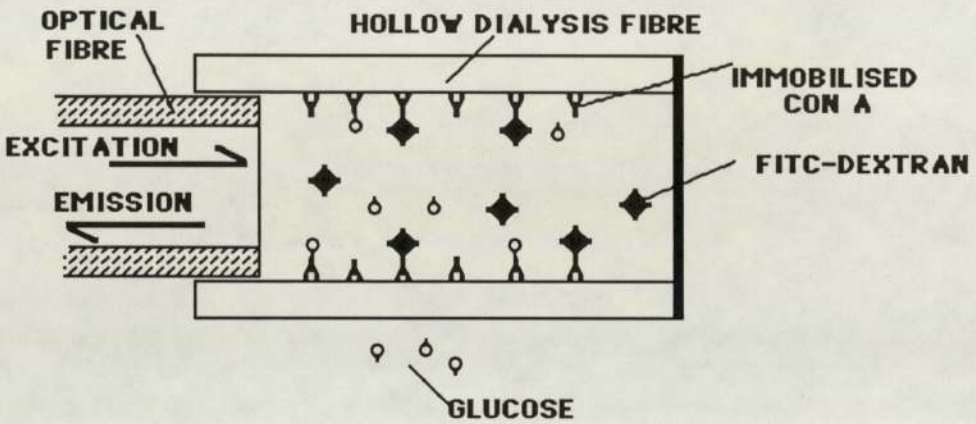
1.7.2 Fluorescence Sensors.

Fluorescence sensors for the detection of Al^{3+}, Be^{2+} and pH have been developed by Saari and Seitz, where the two indicators Fluoresceinamine and morin were immobilised onto cellulose. A sensor of similar design was also reported for the detection of Al^{3+}, Mg^{2+}, Zn^{2+}, and Cd^{2+}, based on the fluorescence of quinolin-8-ol sulphonate immobilised onto an ion-exchange resin[76,77]. This technique is said to be inconvenient, as the reagent needs to be frequently refreshed and immobilisation techniques reduce the fluorescent intensity of the indicator[88-90]. However, it must also be said that some immobilisation techniques have been reported to enhance fluorescent intensity[91].

Other fluorescence sensors are based on the competition between fluorophor labelled and unlabelled antigen (analyte) for a limited number of receptor binding sites. This technique is useful for reactions which do not directly produce optical change[92].

A glucose sensor is shown in FIG 1.8 where the specific glucose binding reagent, concanavalin A, is immobilised on sepharose at the edges of a hollow cell, outside the fibre illuminated volume.

FIG 1.8 Glucose Sensor Using Competitive Binding Fluorescence.



Glucose can diffuse freely through the cell, whereas fluorescene isothiocyanate (FITC)-labelled dextran, the competing ligand cannot. Thus, increasing glucose concentration forces the dextran to be displaced into the illuminated volume and hence the increased fluorescent intensity is detected via the fibre optic[93,94,8]. However, these sensors have been reported to have a response time of about 10 minutes and the limiting factor is said to be the diffusional transport through the hollow fibre membrane[78,86]. Rare earth metal chelates, such as Eu(III) and Tb(III) complexed with b-diketones and EDTA derivatives, can also be used as fluorescent labels and these are reported to have longer life-times than conventional labels, such as FITC and the emitted wavelength is usually shifted from the excitation wavelength by 200-300nm, as opposed to 30nm for FITC. This allows for improved resolution between source and signal radiation, and therefore

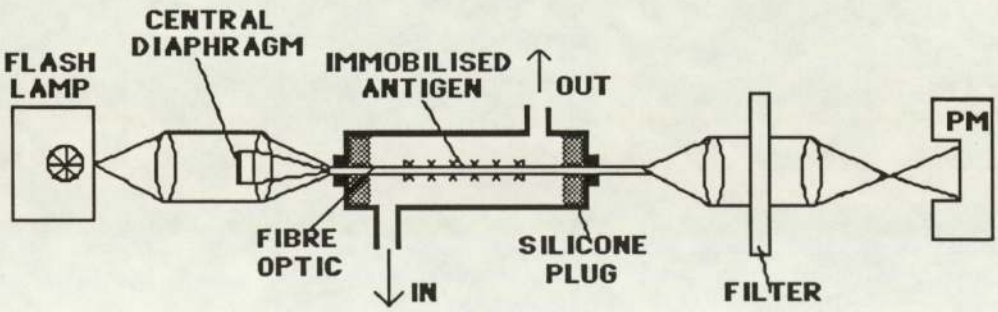
improved sensitivity[95].

Fluorescence quenching can also be used as an analytical tool as illustrated by the production of a sensor for the determination of iodide with Rhodamine-6G immobilised on a teflon film[96]. When pyridine is exposed to organochlorides, the absorption of pyridine changes, due to the formation of a chromophore. This phenomena is referred to as the "Fujiwara" reaction and can be utilised in the determination of organochlorides as water pollutants, when pyridine is immobilised at the tip of an optical fibre, inside a capillary tube. The capillary tube is then capped with a mylar membrane, to keep pyridine in and water out, as well as to allow reasonably rapid diffusion of organochlorides to the reagent. However, mylar is said to be "not the ideal membrane" and "research is continuing to find an optimal membrane"[97].

A probe has also been reported which measures oxygen partial pressure in blood as aresult of the presence of oxygen quenching the fluorescence of solvent green 5, immobilised on XAD-4 resin and contained within a polypropylene membrane[98].

A fibre optic detector utilising the surface along the fibre length, as a coating surface for the reagent phase was developed at the Battelle research centre[99] and is shown in FIG 1.9.

<u>FIG 1.9 Fluorescence Sensor Utilising The Evanescent Field Component Of The Source Radiation.</u>



This was designed for the fluorescence detection of FITC labelled anti human IgG, when bound to immunologically immobilised antigen at a glass-liquid interface. This device uses the evanescent wave component of the incident light as the excitation wavelength and the associated excited wavelength as the signal picked up by the fibre optic. A similar device using fluorophores immobilised in lipid membranes is described in the literature[100,101].

1.7.3 Colourimetric Sensors

As well as fluorescent reagents, colourimetric reagents for use in pH sensors have been reported, where the indicators have been immobilised onto a styrene divinyl benzene copolymer or ion exchange resin and held within a PTFE membrane, at the tip of a bifurcated fibre optic bundle[102-104,54]. In fact this was the technique adopted for the first optical sensor to be reported[105]. The response time of these sensors is approximately 12 minutes and the exchange resin requires regeneration. The performance of these sensors is also affected by the strength of absorption between the resin and the reagent[54]. Another example of an optical sensor using the bifurcated fibre configuration is one of the earliest reported. Here, colourimetric indicators are immobilised onto polystyrene microspheres, which are used to increase light scatter and improve the intensity of the signal to the detector. The cellulosic tubing allows the passage of hydronium ions and small molecules to the indicator dye and hence an increase in absorbance or transmission at a particular wavelength would indicate, for example, a change in pH[106].

A waveguide which utilises the absorption properties of a colourimetric indicator sensitive to ammonia vapours, where the dye is coated along a capillary tube is given in reference[70]. However, no such fibre optic sensors using colourimetric

reagents, coated along the fibre length, have been found in the literature. Sensors which use the energy transfer between a dye and a fluorophore, where the reagents are bound directly onto the fibre tip[107] and onto dialysis tubing[108] are reported.

1.7.4 The Advantages Of Fibre Optic Sensing

Optical sensors, sometimes referred to as optrodes or optodes, can offer many advantages over other sensor types and these are listed below[109,71].

1) In the case of *in vivo* clinical sensors, there are no direct electrical connections to the patient or localised electric fields around the sensor, therefore eliminating the risk of electric shock hazard and blood clots, as is the case with potentiometric sensors.

2) Also, in contrast to potentiometric sensors, no reference electrode is necessary, reducing both bulkiness and cost of the system.

3) In addition to this, the low cost of fibres optics, lends them to the development of throw away devices and their size and flexibility offers the potential of miniaturised, easily manipulated and also remote systems, which in the case of fibre bundles could also be multi-functional[56].

In brief, fibre optic sensors offer the advantages of been potentially cheap, compact and robust and the fact that no direct electrical connections are necessary and therefore, there is no localised electric field at the sensing surface, increases their potential as *in-vivo* multiple sensing devices.

1.8 THE FUNCTION OF MEMBRANES IN DIFFERENT SENSOR TYPES

1.8.1 Introduction: The General Role Of Membranes In Sensing

The most fundamental factor in determining the selectivity and sensitivity of all sensors is either the choice of the membrane/support matrix and/or the reagent.

Since many reagents, other than enzymes and antigens are not highly specific, it is the selective diffusion characteristics of the membrane which determine the sensitivity and selectivity of the sensing device. The membrane can govern the selective diffusion of ions to the sensor and it can also be important in acting as a reagent support, without interfering to more than a minimal degree with it's sensing characteristics. However, the development of highly selective substrate/transport membranes, appears to be an area where little research has been carried out and the incorporation of membrane technology into the design of such systems could lead to the full realisation of their potential as sensing devices[71].

The different types of sensor have been described and illustrated diagramatically in the earlier sections of this introduction and the different types of membrane behaviour and requirements have been referred to, although not in any way that relates structure to performance. The three major types of sensor may conveniently be considered in the way in which they relate to membrane properties. The ways in which different membrane properties impinge on sensor behaviour at the transducer/analyte interface varies for different sensor types and environment.

1.8.2 Membrane Properties

There are three groups of properties that must be borne in mind in selecting or designing polymer membranes for sensor applications. The first of these relates to the transport behaviour of the polymer and encompasses permeability, permselectivity and transmembrane potential. These are important to different extents in different types of sensor (eg. potentiometric, amperometric, fibre optic). Furthermore, there are differing views as to the relative importance of transmembrane potential and permeability in the functioning of potentiometric sensors[110,7].

The second group of properties relate to the role of the membrane as an

immobilisation matrix. This is particularly important in the field of biosensors and may be typified by the immobilisation of enzymes in bilaminar membranes. Often the sensed species is so large (as in antibody/antigen interactions) that the polymer is configured in such a way as to enable diffusion to specific sites to occur in an unmodulated fashion. A good example is the use of nylon mesh as the immobilisation substrate, which may be considered as an extreme case of a macroporous membrane!

The third group of properties relate to interfacial and surface behaviour. These properties are in many ways the most difficult to control without compromising other functions of the polymer, for example permeability. Even so, surface and interfacial phenomena are extremely important in the overall performance of a given sensor, both because of their contribution to transport phenomena and their role in controlling biocompatibility.

Perhaps the most effective way of providing an overview of membrane function in relation to polymer structure, is to consider the appropriate properties in relation to the requirements of different types of sensors.

1.8.3 Amperometric Sensors: The Role Of Permeability

Amperometric sensors provide examples of various aspects of polymer behaviour. It is undoubtedly true however that they provide the clearest illustration of a permeability requirement, since in order to function, they require flow of the electrochemically active species to the electrode surface. The application of a voltage between working and reference electrodes, often in the form of a central wire cathode surrounded by and insulated from a concentric reference anode, electrochemically reduces the transported molecule, thereby generating a flow of electrons.

The role of the polymer membrane in this case therefore, is primarily to

permit adequate flow of the sensed species to the surface of the amperometric transducer. It may equally be required to exhibit a degree of permselectivity, thereby preventing or reducing the flow of other electrochemically active species to the electrode. Additionally, it may be necessary for the membrane to act as an immobilisation matrix for a biologically active species, such as an enzyme, which is required to convert the desired analyte into an amperometrically active species. In this case, multilayered membranes are frequently used and here, the differential transport properties of the various layers enable them to perform their required function.

An ingenious extension of the simple amperometric devices is found in the use of a bilaminar membrane between which an enzyme or alternatively, bacteria or cells, are immobilised. The outermost membrane layer is relatively porous, thus allowing high molecular weight species to diffuse to the enzyme layer. A bio-specific reaction occurs at the interface resulting in the generation of low molecular weight reaction fragments, for example oxygen, carbon dioxide or hydrogen ions. The second membrane is permselective, in that it allows electrochemically active species of choice to diffuse through to the electrode whilst inhibiting the passage of electrode-sensitive interferants.

Early reference to the use of this principle is made by Clarke and Lyons who suggest the use of cuprophane dialysis membrane and polyethylene as the two membrane materials, in conjunction with glucose oxidase[111-118]. Perhaps surprisingly, polymers of widely differing permeability such as poly(4-methyl pent-1-ene) polyvinylchloride, cellulose esters, polyacrylic acid and even polymethylmethacrylate have been used in this connection. A wide range of enzymes and other bioactive species have been described in sensors of this type.

1.8.4 Potentiometric Sensors: Selective Transport or Transmembrane Potential

Whereas amperometric sensors require the flow and consumption of electrochemically active analytes in order to function, this is not so in the case of potentiometric sensors. Here the signal is generated by the production of a potential difference in the system. A useful illustration of the concept is found in the adsorption of hydrogen ions onto the surface of a glass electrode. If the glass is regarded as a membrane for the purpose of the illustration, then it is the generation of a transmembrane potential that gives rise to a signal, whose magnitude is related to the concentration of hydrogen ions at the glass surface. If the electrode is now overlayed with a polymeric membrane, a signal will be generated only if this membrane allows free and rapid transport of the sensed ion to the surface of the glass or if the membrane can act as an extension of the glass. Generation of a transmembrane potential is thus permitted by adsorption of the sensed ion on its own exterior surface. It is without question, the latter approach that has formed the basis of the family of potentiometric sensors now in existence. It is on the basis of ion selective electrode technology, and in particular the ionophores developed for this application that most new potentiometric sensors have been developed.

There is however, a certain amount of disagreement about the relative roles of permeability and transmembrane potential in the functioning of potentiometric sensors. The majority view point, favouring the importance of transmembrane potential, is that propounded, for example, by Buck[7]. Simon[105], however, lays greater stress on the selective transport of individual substrates, through the membrane. It seems clear that in the majority of circumstances a sufficiently rapid response can only

be achieved by rapid establishment of a transmembrane potential and in such circumstances, active transport would merely contribute to signal drift. In principle however, transport across the membrane, if sufficiently rapid, is quite capable of producing an effective potential difference at the electrode surface.

PVC in the absence of a plasticiser is a tough, glassy polymer. It is largely the toughness and physical durability of PVC that has contributed to its success as a material of fabrication. In its unplasticised form however, it is too inflexible to be considered as a coating material and additionally is relatively impermeable to most species. The addition of a plasticiser however produces a tough, yet flexible material, whose glass transition temperature may be reduced (by the plasticiser) to around ambient temperature and which has many useful features as a coating material. In order to be successful as a membrane material with an incorporated charge carrier the plasticiser must, in addition, be an effective solvent for the charge carrier and show resistance to leaching or dissolution by aqueous systems. Plasticisers are often referred to as solvent mediators, serving to dissolve and enhance the mobility of the charge carrier in the PVC.

Initial ion selective membrane studies are still performed on conventional ion selective electrodes[119,120]. Membranes are prepared from a PVC matrix containing an appropriate ion-exchanger or ionophore dissolved typically, in dioctyl phenylphosphonate, o-nitrophenyloctylether or phthalic acid esters, which are the most frequently incorporated plasticisers. The ion-selective electrodes of this type include the potassium electrode based on valinomycin and the sodium electrode based on monensin[33]. In the case of ISE's[5,33,121-123], CWEs[11,12] and ISFETS[13] the undoubted success of PVC as a rigid matrix with liquid membrane properties, together with the incorporated ion carriers has, not unreasonably, localised research almost

exclusively in this area, especially where cation selective carriers are concerned.

The vast majority of membranes studied have contained neutral carriers or complexing agents for carrying cations such as K^+, Ca^{2+}, Mg^{2+}, H^+; Indeed, Na^+ and Ca^{2+} ion-selective materials have occasionally been recovered from commercially available ion-selective electrodes and re-used in ISFET membrane coatings[124]. Other carriers which have been more recently studied have been the crown ethers[121], which provide the selectivity of the membrane in accordance with their crown or ring size. Commercially available ionophores[124,12]; crown ethers[12,125] and nitrogen containing neutral carriers[126] have all been incorporated into ISFET's, however these ranges of products are somewhat limited. There is a vast array of publications associated with neutral carriers designed especially for ion-selective membranes[127]. More recently, this area has been dominated by Simon who has pioneered much of the research into novel neutral carrier ligands suitable for sensor applications[128-132]. Still the most successful of these has been a potassium ion sensor using a PVC support containing valinomycin and a plasticiser.

The production of anion-selective membranes, by the incorporation of either anion-exchangers, positively charged anion carriers or electrically neutral ion-carriers, however, has not been successful[7].

The problems associated with PVC membranes include leaching, complex formation between surfactants and surface components and the interference of counter ions. To try and overcome these draw backs, other polymer matrices have, more recently, been studied. These include polymethylmethacrylate for coated wire electrodes and as a matrix for a nitrate ISE, to determine nitrogen oxides in ambient air. The selectivity profile of an electrode with a poly(vinyl isobutyl ether) matrix with calcium

bis di-2-ethyl hexyl phosphate sensor and decan-1-ol solvent mediator has been found to be similar to a PVC electrode containing the same ingredients.

Urushi, a natural lacquer of Japanese origin, provides a matrix compatible with various liquid ion exchangers for divalent ions, perchlorate, nitrate and other ions. Also, membranes comprising vinyl plastic repair cement plus solvent mediator (dibutyl phthalate or dioctyl phthalate) and a metal complex such as Pb[Hg(SCN)4] give electrodes responsive to lead. Interestingly, membranes such as cellulose acetate, ethyl cellulose and collodion have been considered to be totally unsatisfactory, owing to their hydrophilic character. However, PVA can be adapted by copolymerising with PVC [VAGH copolymer] to give a good functional membrane with organophosphate sensors and dioctyl phenyl phosphate (DOPP) solvent mediator

.1.8.5 Optical Sensors

In optical sensing, selectivity can be determined either by the choice of, or the selective diffusion of the sensed species to, the reagent. Membranes used in optical devices can thus serve two purposes. The first can be to control diffusion into the part of the sensor which contains the reagent phase, eg PTFE and the second to support the reagent phase. A combination of indicators have been trapped behind a teflon membrane in order to produce an ammonia sensor, for waste water analysis, thus allowing the ammonia partial pressure to alter in the indicator solution, via diffusion through the teflon membrane, whilst retaining the indicators at the sensor tip[133].

Also, the reagent can be immobilised directly onto the fibre optic[84,134], especially in the case of antigens where a selective membrane is not necessary. These have also been immobilised on the inside of cellulosic or dialysis tubing or on nylon net, positioned at the end of the sensor[135]. Bromocresol Green has been immobilised on sepharose to produce a membrane which is colourimetrically sensitive to albumin for an

extrinsic sensor[61]. An attempt has been made to resolve the problem of selectivity by immobilising both a fluorophore and an ionophore on silica to produce a sodium selective optical sensor[136]. An alternative technique for producing a cation-selective membrane has been by immobilising the fluorescent reagent, rhodamine 6G onto a Nafion film, which is a perfluorinated polysulphate polymer. Ionophores are formed within the Nafion film, as solvent evaporates and the charged sulphate groups agglomerate in the developing film, providing ionic clusters, randomly distributed across the backbone of polytetrafluoroethylene. Ions diffuse from cluster to cluster via interconnecting channels, through the polymer membrane[9]. Cationic dye molecules can then reside on the Nafion membrane with their organic portions hydrophobically bound to the bulk membrane while their charged groups reside in the ionophore[137]. Rhodamine 6G is also used in the determination of iodide and is immobilised on teflon or ion exchange resins at the end of a fibre bundle[96]. As described earlier, the remote detection of organochlorides can be achieved by the immobilisation of pyridine at the tip of an optical fibre, inside a capillary tube. The capillary tube is then capped with a mylar membrane, to keep pyridine in, water out and allow reasonably rapid diffusion of organochlorides to the reagent[97]. The mass transfer step of the analyte to the reagent is a limiting factor in the response time of fibre optic sensors, as stated earlier, and an attempt has been made to resolve this problem by immobilising congo red onto hydrolysed cellulose acetate. The adsorbtion of various dyes including phthalein indicators onto cellulose acetate is said to have the effect of increasing the dynamic range of the indicator[82]. Cellulose acetate is a polymer which swells in an aqueous environment and is therefore a hydrogel. Other hydrogel membranes which have been used in optical sensor systems are HEMA (hydroxy ethylmethacrylate), HPTS (hydroxypyrenetrisulphonic acid) and acrylamide[91]. This

was in fact used as a sensor for the detection of CO_2, since the dissolution of CO_2 in the hydrophillic environment of the polymer matrix containing copolymerised fluorescent reagents, will alter the local pH and thus the fluorescent intensity of the reagent.

The rate determining step in the majority of these sensing devices is the diffusion of the sensed species into the membrane support matrix. In addition to this factor, which affects the sensor performance with respect to response time, sensing devices generally suffer from a response to interfering ions, or in other words, from a lack of selectivity or specificity. Reagents which are not bound to support matrices but are merely encapsulated within or trapped behind them can produce a drift in the response with sensor life-time and in the case where reagents are bound, the process of binding alters the dynamic response range of the reagent, especially in the case of fluorescent devices. Enzymes which have been introduced as highly specific reagents, suffer from a short shelf life, instability and long reponse times, taking between 10 minutes and several hours to reach an equilibrium response.

1.8.6 Electrochemical And Optical Biosensors

It was stated at the outset of this overview that one of the three major roles played by polymeric membranes, in promoting the function of chemical sensors, is immobilisation. This is important in all types of sensor, amperometric, potentiometric and optical. Although the concept of immobilisation may be associated with the incorporation of an ionophore or charge carrier, it is more usually reserved for biological species such as enzymes cells and bacteria, which take part in very specific chemical reactions with particular analytes, giving rise to more readily sensed species.

The principle of using enzymes as reagents has already been high-lighted in the case of amperometric sensors, where enzymes such as glucose oxidase are commonly

used. Whereas in amperometric sensors the product of the biospecific reaction (hence biosensor) has to be transported through a secondary membrane to the electrode surface, this restriction does not apply to potentiometric devices.

The previously discussed principles of operation of potentiometric sensors apply here and the role of the enzyme is to generate a species capable of giving a potentiometric signal. A range of immobilisation techniques have been used and are described in the literature. These include both physical techniques, in which the enzyme is entrapped and its mobility limited by diffusional restrictions and chemical binding in which the enzyme is covalently linked to a polymeric support. Questions relating to enzymatic stability and the effect of immobilisation on bioactivity lie outside the scope of this review but have been discussed elsewhere[138]. More pertinent here, are questions of membrane permeability and reactivity. These govern effectiveness of physical immobilisation, accessibility of the enzyme to the sensed species, permselectivity of the membrane with respect to the target reaction product and other interferants and the availability of chemically reactive sites that may be used for chemical immobilisation. The situation is very dependent upon the nature of the analyte and biological substrate. In antigen/antibody reactions for example, both the reagents have substantial molecular weights and thus low intrinsic rates of diffusion. In such cases successful physical immobilisation of an antibody would inhibit diffusion of the antigen to the reactive site. This illustrates the type of situation in which chemical linkage, either directly onto the sensing device or into an extremely porous membrane is commonly used. In this sense an extreme case of a very porous polymer membrane is a polymer mesh or net!

These problems become more acute in the case of optical fibre devices, in which an effective reaction has to take place at the fibre/membrane interface. The greatest number of literature references to potentiometric biosensors are found in the

area of field effect transistors, eg the EnFETS. It is certainly true that current growth of literature is greatest in this area.

Enzymes, used as specific reagents, for electrode systems have been either immobilised directly onto standard electrodes, for the detection of pH, NH_4, O_2, and CO_2 or supported on membranes[41,139], such as nylon[140], teflon[141], cellophane[142], cellulose, gluteraldehyde[29] and acetyl cellulose[80],. Immobilisation is either carried out via physical absorption or by chemical binding to a polymer layer[33]. In addition, ISFET devices for the detection of acetylcholine and urea have been reported, where the enzymes have been immobilised on polyvinylbutyral and g -aminopropyltriethoxysilane with gluteraldehyde[45]. Membranes which are used in immobilisation of enzymes are often given trade names, such as Posidyne®, Biodyne® and Pall Biodyne®. Immunoaffinity membranes and others, commonly employed in immobilisation procedures, are poly amide membranes and collagen membranes[39].

As plant and animal tissue naturally contain enzymes, both whole and part tissues have been adapted for use in ISE's to produce enzyme sensors, whilst eliminating the need for enzyme extraction and purification[40-42,143,144],.

1.9 THE ADVANTAGES OF USING HYDROGELS AS MATRICES FOR FIBRE OPTIC SENSING

Hydrogel polymers possess many advantages in relation to the requirements described above and offer the potential of producing a relatively cheap, biocompatible, robust and selective, reversible on-line biosensor. Hydrogel membranes are water swollen polymers, the pore size and water content of which dictate the permeability and permselectivity characteristics, both of which are considered to be limitations in fibre optic sensing[145,146,92]. Various aspects of the properties of these materials have

been described in a series of papers from this research group[147-150].

In the present context useful polymers may be obtained by the copolymerisation of hydroxy alkyl acrylates and methacrylates with hydrophobic monomers, such as styrene or methyl methacrylate. The resultant materials are tough, flexible, polymers, rather than soft gels. In addition, their refractive indices are controlled by monomer composition and water content and enable the design of membranes whose refractive indices interface well with that of polymethylmethacrylate fibres. This is illustrated by the range of refractive indices obtained from copolymers of 2-hydroxyethyl methacrylate (HEMA), with styrene and with methyl methacrylate[151]. The ready control of permeability and permselectivity together with appropriate chemical manipulation leads to the production of ion-selective reactive matrix membranes for use in fibre optic sensors.

CHAPTER 2

MATERIALS AND METHODS.

2.1 INTRODUCTION.

This chapter deals with the preparation of the membranes and sensor coatings studied and lists the reagents involved. It mentions the techniques used to measure the water content of whole membranes and describes the apparatus used for the ion-selective electrode studies. Since the preparation of the fibre optic probes: including chemical etching, coating techniques and flow through cell construction, were techniques developed during the course of this work, they are discussed in the main body of the experimental text.

2.2 SALTS.

All salts were of analytical grade and purchased from Fisons, B.D.H. or Aldrich and used without further purification.

2.3 COLOURIMETRIC REAGENTS

All reagents were supplied by Aldrich and were used as supplied.

2.4 MONOMERS.

All monomers used were purified by reduced pressure distillation with the exception of 2-hydroxyethyl methacrylate, (HEMA), which was obtained in an already pure form (optical grade). This is because HEMA is a very difficult monomer to purify since it readily undergoes disproportionation to ethylene glycol dimethacrylate and methacrylic acid. The purified monomers were then stored in a refrigerator until required.

Table 2.1 Monomers Used.

Monomer	Molecular Weight/g	Abbreviation	Supplier
2-hydroxyethyl methacrylate	130	HEMA	Ubichem Ltd.
methyl methacrylate	100	MMA	B.D.H
styrene	104	St	B.D.H.
N-Vinyl Pyrollidone	111	NVP	Koch-Light Laboratories
N,N-dimethylacrylamide	99	NNDMA	BDH

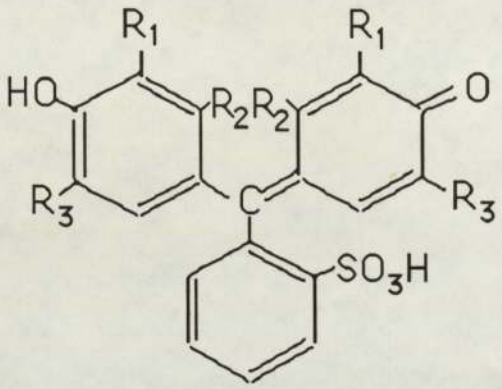
2.5 CROSSLINKING AGENTS.

The crosslinking agents employed for both covalent crosslinking and U.V. cross linking, were used as supplied without further purification. They were stored in a refrigerator until required.

Table 2.2 Crosslinkers Used.

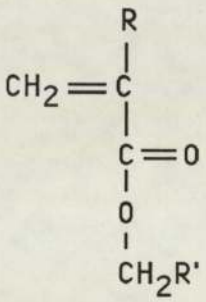
Crosslinker	Molecular Weight	Abbreviation	Supplier
ethylene glycol dimethacrylate	198	EGDM	B.D.H
N-Vinyl Carbazole	193	NVC	Fluka

FIG 2.1 Structure of Colourimetric Reagents



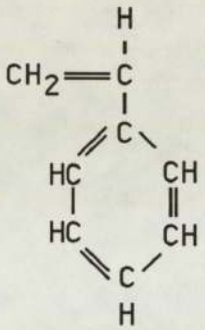
Bromopyrogallol Red	R_1=OH	Bromophenol Blue	R_1=Br
	R_2=OH		R_2=H
	R_3=Br		R_3=Br
Cresol Red	R_1=CH_3	Bromocresol Purple	R_1=CH_3
	R_2=H		R_2=H
	R_3=H		R_3=Br

Figure 2.2 Structures of Monomers.

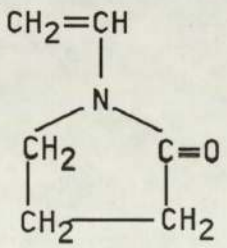


$R = CH_3$ $R' = CH_2OH$ 2-hydroxyethyl methacrylate

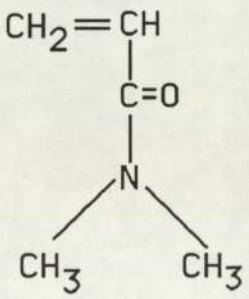
$R = CH_3$ $R' = CH_3$ methyl methacrylate



Styrene

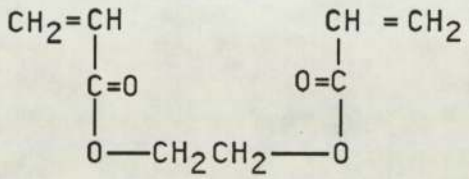


N-Vinyl pyrollidone

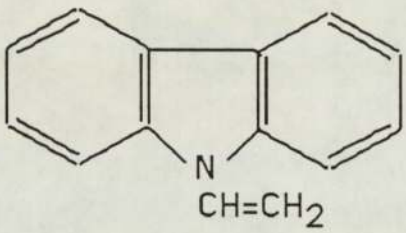


N,N-dimethylacrylamide

Figure 2.3 Structure of Crosslinking Agents.



ethylene glycol dimethacrylate



N-Vinyl Carbazole

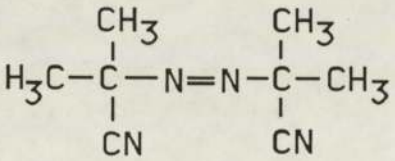
2.6. INITIATOR.

The free-radical initiator was used as supplied without further purification. It was stored in a refrigerator until required.

Table 2.3 Initiator Used.

Initiator	Molecular Weight	Abbreviation	Supplier
azo-bis isobutyronitrile	164	AZBN	B.D.H

Figure 2.4 Structure of Initiator.

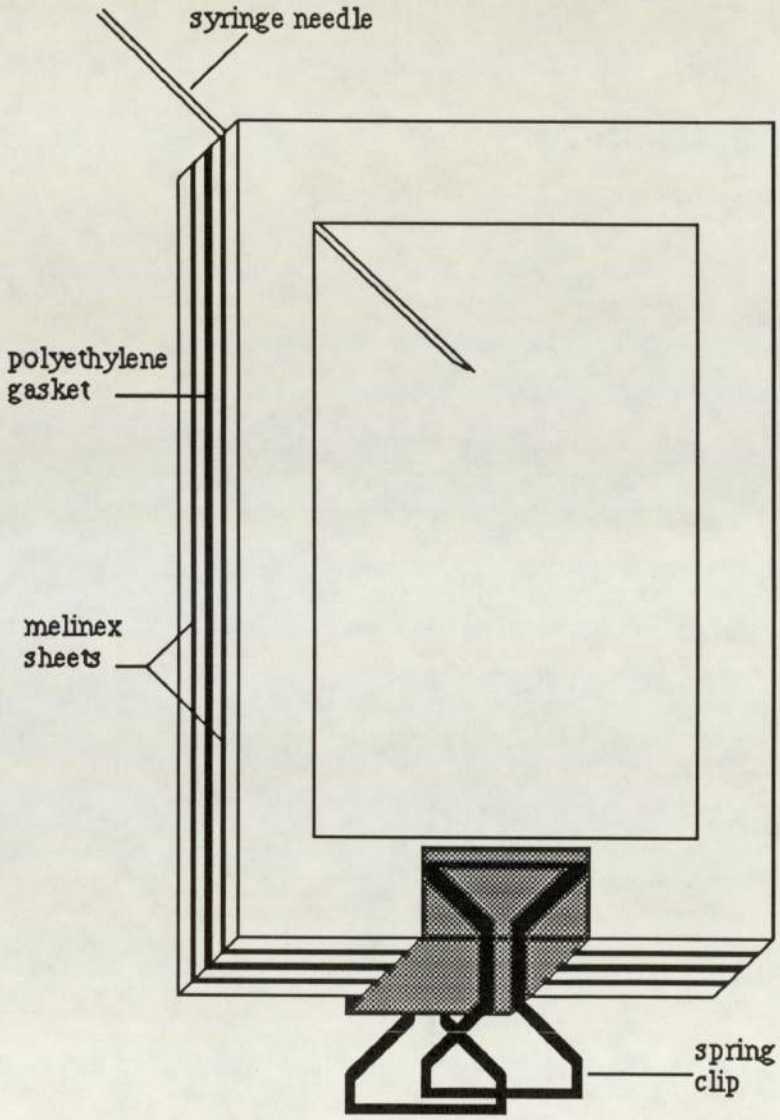


Azo-bis-isobutyronitrile

2.7 MEMBRANE PREPARATION.

Membranes were prepared by the bulk polymerisation of the monomer reaction mixture in a membrane mould[151-153] consisting of one or two polyethylene gaskets (0.2 mm thick, with rectangular hole (6 x 11 cm)) sandwiched between two glass plates (8 x 13 cm). The gaskets were separated from the plates by two sheets of melinex (poly(ethylene terephthalate)) that were present to aid the release of the membrane and give it a smooth finish (figure 2.5) and the complete assembly held together with clips. The reaction mixture was transferred into the mould cavity via the syringe inserted between the melinex sheets. The monomers were then polymerised at 60°C for three days, followed by two hours post-curing at 90°C. On removal from the oven the clips were removed and the glass plates prized off. The gasket was removed and the melinex sheets carefully peeled from the membrane which was then placed in distilled water to hydrate. Membranes were allowed to fully hydrate in distilled water with frequent changes of water to remove any water soluble residue.

Figure 2.5 Membrane Mould.



2.8 PRODUCTION OF LINEAR POLYMER BY SOLUTION POLYMERISATION.

In a typical reaction, a mixture consisting of monomer (10g) and initiator (AZBN, 0.1g) was degassed by bubbling N_2 through and added to a reaction vessel containing 250ml of ethanol, which itself had been similarly degassed. The system was immersed in a water bath at 60°C and allowed to polymerise under a blanket of nitrogen for 8 hours with constant stirring. The polymer was precipitated by adding the solution dropwise to diethylether which had been cooled with solid CO_2. The precipitated granules were then filtered out and washed again with diethylether. The polymer was dried under vacuum and stored in a refrigerator until required.

2.9 DETERMINATION OF THE EQUILIBRIUM WATER CONTENT.

A disc of 1cm diameter (No. 7 cork borer) was cut from the hydrated polymer sheet . The surface water was removed by carefully blotting with filter paper. The sample was placed in a preweighed sample bottle and its hydrated weight, recorded. The disc was dried in a microwave oven for 10 minutes. The Equilibrium Water Content, EWC,was calculated from:

$$\%EWC = \frac{(\text{hydrated weight} - \text{dehydrated weight}) \times 100}{\text{hydrated weight}}$$

This technique has been extensively used in these laboratories and should provide weights which are correct to within +/- 0.01g [153]

2.10 COATED WIRE ION-SELECTIVE ELECTRODE STUDIES.

2.10.1 The Coated Wire Ion-Selective Electrode: Introduction.

The relatively simple construction of coated wire ion-selective electrodes, CWISE's, as well as the ease with which different membrane coatings can be changed, makes these devices a useful test-bed for studying new sensing membranes[152].

2.10.2 Coated Wire Ion-Selective Electrode Construction.

The assembly used, was the detachable-tip CWISE. A sliding terminal blade (250 blade, R.S. Components 0.25") was crimped onto the inner copper wire. The blade was filed down to a width of 0.11 inches to allow it to fit a 110-in-line receptacle (R.S. Components Ltd.). A 2cm piece of Pt-wire, 0.25mm diameter, was crimped into this receptacle, having first folded over 0.5cm of the end of the wire that was being crimped to ensure a secure attachment. A small piece of 1.6mm heat shrinkable sleeving was slid over the Pt-wire leaving 1cm of the wire exposed. 2.4cm diameter sleeving was placed over both the smaller sleeving and the crimped part of the receptacle. Using a hot air blower the sleeving was shrunk to achieve a tight fit. Only the parts of the receptacle that receives the blade at one end and the Pt-tip at the other, were left uncovered. The membrane was dip-coated onto the Pt-wire and the tip wrapped in Nescofilm.

The blade that was attached to the electrode lead was covered by 2.5cm piece of 3.2mm diameter heat-shrinkable sleeving that extended about 1cm beyond the end of the blade such that when the blade was inserted into the in-line receptacle, the sleeving covered the point of attachment thus protecting the assembly from any possible splashes from the test solution. FIG 2.6 shows a CWE which was developed for studies with PVC membranes[152]. This design was altered by replacing the PVC with a bead of linear polymer and NVC. The bead was formed by continually dipping the platinum wire into a

viscous solution of the required linear polymer and NVC in methanol. When the tip of the wire had been coated and the bead was approximately 2mm in diameter, photopolymerisation was carried out under U.V. Further adaption of this design involved coating the very tip of the wire with a bead of epoxy and then over coating with a thin layer of the hydrogel linear polymer. Thus rapid diffusion of ions could take place only through the thin polymer membrane, formed above the epoxy bead.

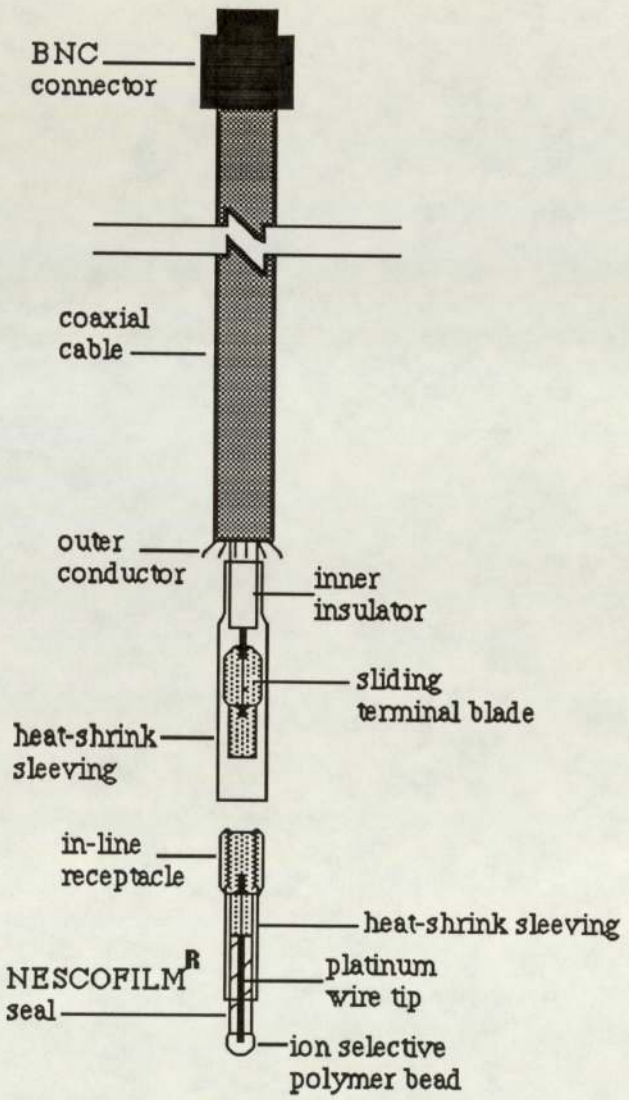


Figure 2.6 Detachable-Tip CWISE.

2.10.3 The Design and Set-Up of the Electrochemical Cell.

The coated-wire ion-selective electrodes were tested on the following electrochemical cell[152]:

Ag | AgCl | 4.0M KCl | 0.1M NH_4NO_3 || test solution | test membrane | Pt

The indicator electrode is shown schematically in fig 2.6 and its construction is described above. In the choice of reference electrode there is the possibility that the electrolyte in the salt bridge may interfere with the test ion determination. This was resolved by use of a flowing double junction Ag/AgCl reference electrode that employed AgCl saturated 4M KCl as the internal reference solution with 0.1M NH_4NO_3 as the outer reference solution.

The reference/CWISE electrode pair were placed close together in a 500ml beaker and the solution was stirred magnetically by using a teflon-coated stirring bar. The potential produced was measured with the aid of a WPA CP460 pH/mV meter.

The CWISE was calibrated for each test solution by measuring the potential produced by the electrode when placed in a series of the salt solutions. The response curve was obtained by plotting the electromotive force, EMF, in mV against minus the log of ion concentration.

CHAPTER 3

REAGENT-MODIFIED HYDROGEL MEMBRANES IN FIBRE OPTIC SENSORS: A FEASIBILITY STUDY

3.1 CONSIDERATIONS TO BE MADE IN THE CHOICE OF APPROPRIATE REAGENTS

The reagent chosen for incorporation into a fibre optic device can be either colourimetric or fluorimetric. However, a colourimetric system would visually lend itself to providing an opportunity to examine the effect of sensed ions on the reagent. These effects could be investigated in complete membranes, irrespective of the stage achieved in the development of the accompanying source and detector systems, required for the construction of a complete sensing device. Colourimetrically sensitive reagents were thus chosen for incorporation into hydrogel membranes and further study.

For the development of a reversible biosensor, the reagent employed must be responsive in an aqueous medium and must not require further elution or titration, as can be the case with some accepted assay techniques[154].

Since most reagents are sensitive to pH and can only detect other ions over a specific pH range, it would therefore be a useful first step, before attempting to distinguish between pH response and that of any alternative detectable species, to attempt to produce a colourimetric pH sensor. Initially it will be sufficient to encapsulate the colourimetric reagent into the hydrogel matrix in order to ascertain whether the matrix will allow transport of detectable species through it, thus allowing the encapsulated reagent to respond accordingly. If this proves to be the case, then as far as sensors for long term use are concerned, the reagent will then have to be bound into the membrane in order to prevent leaching. This is additionally important in view of their potential use as *in-vivo* biosensors. This chapter deals

with a feasibility study of the encapsulation and copolymerisation of a reagent into a complete hydrogel membrane; with its response to pH and with the biocompatibility of the system.

3.2 CHARACTERISATION OF pH SENSITIVE COLOURIMETRIC REAGENTS IN ORDER TO FIND THE REGION OF ABSORPTION.

3.2.1 Experimental

In order to acquire some feeling of the intensity and absorbtion region of this response, known pH sensitive reagents where characterised in solution, using the Unicam SP 1800 ultra violet spectrophotometer. In order to detect changes in pH over a suitably wide range, solutions of pH values varying between around 2 and 10 pH units, containing fixed amounts of the reagents bromocresol purple or bromophenol blue FIGs(3.1 and 3.2) were prepared and their absorption spectra obtained.

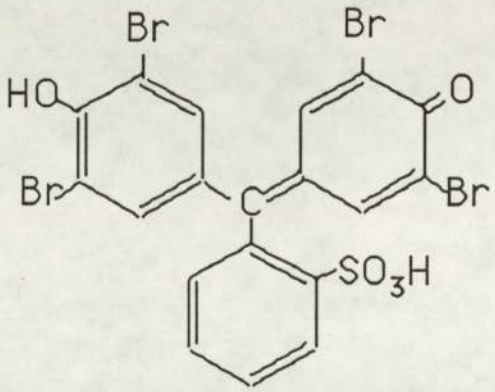


FIG 3.1 Bromophenol Blue

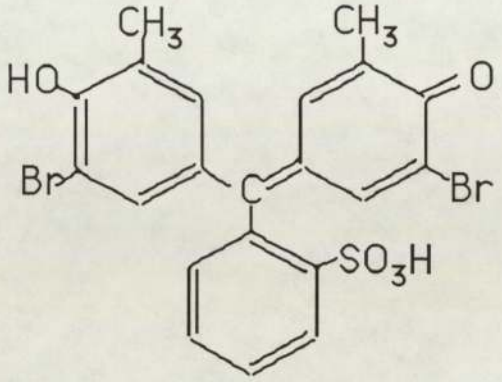


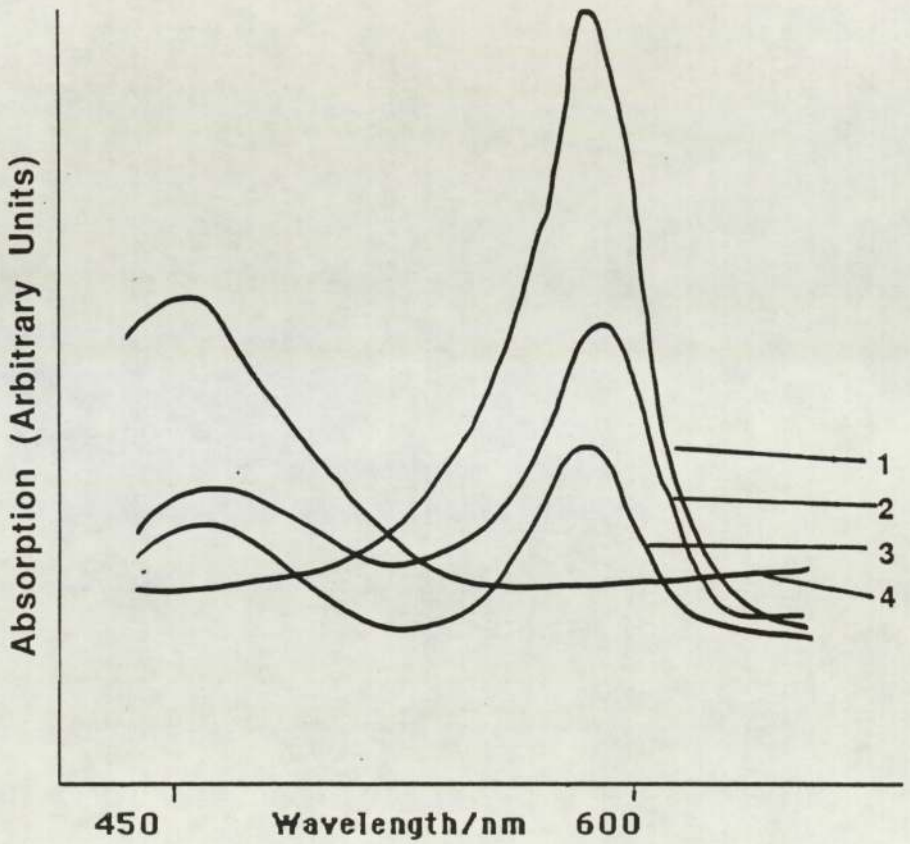
FIG 3.2 Bromocresol Purple

From preliminary experiments, where the absorption spectra of different strengths of reagent solution, in distilled water, were observed for a fixed cell path length of 1cm, suitable absorption intensities were found at reagent concentrations of about 17μM. The pH of the sample solutions was varied by progressively diluting stock solutions of either KOH and of HCl, initially made up to pH values of 10.6 and 2.9 respectively. Reagent was then added to each solution so that its concentration was 17μM and the pH of the resulting sample solution measured using the Kent EIL 3055 Digital pH/Temperature electrode.

3.2.2 Spectra Of Reagents At Varying pH

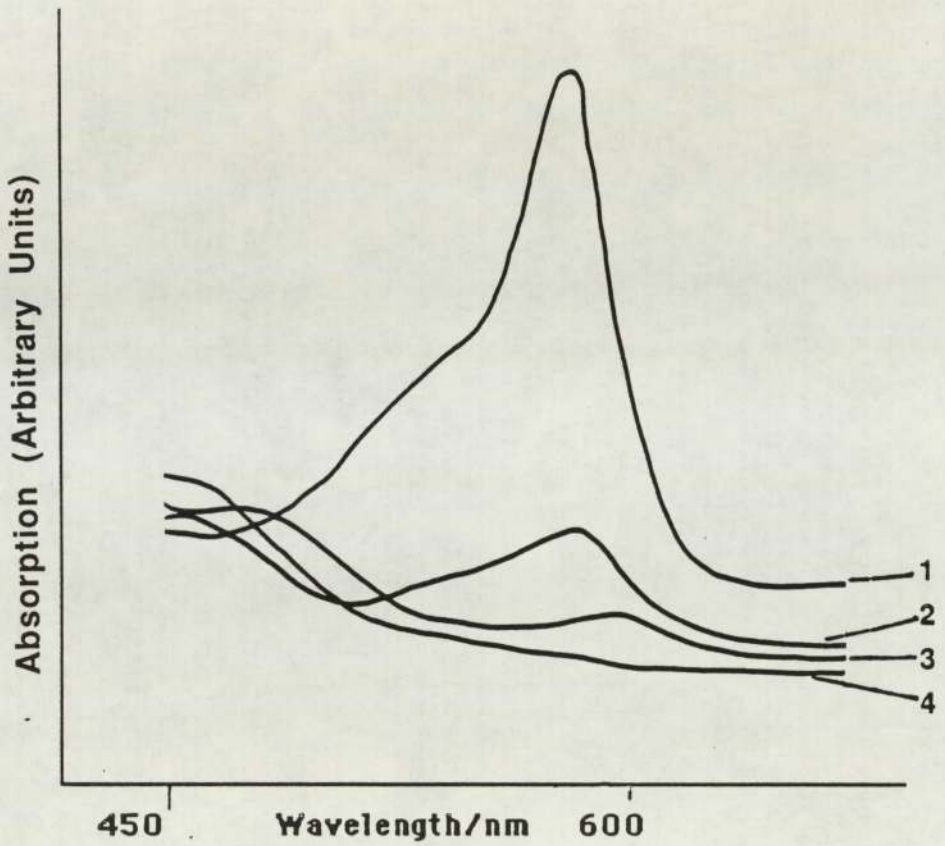
Absorption spectra of the reagent solutions at various fixed pH values were obtained and are shown in FIGs 3.3 and 3.4. The colourimetric reagents, show a strong absorption at about 550-600nm.

FIG 3.3 Absorption Spectra Obtained From Bromophenol Blue At varying pH



pH= 4.18 — 1
3.82 — 2
3.72 — 3
2.91 — 4

FIG 3.4 Absorption Spectra Obtained From Bromocresol Purple At Varying pH



pH= 10.64 — 1
7.14 — 2
4.27 — 3
3.76 — 4

3.3 ENCAPSULATION OF THE REAGENT INTO A HYDROGEL MATRIX

3.3.1 The Choice Of Reagents And Matrix

Having studied the absorption characteristics of the reagents, it was necessary to see whether encapsulation within the hydrogel matrix would affect their response and whether a hydrogel matrix could encapsulate the reagent without any need to chemically bind the reagent within it. A HEMA:MMA (85:15) membrane was taken to be the support matrix, since this would provide a relatively low water content support (around 37%), which would serve to encapsulate the large reagent molecule, whilst not being so low as to restrict the transport of small ions. The equilibrium water content of this membrane should also be relatively insensitive to pH change and the refractive index should be close to that of the polymethylmethacrylate core, if the membrane is adapted for use as a sensor coating. Further details of this aspect of the system design are given both in the introductory chapter and in section 5.1 which deals with the development of sensor coatings.

3.3.2 Encapsulation Of Reagent

Three pH sensitive reagents were chosen to produce a broad range of response, giving thought to their possible combined use. These were bromophenol blue, bromocresol purple and cresol red of pH sensitivity ranges (2.4-5.6), (4.8-7.6) (6.8-9.6) and peak emission wavelengths of 592, 591 and 572nm respectively.

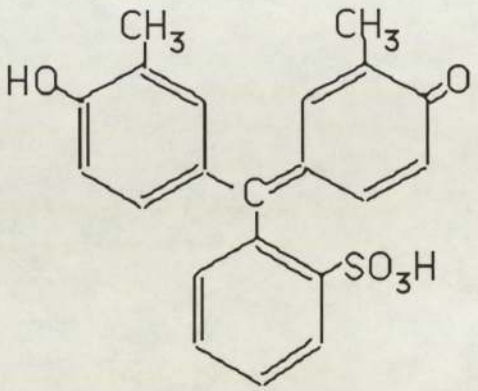


FIG 3.5 Cresol Red

In order to encapsulate the dye within the membrane 0.5g of dye was dissolved in a total of 10g of the mixed monomers plus 0.1g of EGDM as crosslinker and 0.05g of AZBN as initiator. This solution was injected into a mould and then polymerised at 60°C(see section 2.7), in order to produce a colourimetrically sensitive membrane which could then be tested for pH response after hydration.

Frequent changes of water were employed in order to observe the rate of leaching of the indicator and whether or not leaching of reagent would subside or even halt after a period of time. After hydration the membranes were soaked in solutions of various pH values, altered using KOH or HCl dissolved in distilled water. Their response in terms of colour change, time taken and reversibility is discussed.

3.3.3 Results

Membranes were successfully constructed for the three reagents and only a small amount of each reagent leached over the 24hr hydration period. The membranes showed obvious colour changes when placed in solutions of different pH. However, it was noted that the response times varied when changing from acid to

alkali and then vice versa. These response times were of the order of 30 seconds when going from alkali to acid, as opposed to 20 minutes in the reverse case. Most of the reagent had finally leached out of the encapsulating hydrogel matrix over a period of several months.

3.3.4 Discussion And Conclusions

Although it is apparently feasible to encapsulate the dye in the membrane, in order to provide a colourimetrically, pH sensitive membrane; the gradual leaching of the dye means that this would not be a suitable technique for long term clinical and non-clinical sensing, since the response of the sensor would change with time in both cases but more importantly for in-vivo sensing, the leaching of reagent into the patient is unacceptable. However, as far as the more immediate problem of setting up a test bed for the testing of further matrices was concerned, this was, in the short term, an acceptable test matrix.

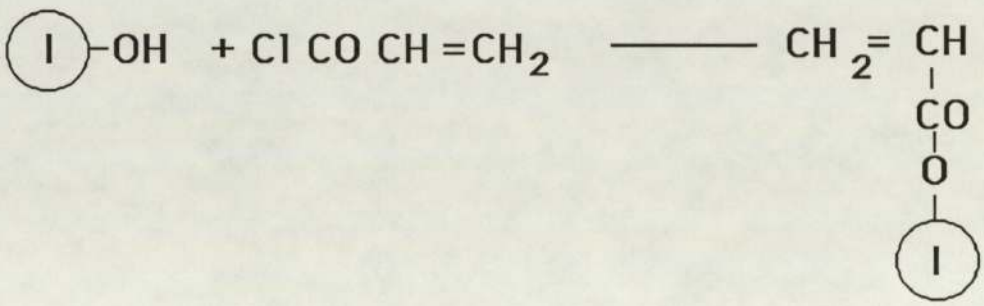
The fact that the membrane changes colour more rapidly in one direction could be a transport related phenomena and one related to the relative hydrated radii of both the anions and cations in the test solutions, which pass through the matrix. The response time observed of around 30 seconds, encountered when the test solution becomes more acid is acceptable and the membrane coatings on the sensors will be invariably thinner than these, also reducing the response times of approximately 20 minutes, noted as the test solution becomes more alkali.

Ultimately, it will be useful to chemically bind the indicator into the matrix, thus allowing a higher water content membrane to be used and hence decreasing the response time of the sensor still further.

3.4 THE FUNCTIONALISATION OF A REAGENT MOLECULE TO PRODUCE A pH SENSITIVE MONOMER WHICH CAN BE COPOLYMERISED INTO A HYDROGEL MATRIX

3.4.1 The Choice Of Reagent

The reagents which were studied, in the earlier parts of this chapter, i.e brophenol blue, bromocresol purple and cresol red FIGS [3.1,3.2,3.5] contain a sulphonic acid group which plays a minor role in colour change, compared to the phenol groups. Although it would be ideal to adapt the sulphonic acid group, in order to copolymerise the reagent molecule into the membrane, an easier reaction would be to convert one of the phenol groups into an ester, using an acid chloride, as described by the following reaction mechanism, where the main body of the reagent or indicator is denoted by an encircled capital " I" with a representative phenol group attatched.



It is the protonation and deprotonation of these groups that result in a change of resonant energy of the molecule, and hence colour change. Thus, the more phenol groups present in the dye molecule, the more successful the conversion of at least one of these groups is likely to be, without seriously affecting the pH sensitivite range of the reagent.

Bromopyrogallol red presents us with a similar structure to the molecules already studied and possesses five phenol groups[154] FIG(3.6). It would therefore be a useful molecule to incorporate into a potential sensor system.

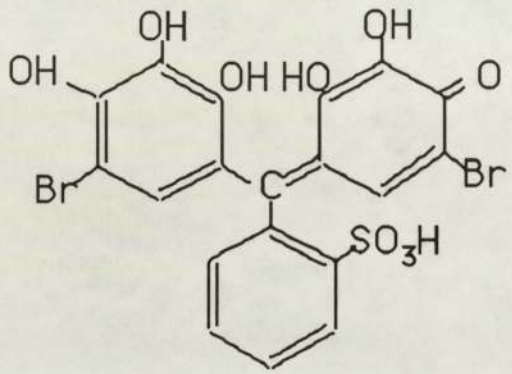


FIG 3.6 Bromopyrogallol Red

The reaction of the phenol group with the acid chloride yields hydrochloric acid, which has to be neutralised by a base in order for the reaction to proceed. Therefore, the reaction is base-catalysed. A suitable base to use is triethylamine since this will yield triethylamine hydrochloride as a product, without reacting with the acid chloride. Similar techniques have been reported[155], which use an excess of sodium hydroxide solution in order to react with the hydrochloric acid but initial attempts using this technique were found to be unsuccessful.

Given a solvent for the reaction, such as acetone, which will not dissolve the quarternary ammonium salt, triethylamine hydrochloride, this product can be removed as a white precipitate and further purification carried out by separation of

the dye into an organic phase and other by-products, such as acrylic acid, into an aqueous phase, after which the product can finally be extracted. The success of the esterification can easily be seen using Infra-red spectroscopy, since there will be a reduction in the broad OH peak at about 3600 cm^{-1} and the appearance of a carbonyl peak at about 1600-1750 cm^{-1}. [156].

3.4.2 Functionalisation Of The Reagent

The reaction described was set up by dissolving 0.5g of the indicator, bromopyrogallol red, in a mixture of 100ml of acetone and 1ml of triethylamine placed in a conical flask upon a magnetic stirrer. 1.0 ml of 20% acryloyl chloride in acetone was added drop-wise to the solution, which was then left to react for 1hr. The remaining excess triethylamine was destroyed with approximately 1.5ml HCl and the solution left to stand for about 2hrs, in order to allow the triethylamine hydrochloride to precipitate. After filtering, the acetone was removed in the rotary evaporator and the product redissolved in water and poured into a separating funnel. The product was then purified by salting it out of the aqueous phase, into iso-butyl alcohol, by using NaCl. The organic solvent was then removed in the rotary evaporator and the product dissolved again in methanol, in order to remove further triethylamine hydrochloride. This last stage of purification was repeated until a dry powder, rather than a tarry product was obtained and a sample was then taken for FT-IR analysis, the results of which are shown in FIGS(3.7,3.8), where the appearance of the carbonyl peak, as described above is apparent indicating that the esterification reaction was successful.

FIG 3.7 INFRA-RED SPECTRA OF BROMOPYROGALLOL RED

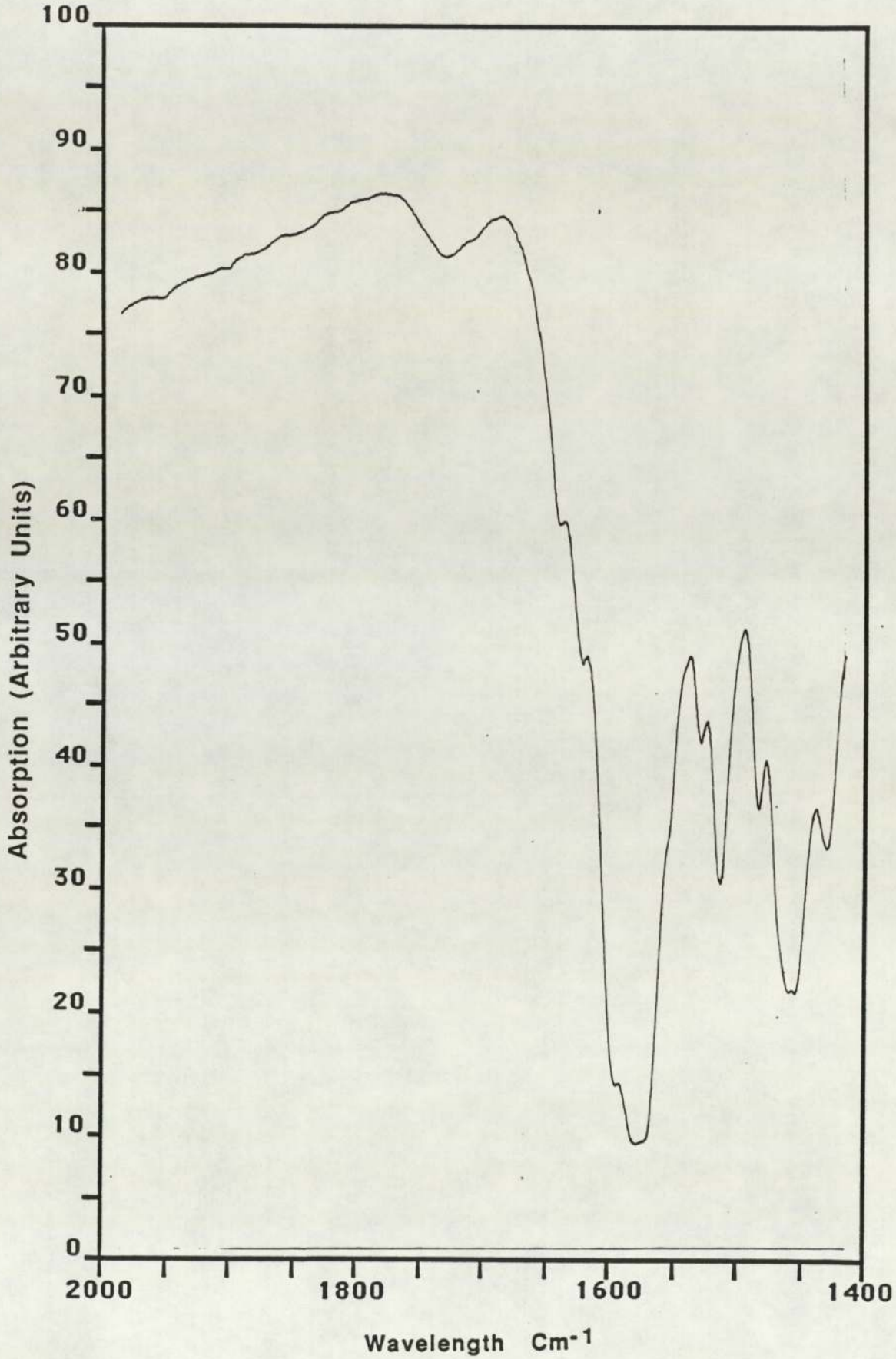
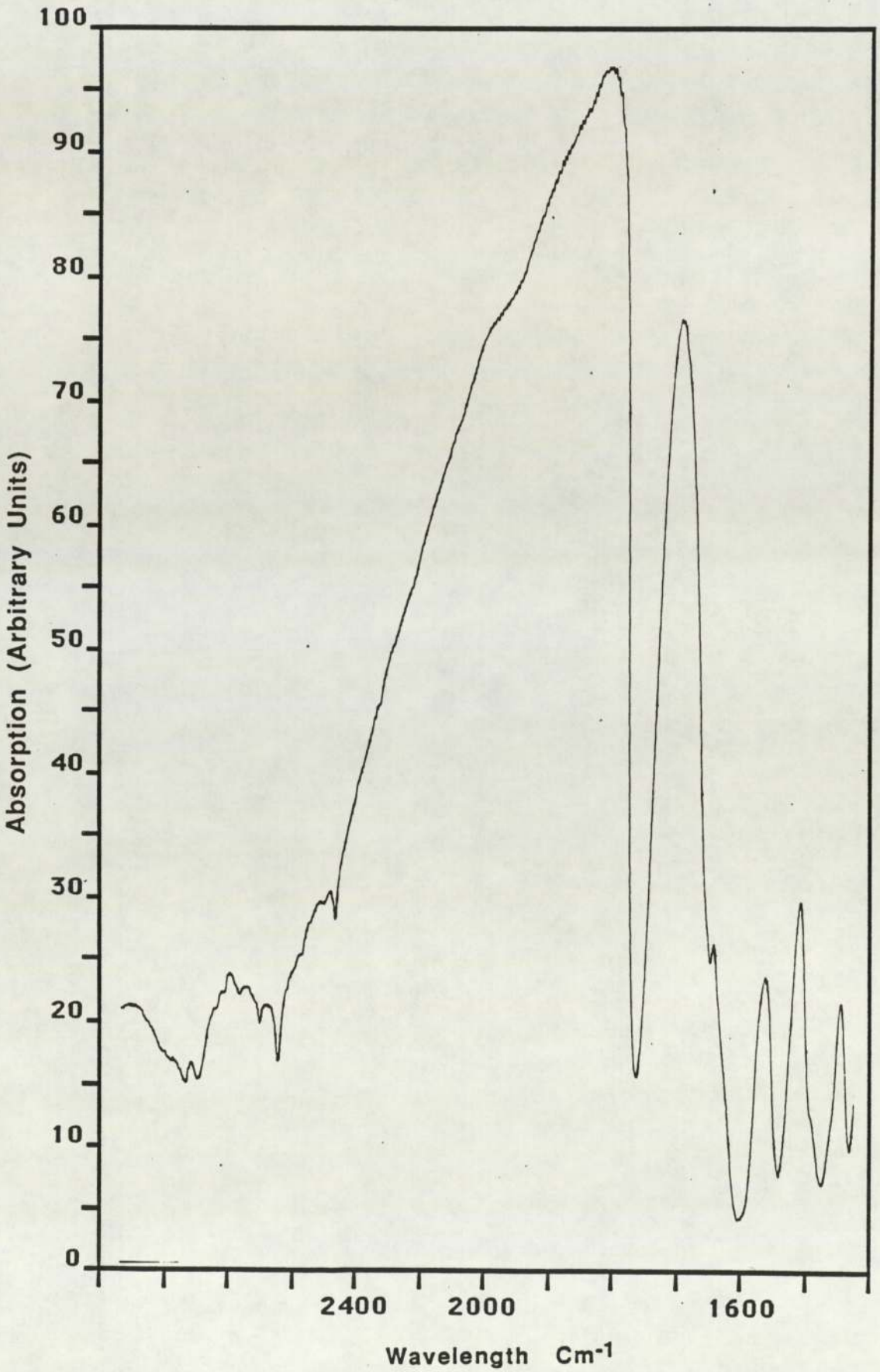


FIG 3.8 INFRA-RED SPECTRA OF ACRYLATED BROMOPYROGALLOL RED



3.4.3 Results And Conclusions

The purification of the product in order to remove triethylamine hydrochloride reduces the yield which appears to be quite high at the separation stage from the intense colour in the organic phase. Therefore an alternative base, such as pyridine, may increase the yield in this respect. Alternatively, if triethylamine is still used then centrifugation of the reaction mixture, once the reaction is completed and the excess triethylamine destroyed, may help to extract more triethylamine hydrochloride, thus reducing the number of purification stages and hence increasing the yield. However as far as this stage of the project was concerned, the technique provided enough product to carry out copolymerisation of the reagent with other monomers.

This technique has proved to be useful in functionalising reagent molecules containing phenol groups and it should also work well on those which contain NH_2 groups, since acryloyl chloride would react far more vigorously in this case. Indicators containing NO_2 groups such as Eriochrome black T can be converted by reduction of this group to NH_2 [157] and then esterification with acryloyl chloride will convert it still further to an acrylic group, thus allowing the indicator to be polymerised.

3.5 THE PRODUCTION OF A COLOURIMETRICALLY SENSITIVE HYDROGEL MEMBRANE CONTAINING THE REAGENT AS A COMONOMER

3.5.1 Introduction

It is important, having produced a reagent monomer, to see if it will copolymerise with a monomer such as HEMA and then to see if the colour of the

resultant hydrogel membrane responds to changes in pH. It is also important to see whether the incorporation of the indicator affects the biocompatible nature of the hydrogel membrane.

3.5.2 Copolymerisation Of The Reagent AcBPR And HEMA In Order To Produce A Colourimetrically pH Sensitive Membrane

Three membranes were produced using the technique described in section 2.7. The monomers used in each case were HEMA together with 0.5%, 1% and 2% acrylated bromopyrogallol red, produced as described in section 3.4; 1% EGDM as crosslinker and 0.5% AZBN as initiator. After polymerisation and hydration, sections of the membrane containing 0.5% dye were equilibrated in buffer solutions of varying pH over a period of several hours. After the subsequent colour change, the membranes were then dried flat between two melinex sheets, with a 1x2 cm window cut concentrically out of both of them. When the membranes were dry their absorption spectra were obtained using the Beckman DU7 UV/VIS spectrophotometer and are shown in FIG 3.9.

3.5.3 A Brief Study Of The Cytotoxicity Of The Membranes

Samples from each of the three original membranes and one from a similarly produced membrane, but containing only HEMA monomer, were taken after soaking in distilled water, for cell adhesion studies, by K.Thomas[158]. The results of these studies indicated that up to 2% dye incorporated into a HEMA membrane does not affect the cytotoxicity of the membrane which to a certain degree reflects its biocompatibility.

3.6 GENERAL CONCLUSIONS

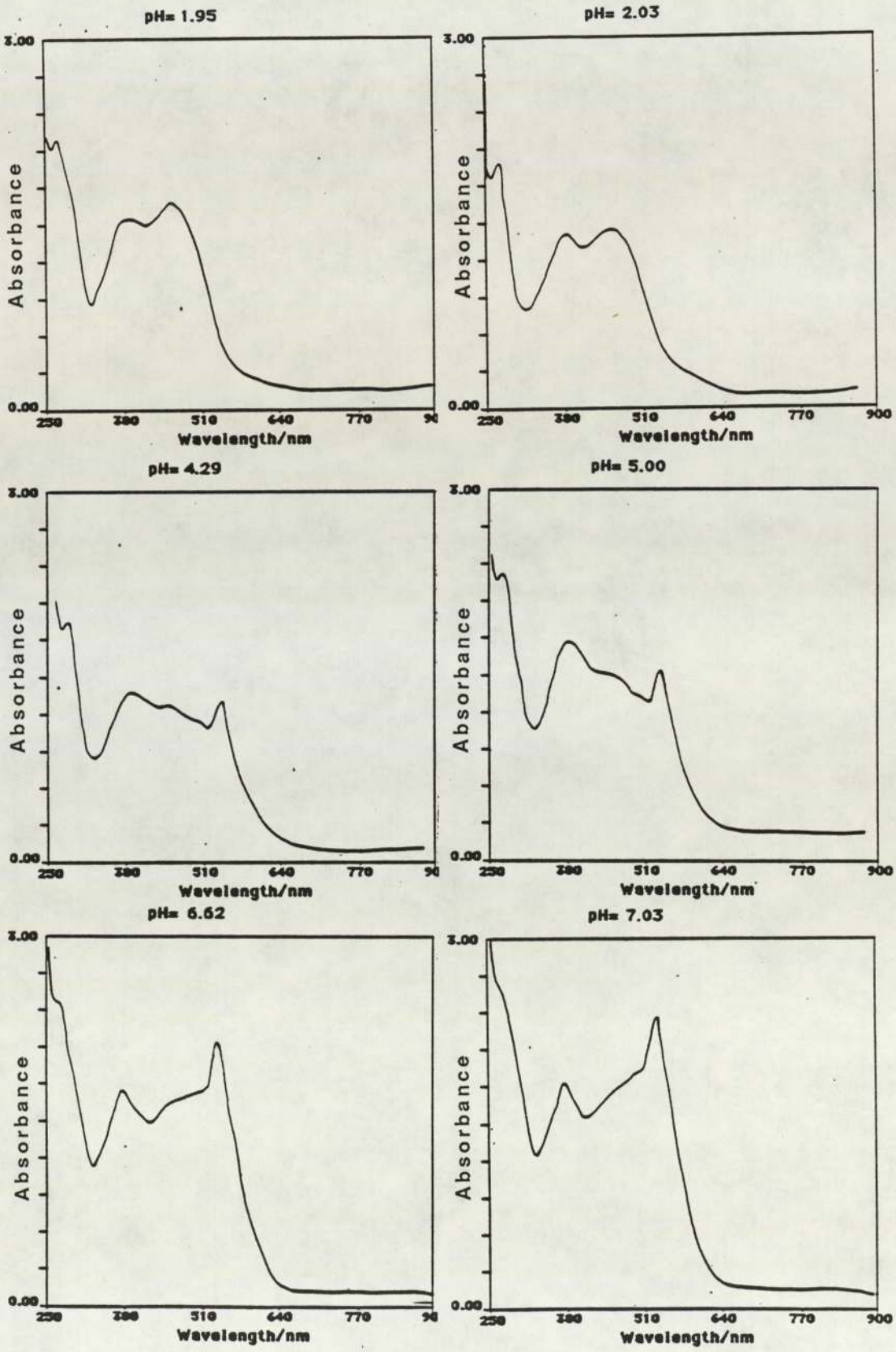
The conclusions drawn from this chapter are that it is feasible to create a

reagent monomer, that can be incorporated into a hydrogel membrane, which still facilitates fairly rapid transport of detectable species. Inclusion of the reagent in the system described above does not affect the cytotoxicity of the matrix. The reduction in thickness of the membrane, when finally applied to a sensor as a coating, may prove to be sufficient in reducing the observed colourimetric response time of approximately 1 hour. However, since we have shown that we can chemically bind the reagent into the hydrogel matrix, the study of even higher water content systems as reagent support matrices is now practicable.

Having achieved the chemical binding of a colourimetric reagent into a hydrogel matrix, it is now practical to begin the development of an optical sensing device. In this respect, the need for the development of a suitable test bed on which to study the use of these membranes as an integral part of a fibre optic sensor, is now evident.

FIG 3.9 UV/VIS ABSORBTION SPECTRA OF HEMA MEMBRANES

CONTAINING ACRYLATED BROMOPYROGALLOL RED.



CHAPTER 4

THE DEVELOPMENT OF A TEST BED IN ORDER TO ASCERTAIN THE SUITABILITY OF HYDROGEL MEMBRANES AS REAGENT SUPPORT MATRICES FOR FIBRE OPTIC SENSORS

4.1 COMPONENTS OF THE TESTBED

The overall sensor test bed system must consist of a source, detector, transducing phase, and a reagent phase, together with a suitable means of interpreting the acquired optical information. In the context of this work, the expression "reagent phase" refers to the reagent modified hydrogel membrane. The initial development of this particular aspect of the device has already been described in chapter 3 and thus, this chapter deals with the aspects of test bed design. The construction and design of the test bed can be divided into four interdependent sections, which are:

1) The development of a compatible source/detector system including a suitable means of data output.

2) The design of the flow through system including the preparation of the fibre optic for coating with the reagent phase.

3) The development of the reagent phase, entailing the immobilisation of the reagent within the hydrogel matrix.

4) The development of coating techniques and application of a reagent phase onto the fibre optic.

The problem in designing a complete system incorporating the above, is that it is difficult to develop both the source/detector system and the reagent phase simultaneously and differentiate between the response elements resulting from each of these interdependent components of the test bed. This chapter however, only deals with the physical development of the source/detector combinations, the means of data output and the preparation of the fibre for coating, as well as the design of the flow through system required for changing test solutions.

4.2 CHOICE OF THE TYPE OF FIBRE OPTIC AND ITS CONFIGURATION IN TEST BED DESIGN

4.2.1 Introduction

It is necessary to decide which type of fibre optic to incorporate into the test bed, and upon the configuration which will best lend itself to the application for which it is intended in terms of ease of incorporation into the test bed, how it is to be connected to the source/detector whilst isolating the coated section of the fibre within a flow through system, and whether any additional optical equipment will need to be implemented.

4.2.2 The Choice Of Fibre Optic

A single core polymethylmethacrylate fibre optic was considered most suitable since it was readily available, relatively inexpensive and was likely to provide good refractive index matching with hydrogel coatings. The configuration which was easiest to implement was that where a dorsal section of the fibre is coated, rather than at the distal end of the fibre. A larger surface area of the fibre is then available to interact with the reagent phase and no extra facilities such as beam splitters are necessary as part of the test bed equipment.

4.2.3 Exposure Of The Fibre Core In Preparation For Coating

The fibre optic cable used consisted of a 0.5mm diameter poly-methylmethacrylate core, with a thin fluoropolymer coating, cladded in a black polythene sheath. In order to allow interaction between the hydrogel matrix and the light travelling along the fibre, this reagent phase must replace the fluoropolymer coating. Thus, the polythene cladding and then the fluoropolymer have to be removed, before coating can commence.

Initially it was sufficient to strip away the polythene and fluoropolymer with a scalpel but this does not provide a reproducible technique. In order to remove the cladding without damaging the core, the fibre was left to soak in petroleum-ether for a period of several hours whereupon the cladding swells and the fibre can be pushed up through its centre. A section of the cladding of about 1cm is then cut away and the remainder cut in half. It is then replaced over the fibre, leaving the middle section of the core exposed. The fibre is then dried in the oven, so that the cladding shrinks back into place, and then placed in chloroform for 7 mins to remove the fluoropolymer from the exposed middle section, which can then be coated and placed into a suitably designed flow through cell as in FIG 4.1. The design of the flow through cell must allow efficient washing out of previous solutions and it must be easily assembled around the appropriate section of the fibre optic, without disturbing the coating or breaking the fibre.

4.3. THE DESIGN AND CONSTRUCTION OF A FLOW-THROUGH CELL

4.3.1 Glass Cells

The first of these cells was made of glass and the metal fibre-optic connectors were glued in with epoxy adhesive at either end, thus allowing the cell to be joined by fibre optic cable to the source and detector, via bulk head connecters FIG4.2. The cell was blacked out with self amalgamating tape and the solutions changed by injection through the inlet and outlet of the glass cell which were then temporarily sealed with plasticine. The fibre optic had to be cut to the exact cell size so that the metal ferrules would fit tightly into the bulk head connectors, providing a good optical connection. The problems encountered in this design arose mainly from the fitting and gluing of the fibre optic into a defined space and from the leakage of

epoxy into the ferrule threads, thus providing neither a good seal at the ends of the cell nor efficient optical alignment in the bulkhead connectors

FIG 4.1 Preparation Of The Fibre Optic For Coating

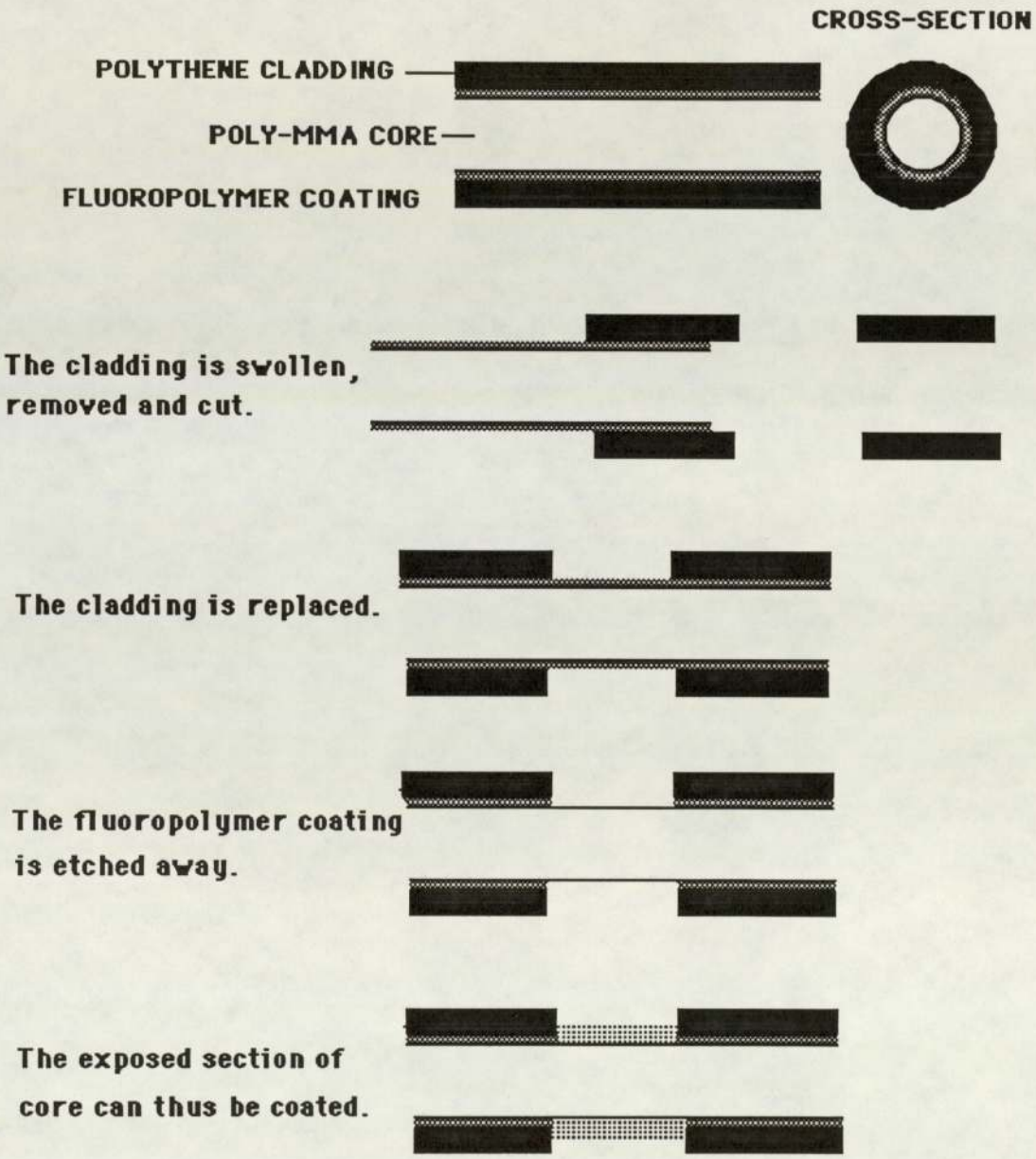
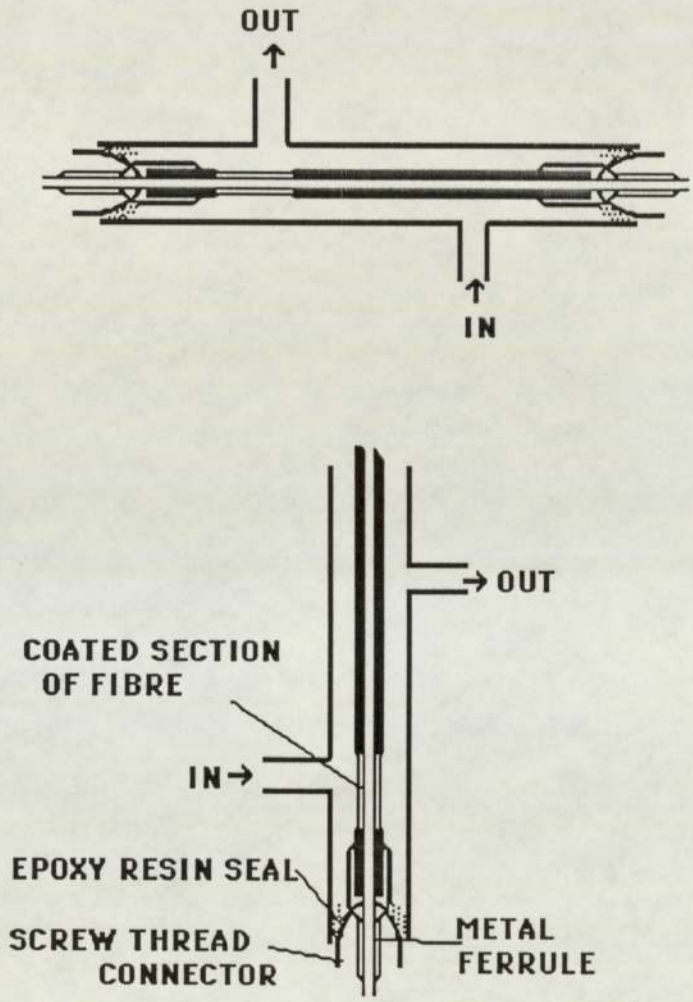


FIG 4.2 GLASS FLOW THROUGH CELLS



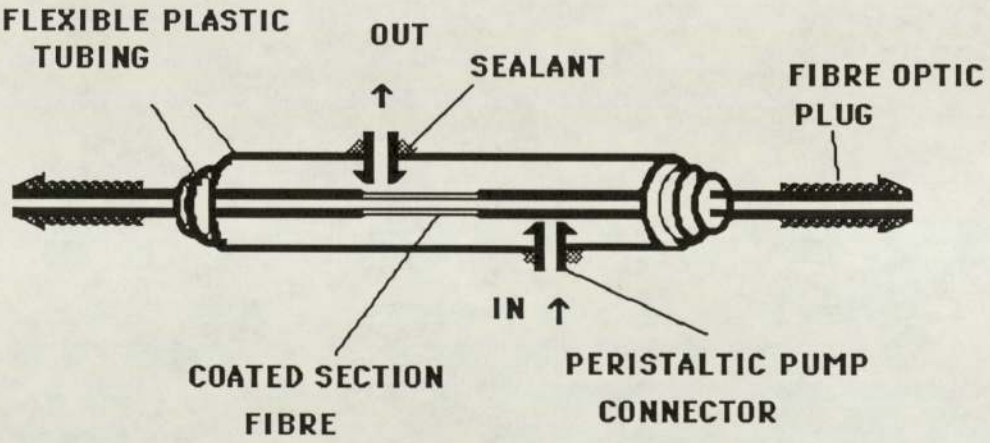
The fact that the cell had to be screwed into place at both ends presented problems with the opposite twisting action of the connections and this, combined with the fact that the cells were made of glass created further problems with their inherent fragility. The cells were not water tight and solution leaked into the bulk head causing the optical interface between fibres to change. This problem was temporarily lessened by standing the cell vertically and only gluing a ferrule at the

base of the cell but a more disposable,water tight and less fragile system needed to be constructed. The optical links required positioning well away from the ends of the cell and the screw thread connectors needed to be replaced by plastic plug-in connectors and bulkheads.

4.3.2 Polythene Tubing Cells

The glass cell body was replaced by a length of rigid polythene tubing FIG 4.3 and made to fit tightly around the fibre optic by using two relatively smaller pieces of flexible plastic tubing, fitted concentrically around the fibre at the ends of the cell. Holes were bored into the cell body at either end and approximately 1mm diameter black polythene tubing glued into the holes to provide an inlet and outlet. The whole cell was then painted black, after sealing the ends and inlet/outlet ports with silicon rubber sealant. Although this began to solve some of the problems already encountered, the tightness of the tubing at the ends of the cell, though providing a good water tight seal, caused probes to bend and break during fitting. In addition, the inlet and outlet connections were weak and did not provide an efficient means of changing solutions and these were thus replaced by peristaltic pump adapters which were plugged into the bored holes and connected to the pump. This provided a vast improvement in the efficiency of changing solutions but the cell body was too narrow and the adapters tended to push against the fibres and disfigure them.

FIG 4.3 Polythene Tubing Cell



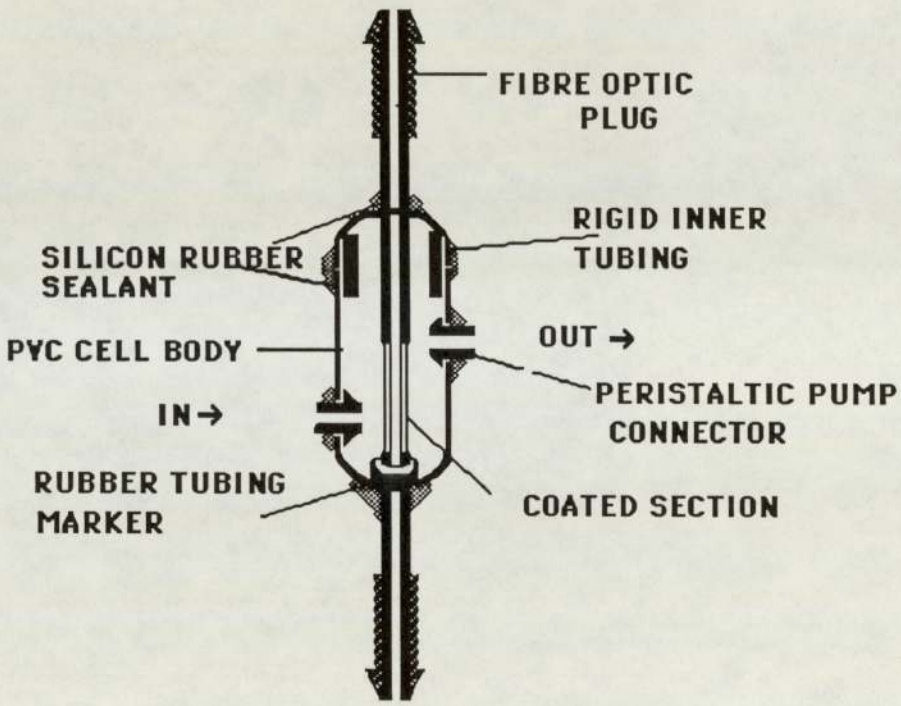
4.3.3 Pipette Teat Cells

A broader cell body, which could be fitted without causing damage to the fibre optic was required. To suit this end, black PVC pipette teats were used FIG 4.4. In order to form the cell body, the open narrow section of the teat was cut away leaving a length of about 3cm and the cell lid was formed by cutting a longer section from a second teat leaving about 1cm at the sealed end. A rigid length of tubing was inserted into the lid, extending about 0.5cm beyond its edge, so that it would both supply support to the cell and allow the lid to be slotted securely into the base, providing a seal.

The flexibility of the cell base allowed the peristaltic adapters to be fitted without touching the fibre coating and holes cut at the sealed ends of the teats allowed the fibres to be easily manipulated into position within the cell base and the lid to be securely fitted without straining the fibre. The stiff plastic tubing was fitted into the lid of the cell allowing it to be locked tightly into place and the

whole cell sealed with silicon rubber sealant and connected to the peristaltic pump. The pump adapters were attached at the bottom and top of the base of the cell and a small piece of tubing slipped around the fibre optic beneath the coated section as a marker, so that it could be pushed well down into the cell, ensuring complete immersion of the coated section of the fibre in solution, when the cell was supported vertically.

FIG 4.4 Cell Constructed From Pipette Teats



4.4. SOURCE AND DETECTOR SYSTEMS

4.4.1 Initial Practical Considerations

The source and detector system incorporated into the test bed design need to be either adapted or designed to house fibre optic links. It is a useful first step in designing a test bed to incorporate a multi-wavelength source so that a comprehensive study of absorption properties of the reagent in the environment of a

hydrogel support matrix can be carried out. The type of detector used must obviously be compatible with the source radiation and be sensitive enough to detect changes in absorption in the region of interest. When the absorption characteristics of a reagent, in response to a changing ion concentration have been studied, it may well be of value to utilise a single wavelength or waveband source, together with a compatible detector system, which operate over an appropriate spectral region. However multiwavelength sources also lend themselves to systems where it may be necessary to sample at several wavelengths in order to interpret changes relating to more than one sensed ionic species.

Having established the source/detector combination it is then necessary to decide on a suitable means of data output. A digital voltmeter provides a suitable output for systems where the behaviour of the reagent is predictable but a chart recorder provides a useful tool for studying on-line response of the device.

The combinations of source/detector and data output device were constantly modified throughout the on-going experimental, in order to suit the requirements of stability sensitivity and general overall sensor performance.

4.4.2 Adaptation Of The Hilger Watts Spectrophotometer.

A Hilger Watts spectrophotometer provided a readily available multi-wavelength system with a photomultiplier tube plus spot galvanometer as the detector and output system. This was modified to host a fibre optic connection at its source and detector ports by removing the specimen mountings and adapting the source and detector ports to hold RS 456-403 screw thread bulk head connectors. This allowed a fibre optic system to be connected between the two. During initial studies it was found that the spot galvanometer was far too unstable, due to its

sensitivity to vibration, and was also prone to the effects of drift and mains interference.

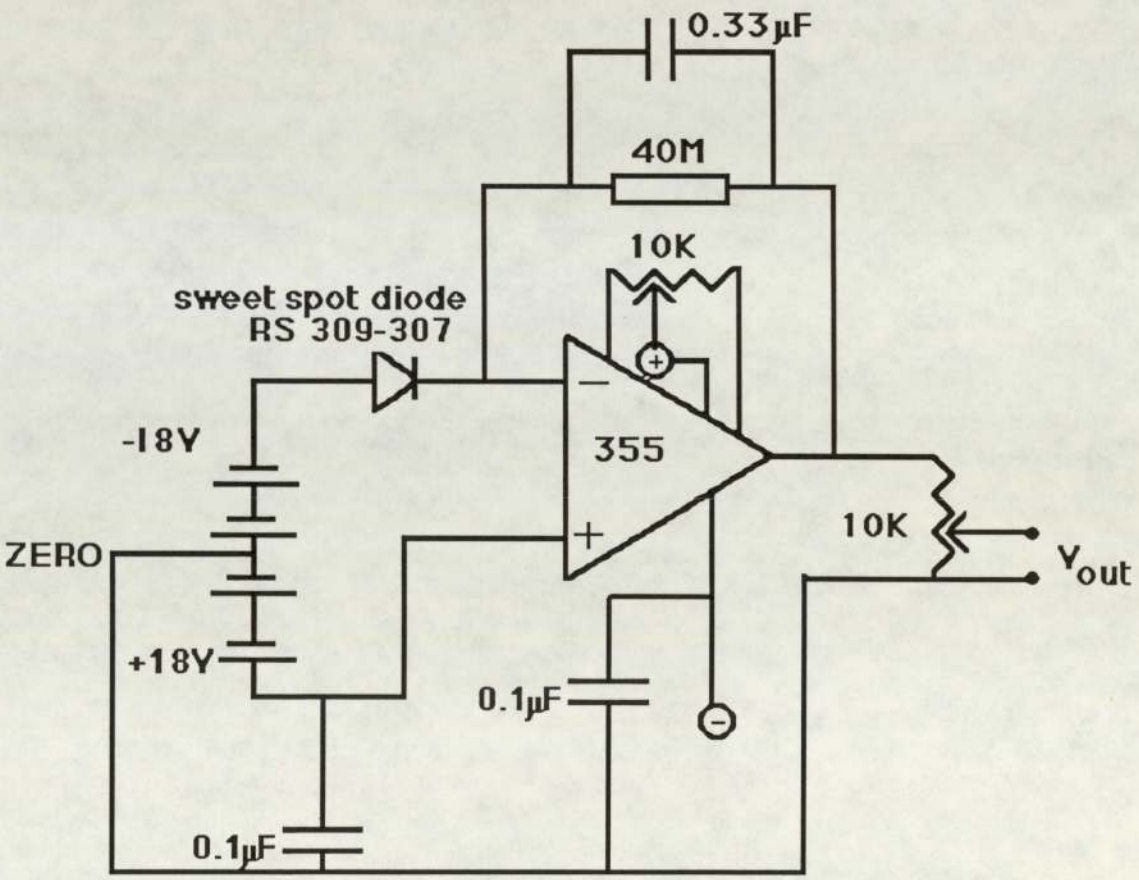
4.4.3 Construction Of A Battery Driven Operational Amplifier Circuit As A Detector In The Infra Red

An electronic amplifier circuit driven by batteries was set up as the detecting system and the Hilger Watts spectrophotometer was used as the light source. A sweet spot infra-red diode RS 309-307, designed to be coupled with the optical fibre, was incorporated into a standard amplifier circuit implementing a J-FET 355 operational amplifier with high input impedance and low offset voltage. The gain of the amplifier was virtually open loop since the feed back resistors incorporated into the design were extremely high. The values of the resistors and capacitors were chosen so as to give maximum output effect and to improve the signal to noise ratio, visible on an oscilloscope. The data was output via a digital voltmeter connected across the output terminals in series with a 10K potentiometer, so as not to overload the meter Fig 4.5.

Although this system removed mains interference, batteries proved to be inconvenient and cumbersome to replace and charge. The system was in fact fairly noisy, perhaps due to the detector picking up stray infra-red, and still suffered from drift because of the high gain of the circuit. Because the spectrophotometer was providing a relatively intense source, the background signal to the detector was too high. This caused the output from the amplifier to swing over to one side and the high gain necessary to detect the change in signal was compounding this problem and was not allowing the amplifier to operate over a wide output range. The signal to noise ratio was too low and infact this system possessed as many problems as that utilising the spectrophotometer detector system, previously described, and did not appear to

be as sensitive. It therefore seemed necessary to revert back to the use of the spectrophotometer but alleviating at least some of the problems by connecting the output across the digital voltmeter, reducing the mains interference by working in quiet periods and taking a zero absorption reading at 350nm at appropriate points in the experimental. This system was by no means ideal but was a useful test bed in the interim period whilst an improved electronic device was being developed.

FIG 4.5 Infra-Red Source/Detector Combination



4.4.4 Construction Of An Operational Amplifier Circuit As A Mains Driven Detector System

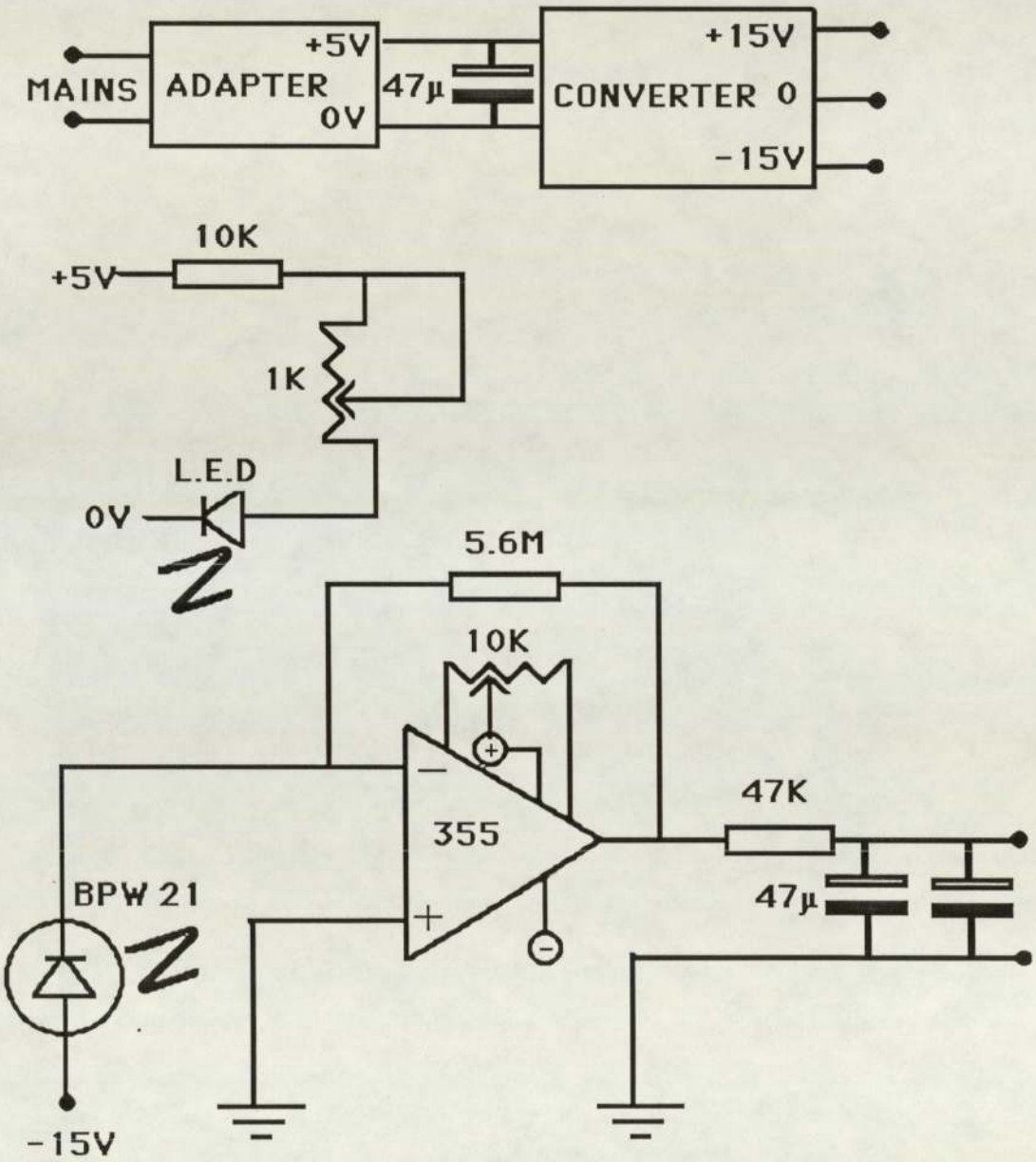
It was necessary to produce a detection system that would run off the mains with less noise and to operate in the visible region. The circuit was adjusted to run from a mains-d.c adapter RS 591-988, through a voltage converter and divider RS 591-304, designed to convert the 5 Volt dc obtained from the mains adapter to +-15 Volts. The photodiode used was a visible range (eye response) photodiode BPW 21 with a peak detection limit at 560nm which is appropriate for the dyes that had been characterised. The light source was an ultra-bright green L.E.D with peak output at 565nm, providing a single wave band source. A 1K potentiometer connected in series with the source allowed the intensity of the source ie the background signal to be varied. A standard amplifier circuit was again employed with a J-FET operational amplifier but the gain of the system was reduced. The output signal was smoothed using a 2.7K resistor and two 47μF electrolytic capacitors, in parallel across the input of the chart recorder, which provided an output giving a visual on-line response. Thus, rise-time, response time, fluctuations and noise would all be recorded FIG 4.6

.4.5 CONCLUSIONS

The most important implications in test bed design, which have been drawn from this chapter are that the source and detector need to emit and detect over a similar waveband, and provide a high signal to noise and change in signal to background ratio across the output. A suitable means of interpreting the data is via a chart recorder and for more fully developed and understood systems, a digital voltmeter. The flow through cell must be easily fitted, without damaging the fibre

FIG 4.6 Source/Detector Combination Incorporating

Eye Response Photodiode



coating and a compromise has to be reached between ease of manipulation during assembly and minimisation of the cell volume. The fibre optic connections must be positioned well away from the main cell body so that solution does not leak into the bulk heads and alter the optical interface. Finally, the complete cell, together with the incorporated fibre optic, must be easily constructed and assembled into the test bed.

We have thus succeeded in producing a suitable test bed, with respect to simplicity and sensitivity. This enables us to proceed in the development of reagent modified hydrogel polymers as fibre optic coatings and their incorporation into an optical sensing device.

CHAPTER 5

THE THEORY OF EVANESCENT WAVES AND ITS APPLICATION TO THE DEVELOPMENT OF PRACTICAL COATING TECHNIQUES FOR WORKING SENSORS

5.1 EVANESCENT WAVES: THEORY AND PRACTICAL IMPLICATIONS.

5.1.1 Introduction.

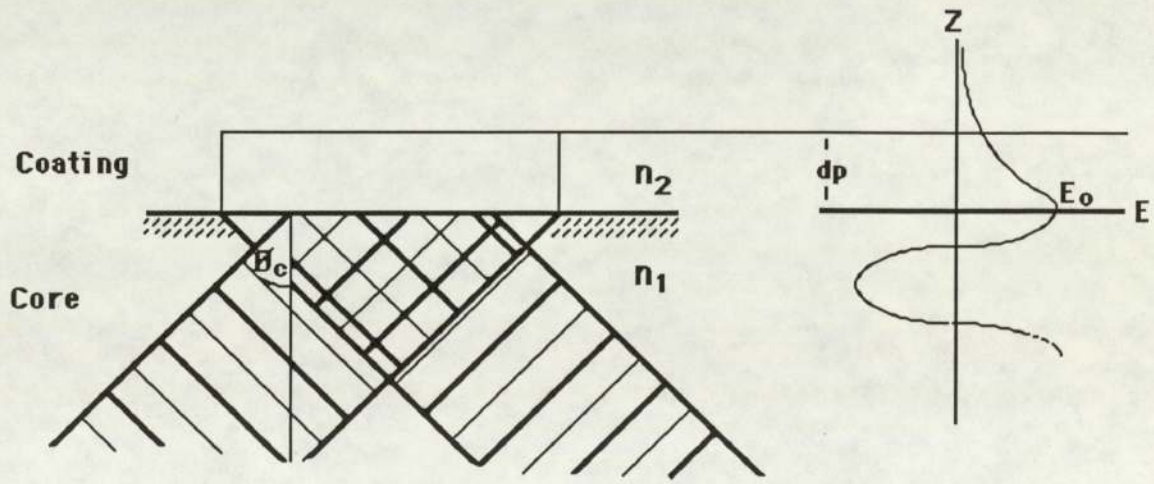
Having developed the test bed described in chapter 4, it is possible to proceed with the development of a suitable technique for applying a reagent modified hydrogel onto a fibre optic. For the reasons given in section 4.2.2, it was decided that the fibre should be coated around the core, thus utilising the evanescent wave component of the source radiation. Hence the theory of evanescent waves and practical considerations to be made in applying the theory are described here[72-77].

5.1.2 The Theory Of Evanescent Waves

When a light beam strikes the interface between two media of refractive indices n_1 and n_2, where $n_1 > n_2$ and the angle of incidence is larger than the critical angle $\emptyset_c$, total internal reflection occurs. $\emptyset_c$ is given as a function of the two refractive indices by the following expression.

$$\emptyset_c = \sin^{-1}(n_2/n_1)$$

FIG 5.1 Evanescent Wave Generation



A standing sinusoidal wave is set up at the interface which penetrates the rarer medium, decaying exponentially. This is the evanescent wave and its penetration depth is given by the equation;

$$dp = (\lambda / n_1) \; / \; 2\pi \; \sqrt{[\; \sin^2 \varnothing - (n_2 / n_1)^2]}$$

Where dp is defined as the distance required for the electric field amplitude, E to decay to exp(-1) of its value at the surface, (E_0) and is usually of the order of a fraction of the wavelength (λ) of the incident beam.

$$E = E_0 \; \exp \; (-Z/dp)$$

Z is the perpendicular distance away from the interface

The important points to be considered from these expressions, are that:

1) The penetration depth decreases with increasing Ø and so, therefore, does the area of the coating sampled at the interface.

2) The critical angle and therefore, the amount of light which is totally internally reflected is dependent on the refractive index matching of the core/coating interface: Ideally, the refractive index of the coating should be as close as possible to, whilst remaining lower than, that of the core.

5.1.3 Essential Properties Of The Fibre Coating

In order to attain minimal light loss along the coated section of the fibre optic, an optically sound interface is required between the fibre core and the hydrogel coating. The coating must be thin enough to allow rapid transport to the reagent, incorporated into the matrix, to take place but not be so thin as to allow the

evanescent wave to penetrate beyond it, thus allowing the properties of the aqueous phase to affect the overall response. In addition, the refractive index of the reagent phase must be lower than that of the polymethylmethacrylate core in order to allow internal reflection to occur at the interface. These indices must be as closely matched as possible to get the maximum penetration depth of the evanescent wave into the matrix.

5.1.4 Coating With Monomer Solutions

Initially an attempt was made to coat the fibres with similar monomer solutions to those used in the construction of whole membranes, as in chapter 2 and to polymerise the coating, under similar conditions, but avoiding the high temperature post-curing at 90^{o}C. The production of coherent even coatings by this technique is difficult because of the low viscosity of the monomers.

5.1.5 Coating With Linear Polymer Solutions

A preferred coating process thus involves the preparation of a soluble linear polymer, of the required constitution of monomers, by solution polymerisation, as described in chapter 2. When a minimum of solvent, such as methanol, is used, the linear polymer solution is very viscous and can be coated onto the fibre, in order to produce an even coating. The linear polymer can be dissolved in the solvent, together with a photocrosslinking agent and polymerised under U.V for about 1 hour, producing an insoluble crosslinked polymer matrix. U.V polymerisation eliminates the degradative effects of the thermal cross-linking process and reduces the time taken to complete the coating proceedure.

Linear polymers of HEMA and HEMA:MMA in the ratios 90:10, 80:20, 70:30 and 50:50 had formerly been produced via this technique and it was necessary to study the reagent encapsulation properties of photocrosslinked linear polymers

and the effect of the addition of the large reagent molecules on the resultant membrane characteristics, in terms of response times to changing pH and of the rate of reagent leaching from the coating after subsequent hydration.

5.2 A STUDY OF THE RATE OF LEACHING OF A REAGENT, ENCAPSULATED IN PHOTO-CROSS-LINKED HYDROGEL COATINGS, AND THE RESULTING RESPONSE TIME OF THE REAGENT TO CHANGING pH.

5.2.1 Introduction

In order to find a suitable sensor coating, containing encapsulated reagent, a study was made of the leaching rates and the response times of several photocrosslinked hydrogel matrices. These consisted of linear polymers of 50:50, 80:20 and 90:10 HEMA:MMA, which would provide a series of polymer matrices, encapsulating the reagent bromocresol purple. The water contents of these hydrogels becomes progressively lower as the concentration of the hydrophobic monomer, MMA, in the linear polymer, is increased. As the water content in a hydrogel decreases, the transport of ions through it becomes physically restricted and therefore, inevitably slower, due to the accompanying decrease in porosity of the membrane with water content. Although, we would wish to limit the pore size within the matrix, in order to arrest the leaching of reagent molecules into the sample solution, it would be inappropriate to limit the diffusion of the sensed ion and its co-ion to the encapsulated reagent. Since the water content of HEMA is approximately 37%, the membranes used in this study have water contents lower than this and therefore are fairly rigid. In addition, the MMA content in the linear polymer will obviously draw the refractive index of the coating, closer to that of poly-MMA. The water contents of HEMA:MMA membranes should also be relatively insensitive to the

pH change in the sample solution and thus so should the refractive index of the hydrogel coating.

It was hoped that the results of this study would reveal a sensor coating which could contain the reagent for sufficient time to obtain results, in response to changing pH, within a reasonably fast response time.

Several coating solutions were made, each consisting of approximately 0.5g of linear polymer of HEMA:MMA in varying ratios, 20% NVC as photocrosslinker and either 10% or 0% of the reagent bromocresol purple, dissolved in about 5ml methanol as described in TABLE 5.1.

Table 5.1 Coating Solutions For Fibre-Optics

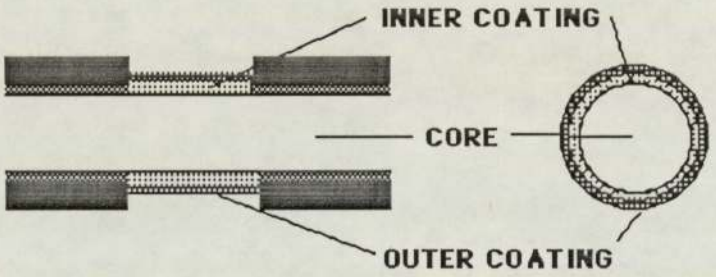
COATING SOLUTION CONTENTS/5ml METHANOL			
Ratio HEMA:MMA in linear polymer	Wt linear polymer/g	Wt Bromo-cresol purple/g	Wt NVC/g
50:50	0.50 0.50	0.06 0.00	0.10 0.11
80:20	0.50 0.50	0.06 0.00	0.10 0.10
90:10	0.50 0.50	0.06 0.00	0.10 0.15

5.2.2 Probe Construction

A 1.5cm section of core was exposed, using the original technique of removing the black polythene sheath from the fibre optic with a scalpel and then

gently scraping away the thin layer of fluoropolymer from the core surface. The probes were coated with two layers of membrane by running several drops of the first linear polymer solution along the exposed section of core, allowing it to dry and then repeating the procedure with the required second solution. The coated fibre optics were then left under UV for 1hr, in order to allow photopolymerisation to take place.

FIG 5.2 Diagram Of Fibre Optic Coatings



The inner and outer layer contained encapsulated reagent in some cases and the outer layer was reagent free in others as described in TABLE 5.2.

5.2.3 The Purpose Of Incorporating An Outer Layer

If the external layer is of lower water content than the inner layer it should restrict leaching of the reagent into the aqueous phase and thus, where the ratio of HEMA:MMA is consistent in the two layers but reagent is not present in the second, this should give some indication as to whether the presence of reagent affects the physical properties of the membrane, possibly by limiting the degree of cross-linking, around this large molecule, during the photopolymerisation stage. This would effectively make the external, reagent containing layer more porous than the internal. Therefore, reagent should leach out of the matrix far more slowly when it is only present in the internal layer, than when it is also present in the external layer (probes 4,5 cf 1,2,3 in TABLE 5.2).

5.2.4 Observations On The Relative Rates Of Reagent Leaching

The probes were first placed into 0.2M dipotassium hydrogen phosphate, pH=8.5, and the rate of reagent leaching was noted. For probes 2 and 3 this was initially very rapid but a faint colouration of the membrane was still noticeable after the initial rate of leaching had subsided. The rate of initial leaching from probe 1 was markedly slower than 2 and 3 and so, from this observation, it is apparent that, as the HEMA content increases at the expense of the MMA content in the linear polymer, the speed with which the reagent initially leaches out of the membrane increases, as would be expected.

When two layers of similar constitution are laid onto the fibre but only the inner layer contains reagent, this reduces the speed at which the reagent escapes, as compared to the probes where both layers contain reagent, implying that the presence of the reagent increases the porosity of the encapsulating matrix and similarly, a lower water content on the outside of a higher water content layer also reduces the chance of the reagent escaping.ie Probes 4, 5, 6 cf 1, 2, 3 in TABLE 5.2.

5.2.5 Observations On The Response Times Of The Probes

When the probes were placed in solutions of 0.2M dipotassium hydrogen phosphate and 0.1M citric acid, it was also noted that probes 3,4,5,6 took approximately 45 minutes to 1 hour to change colour when the pH was being lowered whereas when it was being increased, from 2.5 to 8.5, then the response time was between 15-30 minutes, except in the case of probe 2 where the response time was extremely rapid but the rate of reagent leakage was too fast for the probe to be of practical value. It must, however be noted that these response times are approximate and they cannot, of course, take into account the effects of either continually leaching

reagent or that colour change, close to the core/membrane interface, is not visible.

Table 5.2 Fibre Coatings Used And Observations

PROBE COATINGS			OBSERVATIONS	
No.	Coating Interfaced With Core	Coating Interfaced With Solution	RESPONSE TIME	
			ALKALI-ACID	ACID-ALKALI
1	50:50 HEMA:MMA + Reagent	50:50 HEMA:MMA + Reagent	15 MINUTES	1 HOUR
2	90:10 HEMA:MMA + Reagent	90:10 HEMA:MMA + Reagent	5 SECONDS	45 MINUTES
3	80:20 HEMA:MMA + Reagent	80:20 HEMA:MMA + Reagent	25 MINUTES	45 MINUTES
4	80:20 HEMA:MMA + Reagent	80:20 HEMA:MMA	30 MINUTES	45 MINUTES
5	90:10 HEMA:MMA + Reagent	90:10 HEMA:MMA	30 MINUTES	45 MINUTES
6	90:10 HEMA:MMA + Reagent	50:50 HEMA:MMA	30 MINUTES	1 HOUR

5.2.6 Conclusions

The probe coating which proved to be most practical was probe 4 since the rate of reagent leaching was not too fast to be able to use the probe effectively, although the response time required improvement. It was also considered unsuitable to have the external layer containing encapsulated reagent, since this would not provide an additional interface between the inner layer and solution. Also, as a result

of this, reagent leaching continually from the membrane surface, into the sample solution, might have a more pronounced effect on the output than with an additional membrane present, providing an added barrier.

These response times were also lowered respectively, when the pH was varied using solutions of HCl and KOH over a similar pH change, indicating that the cation and anion hydrated radii also play an important part in the sensor response time, which is a well established diffusion characteristic of hydrogel matrices. The external dye free layer is essential in order to trap the dye in the inner layers but it will also result in an increase in response time. Thus, limiting the thickness of this layer would be advantageous.

The conclusions drawn from this section are;

1) that the presence of the reagent in the hydrogel matrix does appear to result in an increase in porosity.

2) The response time of the membrane coating is dependent on the type of ions present in the sample solution. ie simple ions, such as H^+, Cl^-, K^+, OH^-, appears to diffuse through the membrane at a faster rate than K_2HPO_4, $C_3H_5O(COOH)_3$, which are present in the buffers.

3) From the probes studied, it appears that the optimum leaching rate/response time is achieved at a HEMA:MMA ratio of 80:20 with reagent present in the inner layer only.

5.3 THE CONSTRUCTION OF A PROBE AND A STUDY OF ITS RESPONSE TO CHANGING pH OVER A RANGE OF WAVELENGTHS.

5.3.1 The Probe Construction And Testing

On the basis of the conclusions drawn in the preceding section, a similar

probe was constructed, consisting of two thin layers containing 80:20 HEMA:MMA and the inner layer only, containing bromocresol purple as above. This was incorporated into a test bed as described in section 4.4.3, the source being the Hilger Watts spectrophotometer with an Infra red sweetspot photodiode detector, and a digital voltmeter as the output device.

Solutions of varying pH, measured on the Kent pH electrode were made up from distilled water and HCl or KOH, in order to reduce the response time to a minimum. The solutions were injected into the cell body in order of decreasing pH in order to save time, and were left for 15 mins to allow the membranes to equilibrate. Readings over a range of wavelengths were then taken from the voltmeter, allowing for fluctuations observed over the hydration period.

From preliminary studies, it was noted that the areas of maximum sensitivity were at 710nm and 590nm and that there was little effect with changing pH at 350nm. Thus readings were taken at these wavelengths for different pH values and the response at 350nm was taken to be a base line indicator of mains fluctuations.

5.3.2 Discussion Of Results

The "actual response" of the probe is shown at 350nm and 710nm in FIG 5.3. The response at 590nm is shown in FIG 5.4. Also in FIG5.4 , the response has been corrected by subtracting the response given at 350nm from the actual observed response. However it must be noted from this figure that it is not correct procedure to take the response at 350nm to be a base-line since it is of the same shape as that at higher wavelengths, and thus also shows a response to pH change. A possible explanation of this phenomenon will become apparent in the next section.

FIG 5.3 Actual Response Of Probe to pH At 710 and 350nm

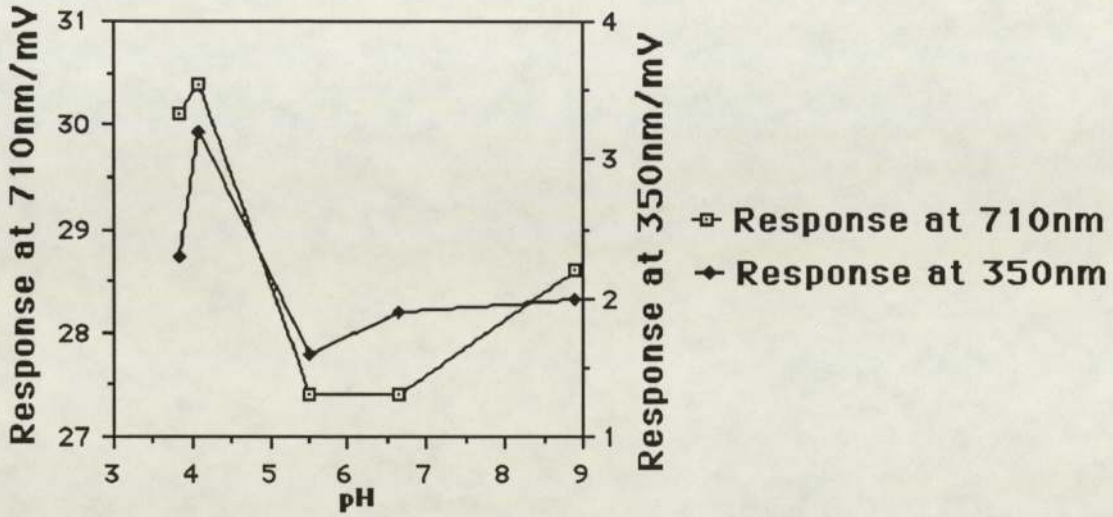
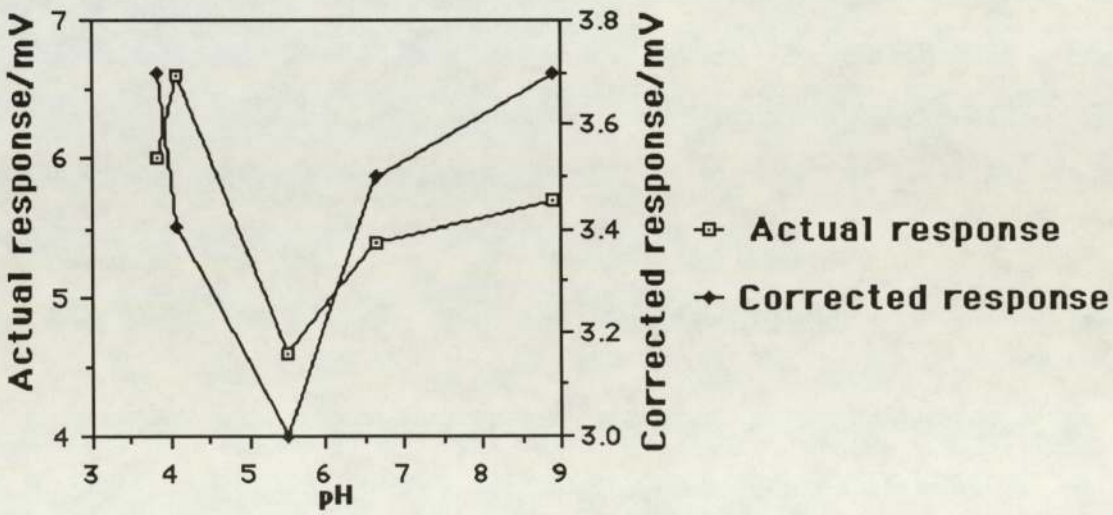


FIG 5.4 Actual Response and Response Corrected for Background at 590nm



5.3.3 Conclusions

These devices have indicated that it is quite feasible to utilise hydrogels as sensor membranes and that the sensor is responding in some way to the change in pH of the aqueous solution. However, it is now obviously imperative to be able to bind the dye into the membrane, thus allowing a high water content system to be used, decreasing the sensor response time and increasing stability and life time.

5.4 THE CONSTRUCTION OF A PROBE CONTAINING COPOLYMERISED REAGENT AND A STUDY OF ITS RESPONSE TO pH AND pCa^{2+}

5.4.1 Production Of The Coating Solution

The acrylated bromopyrogallol red had been developed, as described in section 3.4, by the convertion one of its phenol groups into an ester. A study of the colourimetric response of the chemically immobilised reagent (section 3.5) revealed that this process did not impair the ability of the reagent to change colour with varying pH, in whole membranes. Thus, in order to produce an even, colourimetrically sensitive coating, a linear copolymer of the reagent, HEMA and MMA was produced, using the solution polymerisation techniques described in chapter 2. The linear polymer was made containing approximately 7g HEMA plus 3g MMA and 0.05g AcBPR. The solution polymerisation was carried out at 60^0C for 8hrs, in approximately 250ml ethanol and 1% AZBN was added as initiator.

After the linear polymer had been extracted, 3g of linear polymer and 1% NVC were dissolved in 15g of methanol.

5.4.2 Exposure Of The Fibre Core And Subsequent Coating Of Linear Polymers

A section of core, approximately 1cm in length was exposed by removing the cladding and etching away the fluoropolymer coating, using the technique described in section 4.2.3. The end of the fibre optic was then dipped into the solution and the linear polymer allowed to run over the fibre optic, until several dry layers of linear polymer plus NVC were coated over the etched section of the core. A layer of 50:50 NVP:HEMA was placed over this to provide a low refractive index, high water content external layer. This was included in order to provide an additional boundary between the coating and test solution to both prevent interaction between the evanescent wave and solution and to possibly reflect any lost light back into the core.

The fibre was then placed under UV for 1hr and after subsequent hydration, allowing any impurities or non-crosslinked polymer to diffuse out of the membrane, was then incorporated into the test bed described in section 4.4.3, utilising the Hilger Watts spectrophotometer as source and detector with a digital voltmeter as the output device.

5.4.3 Experimental Technique

Buffer solutions of dipotassium hydrogen phosphate and citric acid, pH range 2.5-8.6 were pumped into the flow through cell in order of decreasing pH, since this was the direction of most rapid response (see section 3.3.3). After leaving the probe for 15 mins to equilibrate, the response of the probe to each solution was taken over a range of wavelengths from 350-750nm. It was apparent that the effect due to changing the pH of the buffer was most noticeable at 565nm and therefore, an expansion of this region of response is shown in FIG 5.5.

FIG 5.5 Response of Probe [(70:30):5% of (HEMA:MMA):AcBPR] To Varying pH In The Region 555-580nm

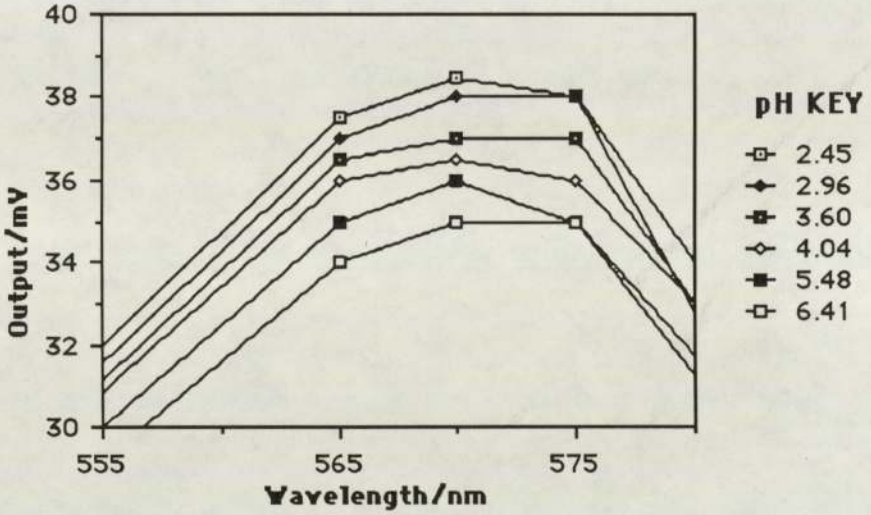
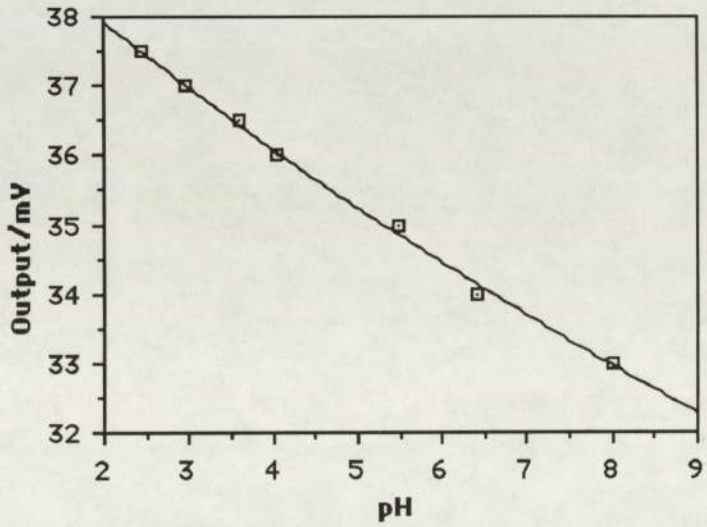


FIG 5.6 Response of Probe [(70:30):5% of (HEMA:MMA):AcBPR] at 565nm to Changing pH



5.4.4 Discussion Of Results

Plotting the probe response at 565nm we have the graph shown in FIG 5.6. The most prominent feature of this response is its near linearity over such a

wide range of changing pH, especially when we consider the response given by complete membranes in the feasibility study described in section 3.5.2. This would suggest that there is another factor influencing the response to pH. One characteristic of the hydrogel that would be expected to vary with pH, is the water content and this would affect the refractive index matching at the interface between the core and reagent phase. It is worthy of note that congo red and other indicators, chemically bound onto porous membranes, similarly exhibited an extended range of sensitivity[82].

5.4.5 Construction Of A Similar Probe With No Reagent Present In The Linear Polymer Coating

In order to study the effects of this phenomenon, a similar probe to the one described above, but for the fact that the inner layer was only a linear polymer of 70:30 HEMA:MMA containing no reagent, was constructed and the parallel set of results obtained are shown in FIGS 5.7-5.8.

FIG 5.7 Response of Probe [(70:30) of (HEMA:MMA)] in the Region 555-580nm

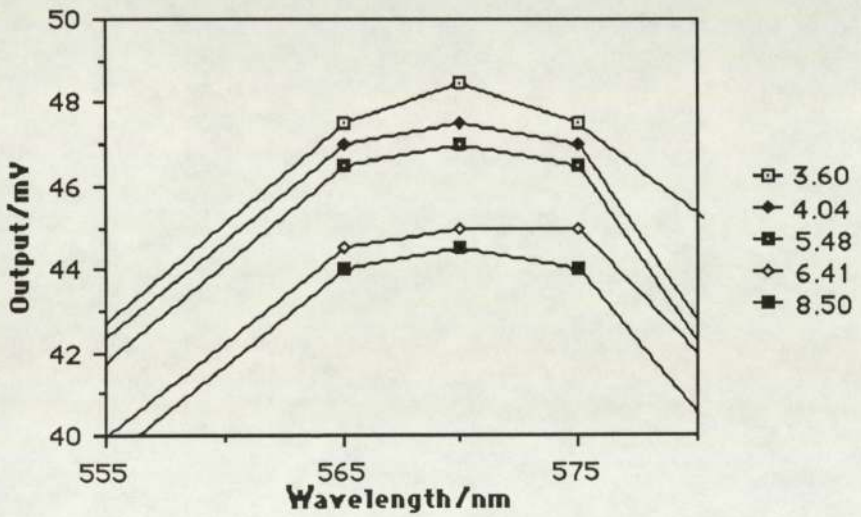
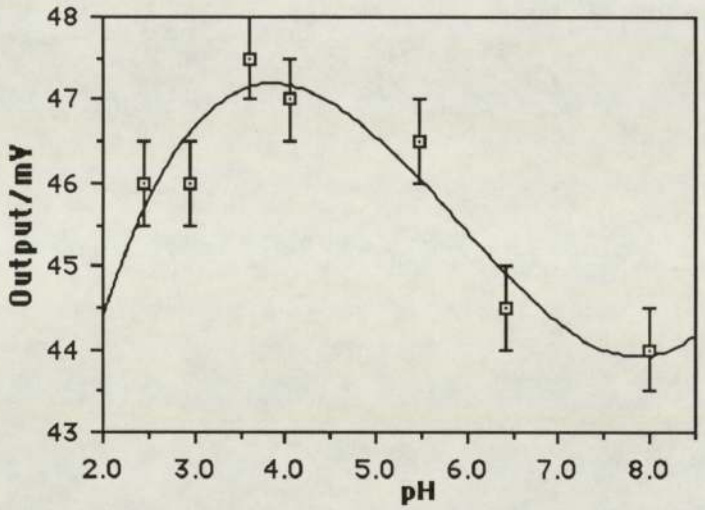


FIG 5.8 Response Of Probe[(70:30) of (HEMA:MMA)] At 565nm



5.4.6 A Study Of The Probe Response To Calcium

Varying concentrations of calcium chloride solutions, containing bromopyrogallol red had been noted to undergo small colour changes, although only altering between pink and red. The probe response was therefore tested over a range of calcium chloride solution strengths which were made up between pCa^{2+}=1.7-4.7. The pH change over this concentration range was measured and found to vary by only 0.6pH units. The results were obtained in the same manner as for pH, described above. The results are shown in FIGS 5.9-5.11.

FIG 5.9 Response of Probe [(70:30):5% of (HEMA:MMA):AcBPR] to Calcium Chloride in the Range 565-590nm

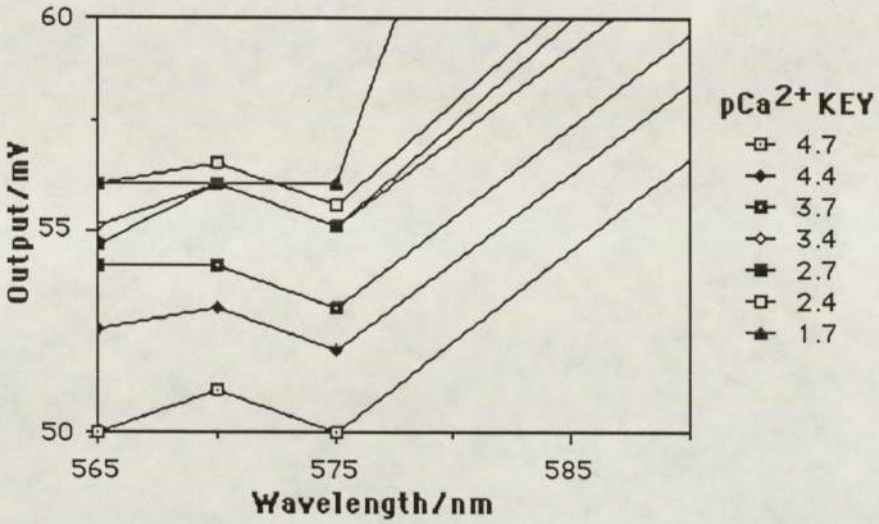


FIG 5.10 Response Of Probe [(70:30):5% of (HEMA:MMA):AcBPR] To Calcium Chloride At 565nm

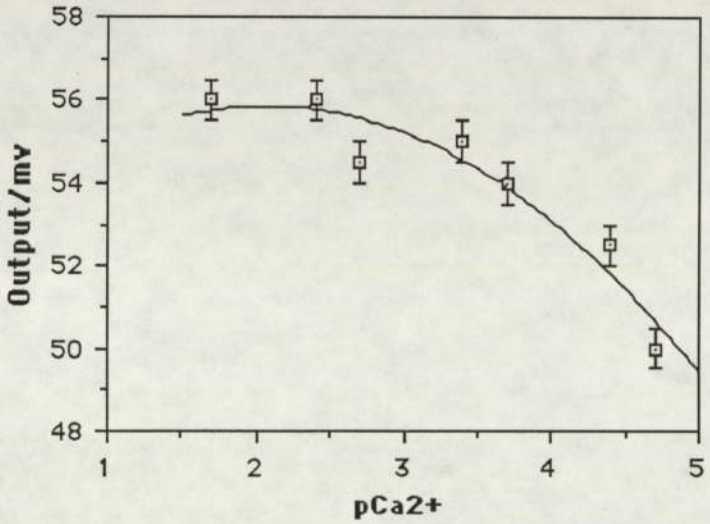
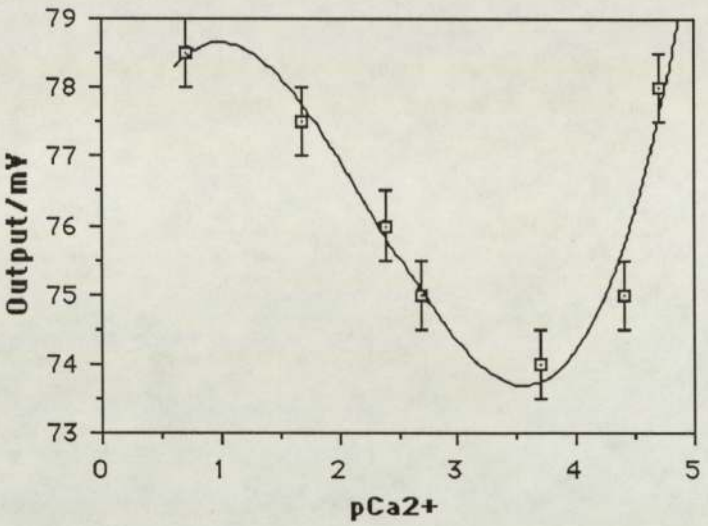


FIG 5.11 Response Of Probe [(70:30) of (HEMA:MMA)]

To Calcium Chloride at 565nm



5.4.7 Discussion Of Results And The Function Of The Membrane And Reagent

It now becomes even more apparent that the actual matrix is behaving in a reactive manner because it is not likely that a reagent would respond colourimetrically, over such a wide range of either changing pH or pCa^{2+} and the results from the control probes, which do not contain reagent, indicate that the observed phenomena are indeed related to the type of support matrix used. Although the presence of the reagent exerts some influence on the response, the over-riding effect seems to be as a result of the changing physical properties of the hydrogel matrix. It is highly likely that the interface properties between the core and the hydrogel are being altered as a result of changing refractive index of the hydrogel with pH and pCa^{2+}. Although the response we are seeing to both pH and pCa^{2+} is not

colourimetric, both the protonation of the reagent with increasing pH and the possible formation of a chelate between the reagent and the calcium ions, are affecting the transport properties of the matrix. This phenomenon, in both cases is manifested as a change in water content with ion concentration and therefore a change in refractive index of the coating.

The response of the probes, shown on the digital voltmeter, fluctuated greatly and it was not understood whether this was due to the changing water content of the matrix or whether it was electronic noise, inherent in the test bed circuitry. The probes also seemed to take a long time to reach equilibrium (about 20 minutes) and this could have been due to the inner membrane having too low a water content. As the response of the probes was heavily dependent on water content change with pH and pCa^{2+}, it was necessary to find a membrane system that would have a high water content, to allow rapid equilibria, but was rigid enough for the water content not to change with pH, in order to see if a colourimetric response of the reagent, could be isolated.

5.5 INTERPENETRATING POLYMER NETWORKS AS SENSOR COATINGS

5.5.1 The Properties Of Interpenetrating Polymer Networks With Respect To Sensor Applications

In order to improve the response time of the sensors and perhaps decrease the differing responses, when changing from acid to alkali and then vice versa, it was necessary to produce a membrane of high water content but with a fairly rigid structure so that the ability of the membrane to swell and therefore to change water content with pH would be restricted.

Interpenetrating polymer networks[153], should provide the facilities of being a high water content but rigid network. However, since coating monomers onto the fibre had previously proved to be unsuccessful, it was considered to be more practical to use semi-interpenetrating polymer networks, which are a combination of linear polymer chains, entangled in a cross linked polymer matrix. This was achieved by coating a solution of the reagent containing linear polymers, plus a combination of monomers which could be thermally crosslinked with EGDM, thus entangling the linear polymer chains. It was also hoped that the rigidity of the matrix would dispose of the difference in response time of the probe when the pH is varied in alternate directions.

5.5.2 The Construction And Study Of Various Semi-Interpenetrating Polymer Network Systems

Thus, several semi-interpenetrating polymer network membranes were produced using a combination of the linear polymers, already made via solution polymerisation, including HEMA: AcBPR(0.5% and 2.0%) and (70:30 HEMA:MMA): AcBPR(2%) and monomers such as NNDMA, HEMA, NVP and MMA, TABLE 5.3. Their characteristics such as water content and response time were noted. NVP and NNDMA were chosen because they are very hydrophilic and were thus likely to increase the water content of the resultant hydrogel. However, HEMA and MMA were used to moderate this effect and keep the rigid properties of low water content membranes.

Table 5.3 Membranes Constructed For Semi-Interpenetrating Polymer Network Studies

SEMI-INTERPENETRATING POLYMER NETWORK MEMBRANES			
No	LINEAR POLYMER CONTENT	MONOMER CONTENT	EQUILIBRIUM WATER CONTENT
1	0.5g HEMA:MMA:AcBPR (70:30):2	4.5gNNDMA	85.3%
2	0.5g HEMA:AcBPR 98:2	3g NVP 1.5g NNDMA	85.1%
3	0.5g HEMA:AcBPR 98:2	3.5g HEMA 1.0g MMA	27.0%
4	0.5g HEMA:MMA:AcBBR (70:30):2	4.5g HEMA	36.8%
5	0.5g HEMA:AcBPR 98:2	4.5g HEMA	38.0%
6	1.0g HEMA:AcBPR 98:2	2.5g NNDMA	82.0%

5.5.3 Discussion Of Results.

Although solution 1, Table 5.3, provided a rigid membrane with a high water content, it was too brittle and easily fell apart. The membrane formed from solution 2 possessed a high water content but upon hydration swelled to approximately four times its original size and when coated onto a fibre, lifted away from the core and the colour of the membrane was not visible when viewed down a distal end of the fibre. The membranes produced from solutions 3, 4 and 5 were very rigid and little colour change took place when the membranes were placed in

either 0.1M citric acid or 0.2M dipotassium hydrogen phosphate. Solution 6 showed the most promise as a sensor coating as it had a very high water content, reducing the response time, either from acid-alkali or vice-versa, to well under a minute, was quite rigid and when coated onto a fibre optic, an intense colour was visible when viewed along the fibre. Unfortunately NNDMA is a solvent for MMA and although this assisted in providing a high water content coating, which was firmly attached to the fibre surface, the resulting low water content at the core/coating interface prevented colour change from occurring rapidly, other than at the outer most surface of the coating. However, the outer layers were very swollen and broke up with continual use. Also, these solutions presented problems when trying to use them as coatings since, as with the monomer solutions in section 5.1.4, the resultant coating was uneven, which would later present problems with the reproducibility of the fibre response.

5.5.4 Conclusions

Although the complete membranes produced did possess the properties of being fairly rigid but allowing extremely rapid response times, semi-interpenetrating polymer network solutions do not provide convenient coatings for the poly-MMA fibre optics, mainly due to their oily nature, but also because of the length of time required to complete thermal polymerisation. The most rapid and successful technique of obtaining an even, thin coating on the fibre optic remains to be via linear polymer solutions and UV polymerisation. In order to maintain a rapid response time of the membrane and therefore sensor, high water content systems require further investigation.

5.6 CONSTRUCTION OF PROBES WITH HIGH WATER CONTENT, LINEAR POLYMER COATINGS AND A STUDY OF THE EFFECTS OF AN EXTERNAL COATING OF HIGH WATER CONTENT AND LOW REFRACTIVE INDEX

5.6.1 Introduction.

Although the study of semi-interpenetrating polymer networks, in the previous section, proved them to be not entirely satisfactory as sensor coatings some positive aspects of solution1, table 5.3 emerged: This solution provided a fairly rigid membrane with a rapid colourimetric response. When applied as a sensor coating, despite the mechanical difficulties encountered upon hydration, an intense colour was viewed along the fibre optic. In order to provide an even coating of slightly lower water content and thus increase the rigidity of the coating, a linear polymer of similar constitution as this semi-interpenetrating polymer network but containing less NNDMA, was produced. This was coated onto the fibre in the manner described in section 5.1.5. The intense colour was still visible and it was hoped that this would have a greater influence on the overall sensor response than membrane water content, than had been previously experienced.

In addition, an external layer of lower refractive index was also added, since it may assist in reflecting stray light back to the fibre optic core, via the lossy, high water content internal layer, thus improving the output signal obtained. The response of the probe and the effectiveness of the external layer were examined.

5.6.2 Test Bed And Sensor Construction

A sensor was constructed using a solution of the linear polymer, consisting of 50:50 HEMA:NNDMA and 5% AcBPR, with an exterior coating of 50:50

HEMA:NVP linear polymer containing 5% NVC (see section 5.5.3), in order to provide a low refractive index on the outside, so as to reflect any stray light back through the reagent containing layer and into the fibre optic. The results of this were determined on the test bed described in section 4.4.4, consisting of an ultra bright green LED and an eye-response photodiode as the source/detector and the results are shown in FIGS 5.12,5.13.

5.6.3 Construction Of A Sensor With No External Coating Layer

A second probe was constructed where the outer layer of HEMA:NVP was not present, in order to see whether this layer does have an effect on the probe response and whether it is advantageous to have the external layer of high water content present.

5.6.4 Results

It is apparent that the sensor outputs do not return to their original value at pH=8.6 after being tested at a lower pH. This may be due to the swelling of the membrane as water content changes with increasing pH followed by an inability to shrink back to a lower water content. Also, the response of the probe is not typically colourimetric since it is sensitive over too wide a range of pH change, as was previously shown to be the case. This again implies that the probe is responding to the change in refractive index of the hydrogel.

The results, shown in FIG 5.13, indicate that when the outer layer of HEMA:NVP is not present, the response seems to be less reversible. The presence of the outer layer is completely changing the way in which the probe responds to pH, rather than merely increasing the intensity of response.

FIG 5.12. Response Of Probe With High Water Content Exterior Layer.

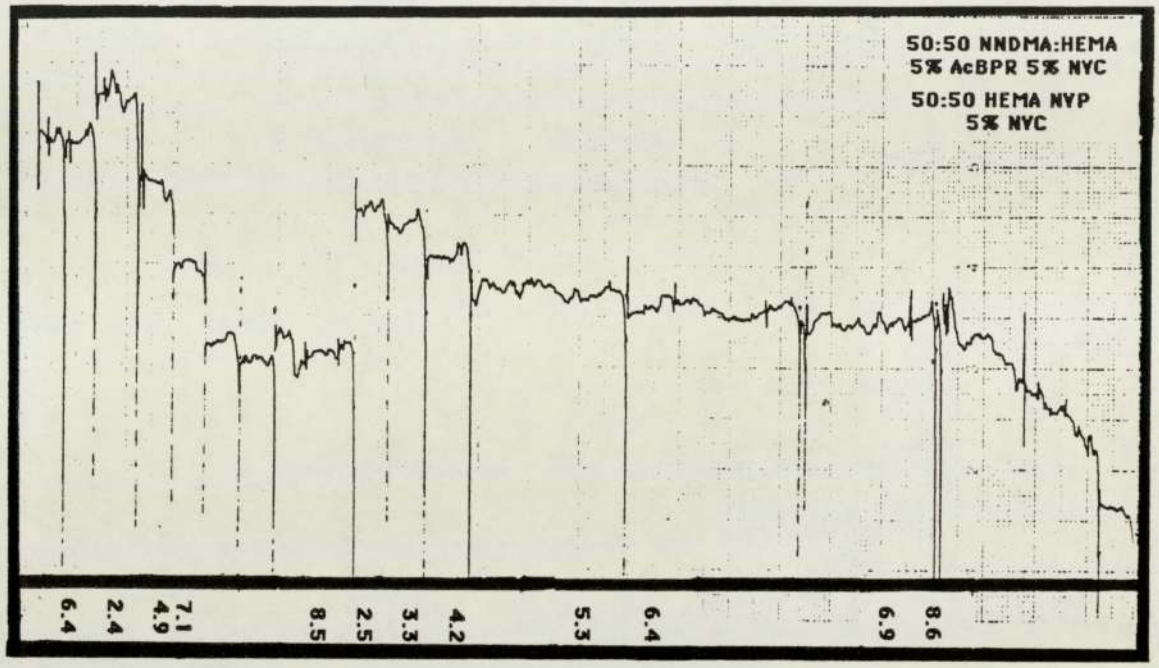
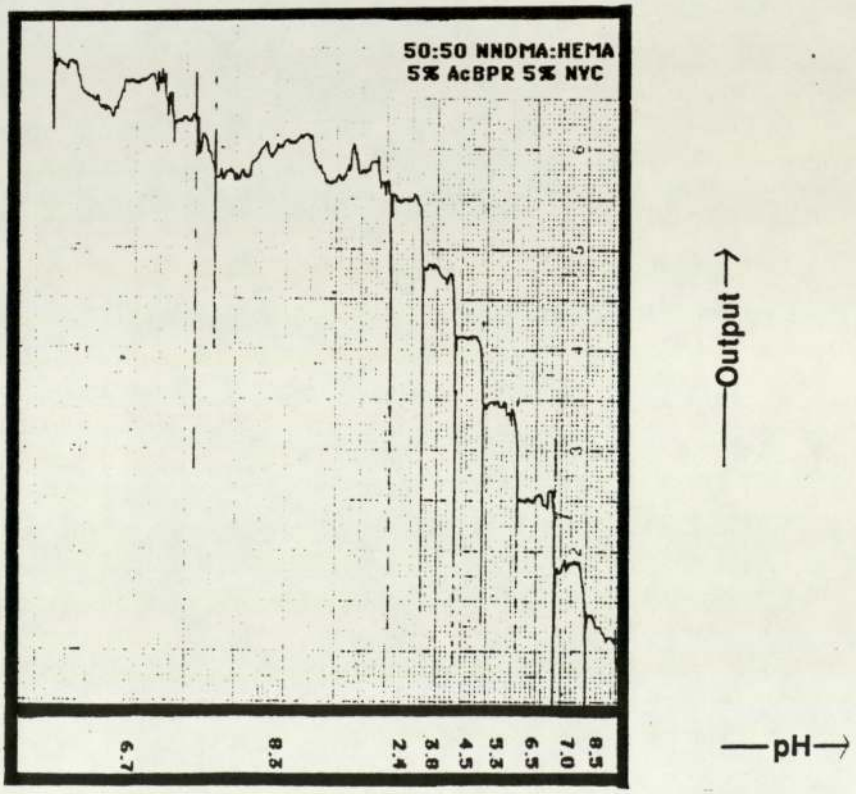


FIG 5.13 Response Of Probe Without High Water Content Exterior Layer.



5.6.5 Conclusions

It is unlikely the non-reversibility of the response is due to any colourimetric change and most likely that it is due to a physical change in the membrane. These high water content coatings may not be able to under go large and rapid changes in water content. There may also be some lifting of the membranes from the surface of the fibre core, thus also creating a non-reversible change in the optical properties at the interface.

Lowering the water content of the membrane may reduce the degree of change in water content with pH and only a colourimetric effect might then be visible and since it appears that there is, in fact sufficient optical interaction at the first interface, to produce an appreciable response, the second only serves to confuse the issue.

5.7 GENERAL CONCLUSIONS

This chapter has demonstrated that via the production of linear polymers, reagent modified hydrogels can be easily and quickly applied to a fibre optic core. As an integral part of the test bed system, a single coating of the hydrogel matrix produces a visible but not colourimetric, on-line response to pH and pCa^{2+}. Thus a further study of both isolated and coated hydrogel membranes can now be made, in order to attempt to distinguish between the different effects contributing to the overall sensor response. This will enable us to optimise the components of the membrane in order to produce a rapid and reversible system and therefore, expands into a broader and more in-depth study in the next chapter.

CHAPTER 6

AN ANALYSIS OF THE FACTORS CONTRIBUTING TO THE OVERALL SENSOR RESPONSE

6.1 THE COLOURIMETRIC RESPONSE OF A SOLUTION OF REAGENT IN BUFFER AROUND AN EXPOSED FIBRE CORE.

6.1.1 Introduction

It is apparent, from previous chapters, that the interaction between the reagent phase and the core is complex. It certainly is not a completely colourimetric response but the presence of the reagent contributes in some way towards the total effect in detecting a change in either pH or tonicity of the sample solution. Thus, an attempt must be made to establish the individual factors which contribute to the nature of this interaction and hence the probe response.

6.1.2 Colourimetric Response Of Reagent In Buffer Solutions

Thus the probe response elements were broken down by first attempting to produce a purely colourimetric response using buffer solutions containing the reagent bromopyrogallol red. These solutions were made up by dissolving approximately 0.5g BPR in 250ml of distilled water and taking 2.2ml of this solution and mixing with 50ml of buffer. The pH was then checked using the Kent pH electrode. The resulting test solution was passed through the flow through cell, around an etched section of fibre optic, as in section 4.2.3, exposing a 1cm length of the core. The results of which are shown in FIGs 6.1 and 6.2.

The most noticeable feature of the response created by the buffered reagent solution, surrounding an exposed fibre core, is that changing pH virtually has the reverse effect on the observed absorption characteristics, to that where the reagent is copolymerised into a hydrogel coating, (FIG 5.6). This further suggests that the change in colour of the reagent does not directly create the change in the absorption of the incident light. However, as discussed in section 5.4.7 the

deprotonation of, or the possible formation of a chelate with, the reagent molecule may alter the transport properties of a reagent containing hydrogel matrix.

FIG 6.1 Bare Fibre Response To Bromopyrogallol red In Buffer.

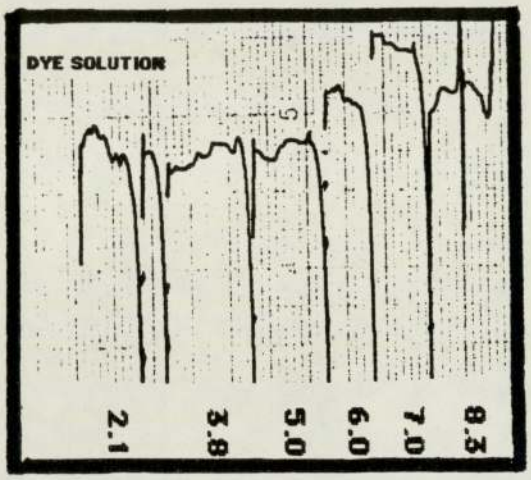
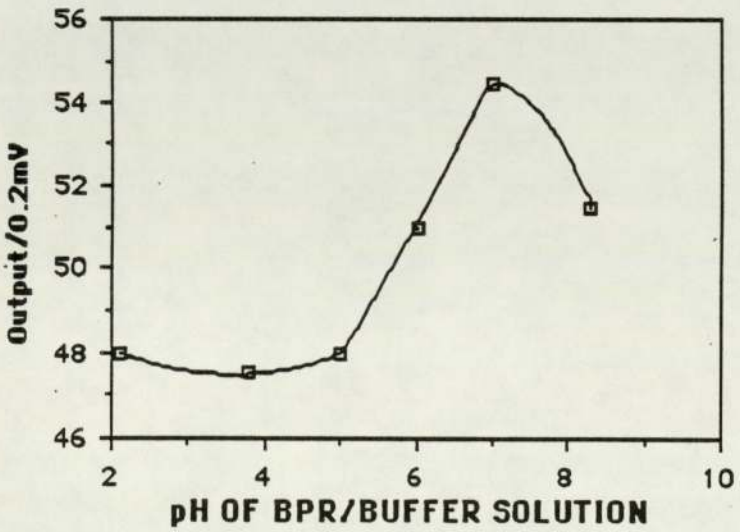


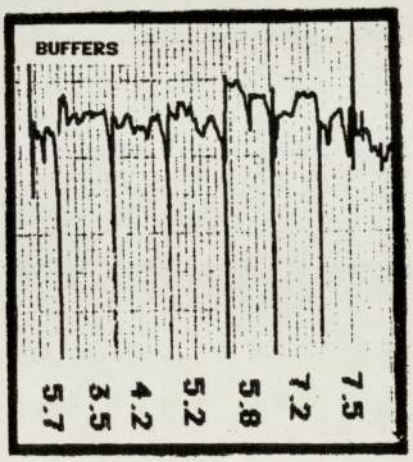
FIG 6.2 Bare Fibre Response to Bromopyrogallol Red In Buffer Solutions



6.1.3 Response To Changing Refractive Index Of Buffer

The response of the fibre to changes in refractive index of the buffer solution was also examined by a similar technique but with no reagent present. The results of this response show this effect to be negligeable. FIG 6.3

6.3 Bare Fibre Response To Buffer Solutions.



6.2 THE EFFECTS OF AN INCREASING PERCENTAGE OF HYDROPHOBIC MONOMER IN THE LINEAR POLYMER.

6.2.1 The Effect Of Water Content On The Performance Of A Membrane As A Sensor Coating

As the response of former probes seems to be dependent on the changing water content of the hydrogel membrane with pH, the ratio of hydrophobic to hydrophilic monomers, in the linear polymer, is thus likely to be of some consequence in determining the response of the probe, in terms of reversibility and the range and degree of change detectable, since the ratio of these monomers will dictate the rigidity or flexibility of the hydrogel matrix. We have already discovered that a membrane of very high water content is impractical as a sensor coating since it does not possess reversible characteristics, in response to changing pH. However, a high water content allows for rapid transport of detectable species through the membrane to the reagent. Also, as a result of the increased ability of the membrane to swell, higher water content membranes are likely to be more sensitive to smaller changes in pH.

It may also be the case that should we reduce the ability of the membrane to change water content, in response to a changing pH, by increasing the hydrophobic constituent of the polymer, we may be able to see a response of a colourimetric nature when a suitable reagent is present.

In addition to the effect of changing water content, as the MMA content in the linear polymer increases, the refractive index of the coating will become closer to that of the fibre core. Thus, since the critical angle will be decreasing, the amount of light which is totally internally reflected will increase and so therefore, will the

output of the detector. The refractive indices and water contents of some HEMA:MMA copolymer membranes which were covalently cross-linked are given below[151].

Table 6.1 Refractive Indices and Water Contents of Various HEMA:MMA Cross Linked Hydrogel Membranes

RATIO HEMA:MMA	EWC	REFRACTIVE INDEX
0:100		1.490
50:50	13%	1.480
60:40	16%	1.477
70:30	22%	1.465
90:10	31%	1.447
100:0	37%	1.435

6.2.2 The Effect Of Hydrophobic Monomer Constituents On Response To Changing pH

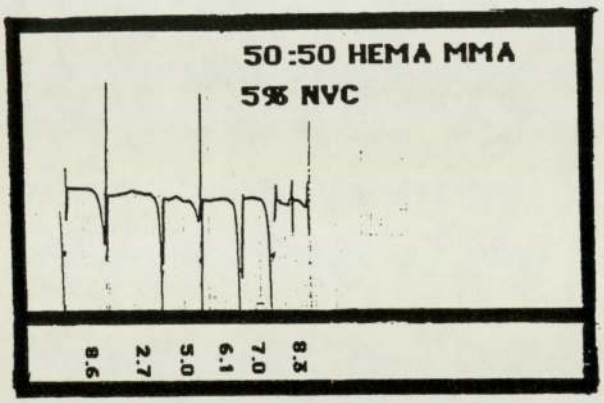
A number of linear polymers, varying in ratio of HEMA:MMA were available and since some success had previously been attained using a combination of these monomers it was a logical step to study the response of these membranes as sensor coatings. Linear polymer solutions, containing 1g of the hydrogel linear polymer and 5% NVC, dissolved in 5ml of methanol were produced. The linear polymers used were:

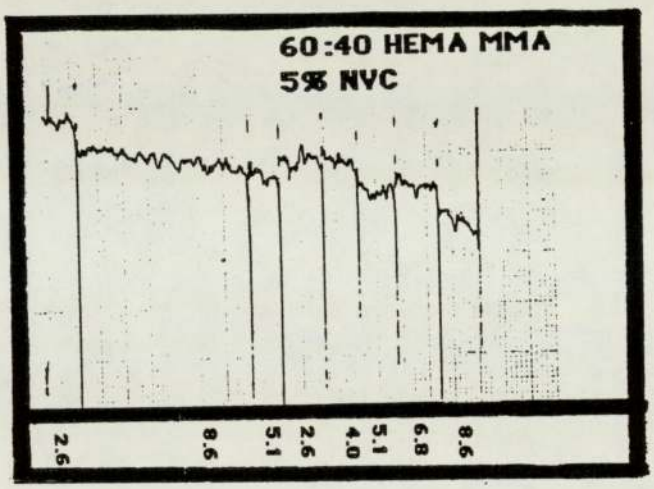
1) 70:30 HEMA:MMA
2) 60:40 HEMA:MMA
3) 50:50 HEMA:MMA

Buffer solutions, pH range 2.5-8.5, were made from 0.1M citric acid

and 0.2M dipotassium hydrogen phosphate. Approximately 25ml of buffer was washed through the flow through cell at each change in pH, after initially soaking the probes, constructed from the coating solutions, as described in sections 4.2.3 and 5.1.4, in dipotassium hydrogen phosphate overnight. In order to determine the reversibility of the sensor, the sample solutions were cycled from pH 8.5 to 2.5 and back to 8.5 and, where an appreciative response to changing pH was apparent, this was repeated for up to four consecutive cycles. Whilst each sample was being pumped into the cell, the chart recorder was turned off, since the response of the probe is possibly also dependent electrical noise or on vibration, as a result of the fibre bending and altering the absorption or loss of light at the core/coating interface. This interim period was no more than 1 minute and the chart recorder was then allowed to run for a period of three minutes, or longer in the case of the higher water content membranes, such as 70:30 HEMA:MMA, which appeared not to have reached a state of equilibrium at the end of this time interval. The chart recordings are shown in FIG 6.4

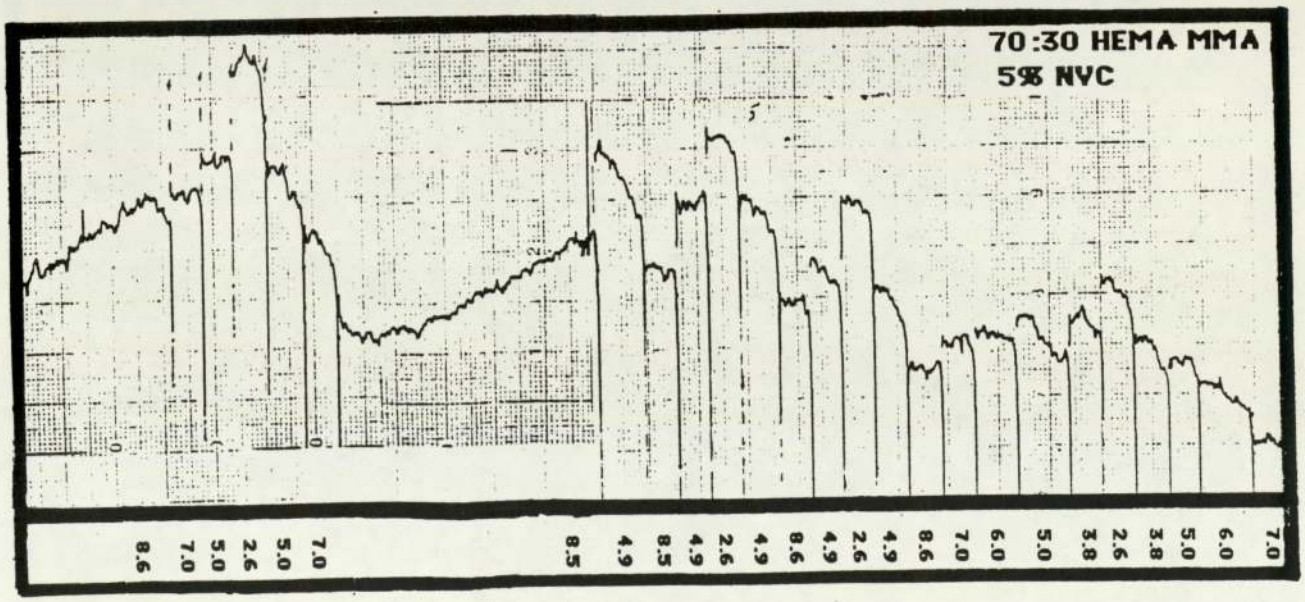
FIGS 6.4 The Effect Of Varying The Ratio of HEMA:MMA In The Sensor Coating Solution, On The Response To Changing pH.-Chart Recordings





—Output→

—pH→

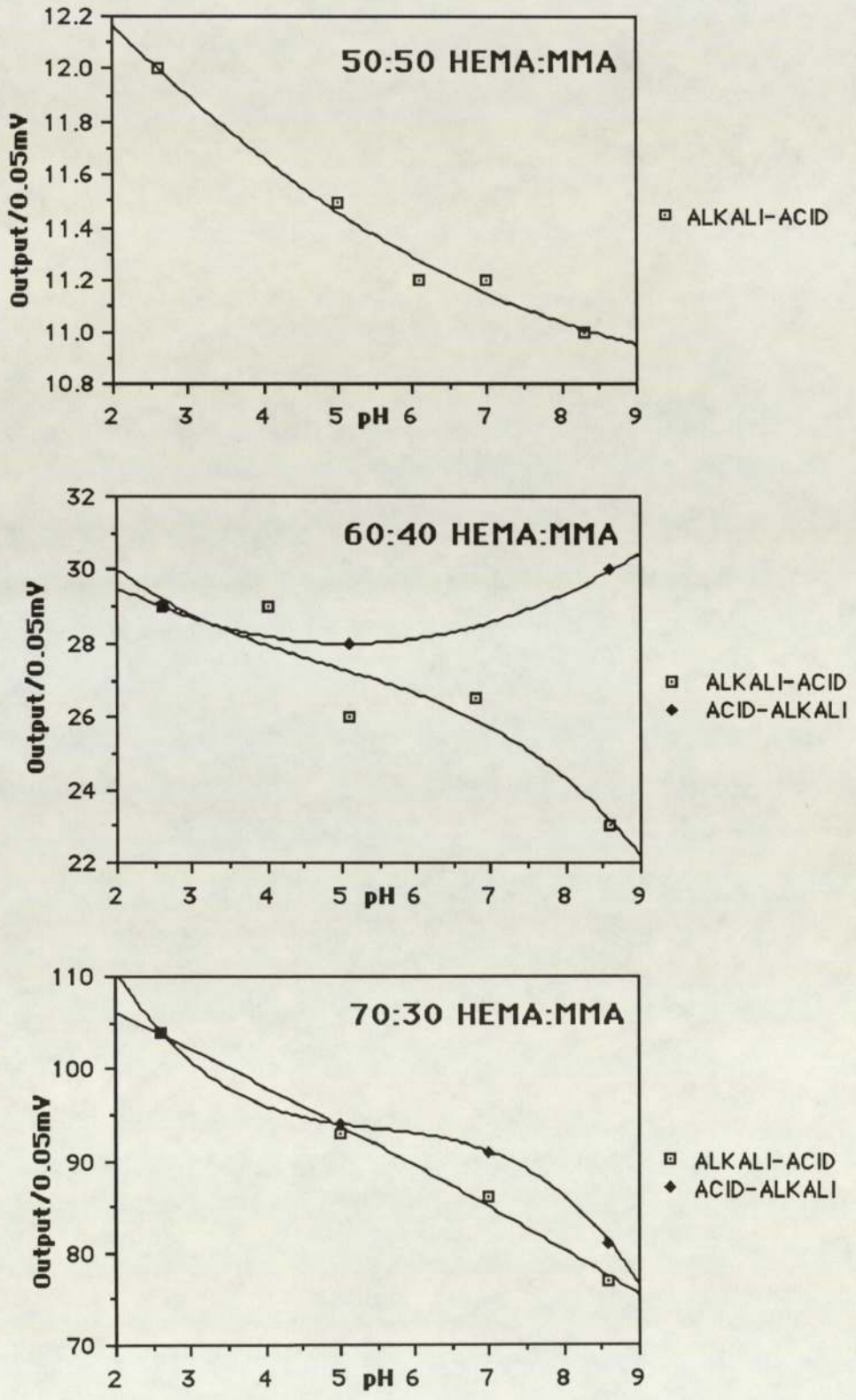


6.2.3 Discussion Of Results

From these chart recordings, it is apparent that the hydrophobic content of the membrane does have some affect on the response of the probe. It is also evident that the noise in the response seems to subside with the addition of the hydrophobic monomer. Also, that which was previously considered to be electrical noise, becomes more pronounced as the HEMA content of the membrane increases and is hardly visible in the case of the 50:50 HEMA:MMA membrane where little change with pH, and therefore little transport of ions through the membrane, is taking place. The effect of pH on changing water content in the 50:50 HEMA:MMA membrane is limited and it appears that generally, as the percentage of hydrophilic monomer increases, so does the sensitivity of the probe response.

If we take readings from the chart recordings, over the range of changing pH, in both directions, over the last or only cycle, which will be on the left hand side of the figures shown, we can see more clearly the reversible or non-reversible nature of the response and the range of changing pH over which the response is effective. Graphs of these responses are shown in FIG 6.5

FIG 6.5 The Effect Of Varying The Ratio Of HEMA:MMA In The Sensor Coating Solution, On The Response To Changing pH



Although the response from the 50:50 HEMA:MMA membrane, initially seems to be non-existent, from the chart recordings, on close inspection there is a small response to pH. We would have expected the 60:40 membrane to show more reversible characteristics than the 70:30, due to its lower water content, it appears from these graphs that the changing water content of the 70:30 membrane is in fact of a more reversible nature. Also, the percentage error in the former is far greater, since it is approximately 25% of the total change in response over the last cycle. For both of these sensor coatings the amount of error or "noise" is of the same order of magnitude, whereas for the 50:50 membrane it is negligeable.

6.2.4 Conclusions

Generally, the range of output decreases with increasing MMA content and this would be expected since the refractive indices of the core and coating become closer together as the fraction of MMA in the coating becomes greater, thus reducing the range over which the index of the coating can vary, before it becomes close to that of poly-MMA.

6.3 THE EFFECT OF PERCENT NVC ON THE WATER CONTENT AND THEREFORE RESPONSE OF HEMA COATINGS, TO CHANGING pH

6.3.1 Introduction

NVC itself is a hydrophobic monomer and although it has been assumed that its presence has little or no effect on the water content of the membrane it is a factor which has to be investigated. Previous photopolymerisation work, carried out in these laboratories, has utilised 20% U.V photocrosslinking agent and this has been shown to have little effect on the water content of complete membranes, hydrated in

distilled water. However, since the system is evidently highly sensitive and originally was not expect to detect changes in membrane water content with pH, the effects of the presence of NVC must be determined. As 70:30 HEMA:MMA had shown an appreciable and relatively symmetric response to changing pH, pure HEMA membranes were chosen to study the effects of varying the amount of photocrosslinking agent used. This was because the difference in response over the same pH range, 2.5-8.5, would be greater for HEMA than for 70:30 HEMA:MMA, due to its higher water content. From the membranes discussed earlier, we would also expect less symmetry and reproducibility in the response as we cycled through this pH range. Hopefully any effect that the NVC had contributed would therefore, become more outstanding and the fact that we used combinations of HEMA:MMA in order to study the effects of adding a hydrophobic monomer, in section 6.2, will mean that these results will be directly comparable.

6.3.2 Composition Of Coating Solutions

Linear polymer solutions of 1g HEMA, dissolved in approximately 5ml methanol were made to contain 5%, 12% and 20% NVC. Probes were constructed from these solutions as in sections 4.2.3 and 5.1.4 and results obtained as described previously in section 6.2. The chart recordings are shown in FIG 6.6

6.3.3 Discussion Of Results

It becomes immediately obvious that the addition of NVC is an important parameter in determining the type of response obtained from the probe, with varying pH. Generally, the addition of increasing amounts of the photocrosslinking agent seems to stabilise the response, since the membrane seems to come rapidly to equilibrium, in the case of 12% and 20% NVC but where only 5% NVC is present,

the response of the probe is prone to noise and drift. However, the response for 12% NVC appears to be less noisy than for 20% but the latter seems to respond over a larger output range. The results obtained for the HEMA membrane, containing 5% NVC, can be directly compared to the results obtained for HEMA:MMA membranes in section 6.2.2 and this does in fact reinforce the points made in the associated discussion of these results. In the case of both hydrophobic monomers, MMA and NVC, the effect of their presence, up to a point, is generally to stabilise the response of the probe, in terms of improved symmetry of response, when cycling through a pH range and to reduce the passage of ionic flux through the membrane, thus decreasing the associated fluctuations of the output signal.

FIG 6.6 The Effect Of Varying The Percentage NVC in HEMA Coating Solutions, On The Response To Changing pH-Chart Recordings.

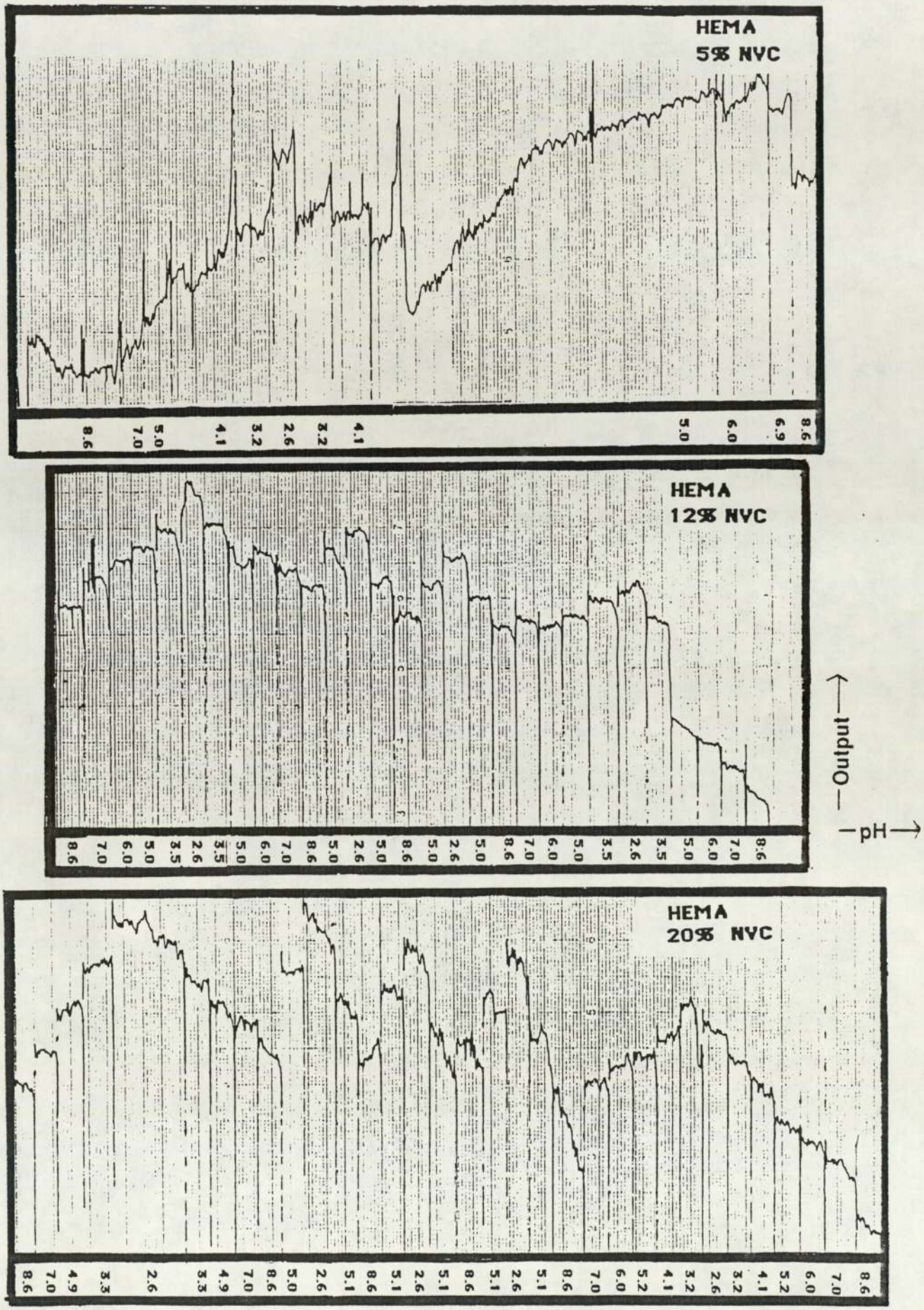
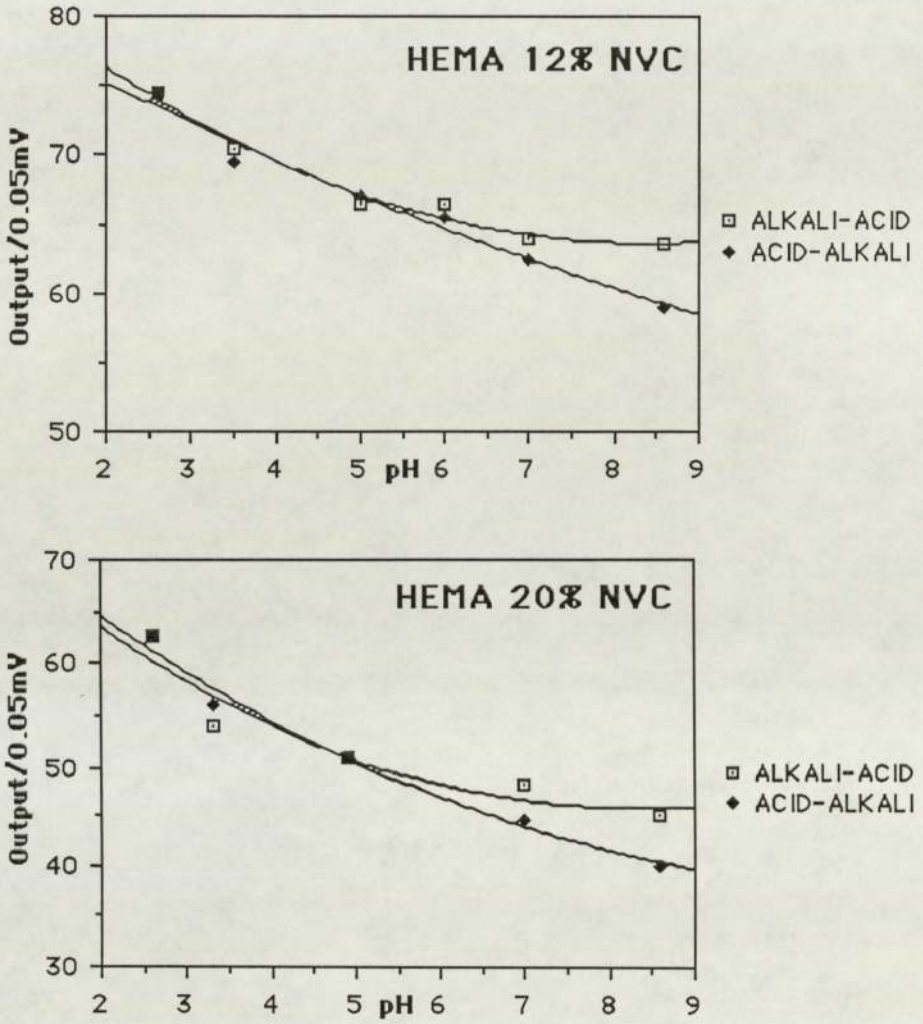


FIG 6.7 The Effect Of Varying The Percentage NVC In HEMA Coating Solutions, On Sensor Output in Response To Changing pH.



6.3.4 Conclusions

Although increasing proportions of MMA reduce the output range of the response, where NVC is concerned, the range of response increases as the percentage NVC increases from 12 to 20%, for HEMA membranes. The range of pH to which the probes respond does not seem to change in either case between the limits of 2.5 and 8.5 but the level of sensitivity or the difference in output at these two values, alters with hydrophobic monomer content.

It appears therefore, that we need a low water content membrane, as a

sensor coating, in order to reduce the noise factor and improve the symmetry and thus, the reproducibility of the response. However, we must also balance this effect against that of reducing the sensitivity range of the device. Another effect which should play an important part in improving the sensitivity, is the matching of the refractive indices of the sensor coating with the fibre core. In this case, since the core is polymethylmethacrylate, reducing the water content by the addition of methylmethacrylate to the constituent monomers of the linear polymer will also improve the refractive index matching between these two media.

We now need to attempt to establish the reason for NVC having the opposite effect to MMA on the output range of the sensor. Since we suspect that membrane water content has a strong influence on the sensor response, the measurement of water content at different pH values may reveal the answer.

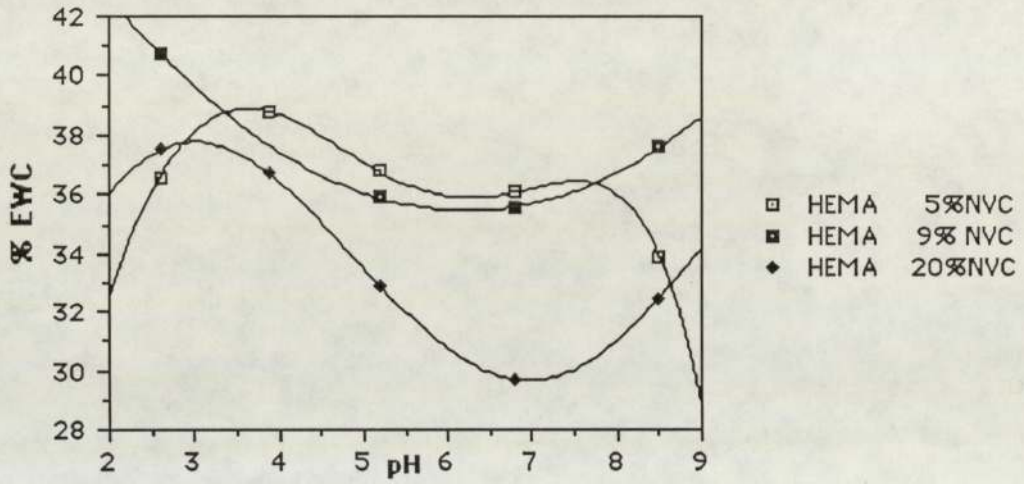
6.4 A STUDY OF THE CHANGE IN WATER CONTENT WITH pH OF LINEAR POLY-HEMA,CONTAINING VARYING PERCENTAGES OF NVC AS PHOTO-CROSSLINKER AND OF COVALENTLY CROSS-LINKED POLY-HEMA CONTAINING AcBPR

6.4.1 Membrane Construction For Water Content Measurements

In order to ascertain whether the response we were seeing corresponded to a change in water content of the membrane with pH; complete membranes were constructed from the same solutions which were used for coating the fibre optics. Sections of these and of HEMA membranes, containing varying amounts of NVC, were soaked overnight in buffer solutions made from citric acid and dipotassium hydrogen phosphate, over a pH range 2.5-8.5. The equilibrium water contents were then measured using the method described in chapter 2 and a graph of pH against water

content plotted as in FIG 6.8. The same procedure was carried out for both cross linked HEMA and HEMA plus 0.5% reagent membranes as shown in FIG 6.9.

FIG 6.8 The Effect of NVC On The Water Content Of Photocrosslinked HEMA Membranes

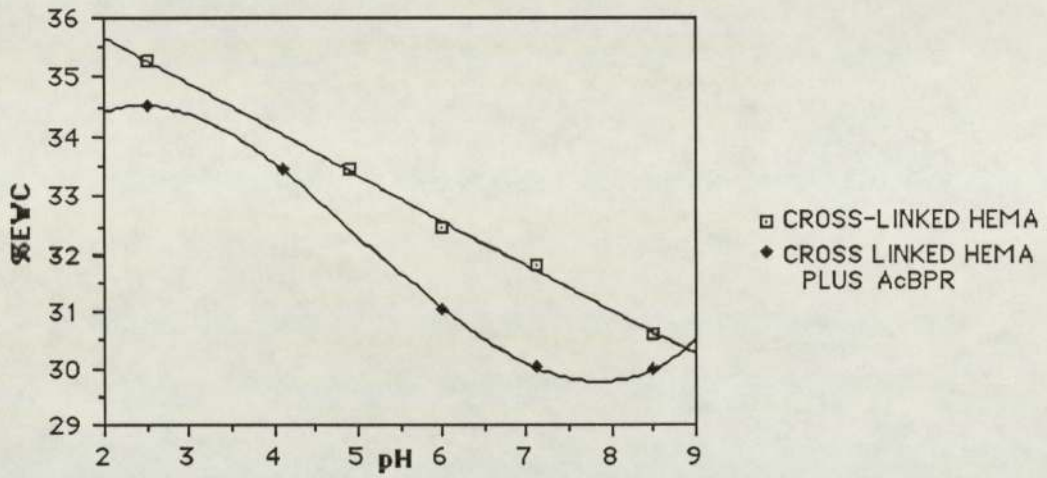


6.4.2 Discussion Of Results

The membranes show a general increase in water content with decreasing pH. The amount of NVC present also seems to affect the shape of the curves as water content varies with pH and at 20% NVC there is a marked increase in the sensitivity of membrane water content to pH. At 5 and 9% NVC, the water content is between 5 and 10% higher than that of cross-linked HEMA but at 20% NVC the water content approaches similar values. The increase in the response of water content to changing pH, as the percentage of NVC is also increased, explains why we were seeing a corresponding increase in the sensitivity of probes, containing similar proportions of NVC in the sensor coatings, rather than a decrease as would be expected with the decreasing water content, due to the addition of this hydrophobic monomer. It is therefore reasonable to assume that the percentage NVC in the coating solutions is an

important parameter in deciding the type of response and that the variation in water content, over a pH range, bears direct significance on the sensitivity of the response.

FIG 6.9 Variation Of EWC With pH For HEMA And HEMA:AcBPR Membranes



6.4.3 The Effect Of Changing pH On The Water Content Of Covalently Cross-Linked Reagent:HEMA Membranes

The variation in water content of cross linked poly-HEMA, as compared to that of a similar membrane, containing 1% copolymerised Acrylated bromopyrogallol red, shows that the presence of this reagent, although at only a small concentration also has an effect on the shape of the water content curve with varying pH, FIG 6.9. In fact, this curve is of a similar shape to that obtained from linear poly-HEMA when photocrosslinked with 20% NVC. The presence of the reagent however, seems to result in a general reduction in the water content of the membrane.

6.5 CONCLUSIONS

The four major conclusions drawn from this chapter are that:

1) The refractive index change in the buffer solution has little effect on the sensor response, as does the change in colour of buffered solutions of bromopyrogallol red, at varying pH.

2) In membrane coatings of linear poly-HEMA, photocrosslinked with NVC: An increase in the percentage NVC results in an increase in sensor output range.

3) In membrane coatings of linear poly-HEMA:MMA.;20% NVC: An increase in the percentage MMA results in a decrease in sensor output range.

4) The response of the fibre optic sensors is most likely attributed to a change in membrane water content and thus refractive index with changing pH. This effect appears to be more reversible in lower water content systems.

Thus, we now need to study specific membrane systems in order to find an "ideal" constitution of component monomers, which will provide us with a rapid but reversible on-line response. We have established that the major effect on response, in the hydrogel coatings used to date is due to a changing water content with either tonicity or pH. The study of very low water content membranes, may therefore reduce this effect and additionally result in a sensor response which is of a colourimetric nature.

CHAPTER 7

THE PROTOTYPE pH PROBE: OPTIMISATION OF MEMBRANE PROPERTIES.

7.1 THE CHOICE PRODUCTION AND USE OF LINEAR POLYMERS FOR SENSOR COATING SOLUTIONS

7.1.1 Introduction

In chapters 5 and 6, it was discovered that the main factor contributing to the sensor response was the change in hydrogel water content with pH. It was proposed that the study of low water content membranes may result in a reversible and possibly colourimetric response. Several linear polymers were made via solution polymerisation in order to provide low water content coatings with refractive indices close to MMA. Although the cut off point for transport was found to be 50:50 HEMA MMA, section 6.2.2 very much earlier studies, section 5.2, where the reagent was only encapsulated, had revealed that it served to produce a more porous network and therefore, it was considered feasible to attempt to reduce the HEMA content of these solutions still further and expect sufficiently rapid transport to still be possible. In view of previous studies in section 6.3, the effects of varying the percentage of NVC in each linear polymer coating was also studied.

7.1.2 Linear Polymers Produced For Sensor Coatings

The linear polymers were produced using the solution polymerisation techniques described in chapter 2 and are listed in the order in which they were produced:

1) 60:40 MMA HEMA 5% AcBPR,

2) MMA 5% AcBPR,

3) 75:25 MMA HEMA 5% AcBPR,

4) 80:20 HEMA STYRENE 5% AcBPR,

The ratio of the components were chosen such that the refractive indices were

approximated to be just lower than that of the fibre optic core (see section 5.1.1). The refractive indices and water contents of similar hydrogel polymers which were covalently cross-linked and do not contain reagent are listed below[151].

Table 7.1 The Refractive Index And Water Content Of Polymers Used As A Basis for Sensor Coatings

POLYMER	EWC	REFRACTIVE INDEX
MMA		1.49
60:40 MMA:HEMA	10%	1.482
75:25 MMA:HEMA	5%	1.485
80:20 HEMA:STYRENE	12%	1.485

Since these were very low water content membranes, they were coated thinly on to the fibre in order to allow more rapid ionic transport, through the hydrated matrices. The fibres were coated by allowing a solution of 0.5g of linear polymer in approximately 3ml of methanol, containing between 5 and 20% NVC, to run along the etched length of fibre. This was then UV polymerised for 1 hr to allow the NVC to cross link with itself, entangling the linear polymer chains.

The polymerisation between MMA and AcBPR was carried out, as described in section 2.8. However, it was noticed during the course of this reaction that polymer was dropping out of solution. After the reaction had run for 8hrs, this polymer, together with that still remaining in solution was extracted and the response of the resultant probe was studied. During an attempt to repeat the solution polymerisation in methanol, as an alternative solvent to ethanol, it was discovered

that the reagent would not dissolve in the MMA monomer, until the solvent was added. This suggested that there may be some incompatibility between these two monomers, possibly confirmed by the small yield of polymer obtained from the original reaction. When a small amount of HEMA monomer was added to the MMA and AcBPR monomers, the reagent dissolved and the monomers of HEMA and MMA were miscible. It was found that the minimum amount of HEMA, which could be added to this system was 25% and therefore a linear polymer of 75:25 MMA:HEMA and 5% AcBPR was produced.

7.1.3 Sensor Construction.

After the linear polymers were produced, the coating solutions were made as described in section 6.3.2, with a range of variation in percentage NVC for each type of linear polymer coating solution. The sensors were constructed using the etching and coating techniques described in sections 4.2.3 and 5.1.4. Each sensor response was tested over a range of pH values, provided by citric acid/dipotassium hydrogen phosphate buffer solutions. The results were taken as described in section 6.2.2 and are shown at the back of this chapter.

7.2 DISCUSSION AND ANALYSIS OF RESULTS.

7.2.1 40:60 HEMA:MMA;5% AcBPR

From these recordings, a graph of the last response cycle can be plotted, as in FIG 7.2 and it can be seen that the hydrogel coatings possess a near reversible nature in respect of their changing water contents, with pH. However, the range of response is not very great but it does show that transport, even at these high concentrations of methylmethacrylate, in the linear polymer is occurring. An interesting phenomena appears at a concentration of 13% NVC where there is a sudden increase in the sensitivity of the probe, rather than a gradual increase or

decrease in the range of sensitivity with the addition of NVC to the matrix, as was witnessed earlier in section 6.3. However, since we had obtained some response an attempt was then made to produce a linear polymer of MMA:AcBPR and to study the response characteristics.

7.2.2 25:75 HEMA:MMA;5% AcBPR

The response of the probes made from coating solutions of 75:25 MMA:HEMA and 5% AcBPR are shown in FIG 7.3. It is immediately obvious that a drastic increase in the sensitivity of the membrane with changing pH has resulted from an increase in the MMA content from 60% to 75%. This would not be expected, since the water content should have been lowered and therefore, the output range reduced. The addition of an increasing percentage of NVC to the coating solutions however, does result in a predictable lowering of the output range of the probe. From the graphs of the response in FIG 7.4 plotted from the last pH cycle, in each case, it can be seen that the reversible nature of the hydrogel coating is improving, with increasing NVC content. In fact, at a concentration of 20% NVC we have achieved the construction of a probe which possesses the characteristics of reversibility and virtually immediate response to pH.

7.2.3 80:20 HEMA:MMA;3% AcBPR

The 80:20 HEMA:STYRENE linear polymer was chosen, since we would expect this membrane to have a low water content and the refractive index should be extremely close to MMA, since the addition of styrene will drastically reduce the water content of a HEMA membrane. The results show that there is little response to pH change and this could possibly be due to either a physical characteristic of the membrane or that its refractive index is either too close to or higher than that of poly-MMA.

7.2.4 MMA;5% AcBPR

The results in FIG 7.5 indicate that a small response to pH is evident and that the addition of NVC reduces the sensitivity of the membrane to changing pH. The results indicate that this polymer did allow transport through the matrix and the chart recording is shown in FIG 7.6. After the first cycle through the pH range, the output drifts to a very low value and then shows a small response to pH change over the second cycle

7.3 GENERAL DISCUSSION AND CONCLUSIONS

7.3.1 The Role Of NVC And MMA

NVC has a part to play in the response characteristics. It seems to have a similar effect as adding any hydrophobic monomer such as MMA. ie Improving the symmetry of the response and reducing the fluctuation in the output.

In addition, the shape of the response of changing water content to pH is altered with NVC content (section 6.4.1) and therefore we would expect to see a parallel change in the response curves of corresponding sensor systems.

In the case of these low water content membranes, increasing amounts of NVC reduced the output range of the sensor, as opposed to its addition to HEMA where the output range was increased with increasing NVC content (section 6.3). This could be due to the fact that the increase in optical sensitivity, as the refractive index of the coating approaches that of polymethylmethacrylate, over-rides the effect of the decrease in the sensitivity of the membrane water content to pH change.

With both covalently cross-linked and photo-cross-linked HEMA membranes, the refractive index is much less than that of MMA, due to the respectively higher water content, and thus the latter effect becomes the major contributor to the overall response.

In order to investigate, more clearly, the effects of increasing amounts of both NVC and MMA on the output range, we can approximate the output on the last pH cycle to a straight line, where feasible, and plot the percentage monomer against the gradient of the line. The results of such plots, are shown in FIG 7.7 for

60:40 MMA:HEMA;5% Ac BPR with varying %NVC

75:25 MMA:HEMA;5% Ac BPR with varying %NVC

HEMA;5%NVC with varying %MMA.

Generally, the increase of either MMA or NVC results in a decrease in the range of response, which is dependent on both the sensitivity of the membrane water content to pH change or the optical sensitivity of the overall device. The latter will be of greater importance for low water content systems, especially where MMA is the hydrophobic monomer component, and the former in higher water content systems. Therefore the most sensitive system would be one where the water content is high but the refractive index close to that of MMA. However, the high water content of such a system may decrease the reversibility and stability of the response.

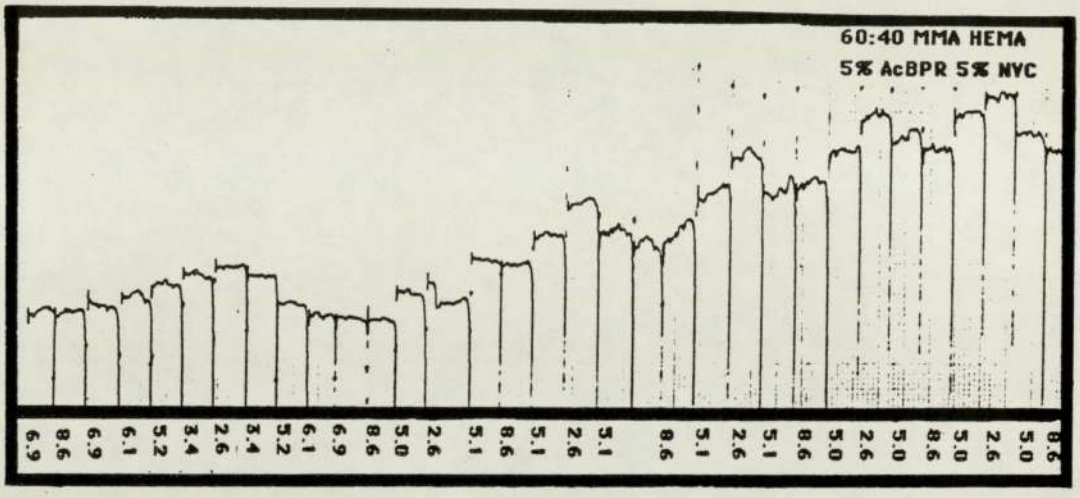
7.3.2 Deviations From Predicted Behaviour

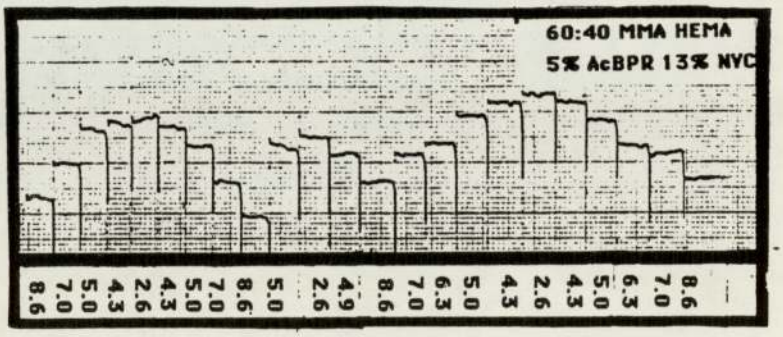
Although generally, we are close to being able to predict the possible effects, on response, of adding hydrophobic monomer to the linear polymers or increasing amounts of NVC to the coating solutions, we have observed deviation from predictability in both the increase in the sensitivity of this response when we increased the MMA content to 75% from 60% and, in the latter, when the NVC content was 13%. The response of the 60:40 (HEMA:MMA);5% reagent shows an optimum response, rather than the general response described earlier.

7.3.3 Physical Characteristics Of Coatings And Possible Relevance To Results

Since the membranes are opaque, they are either macroporous and/or multiphase systems. It is plausible to suggest that where the observed results deviate from a regular or expected pattern that this is due to a fundamental change in the physical characteristics of the membrane. This hypothesis is enforced by both the observations made in section 7.1.2 that the reagent and MMA may not be compatible and also by the fact that NVC dissolves far more readily in a combination of HEMA:MMA than HEMA, suggesting that it may be more compatible with MMA than HEMA: If this is the case, and either a macroporous structure or a block copolymer, containing sections of high water content is present, this would explain why transport is observed at such a low concentration of HEMA, only when reagent is present in the system. When probes were made of linear polymers of HEMA:MMA of varying ratios, these indicated that transport should stop at a concentration of 50:50 HEMA:MMA whereas the "colourimetric" probes are showing transport at water contents less than 12%. The reagent therefore, is playing some part in the sensor response if only altering the transport properties of the membrane. Water content and hence refractive index change is still the major factor contributing to response even at these very low membrane water contents. Evidently, we need to study the physical structure of the coatings in order to draw more definite conclusions in support of the hypothesis that the effects seen are due to either an inhomogeneous or macroporous system.

FIG 7.1 Response to Changing pH: 40:60 HEMA:MMA:5% AcBPR Coatings Containing Varying Percentages Of NVC Chart Recordings.





Output

pH

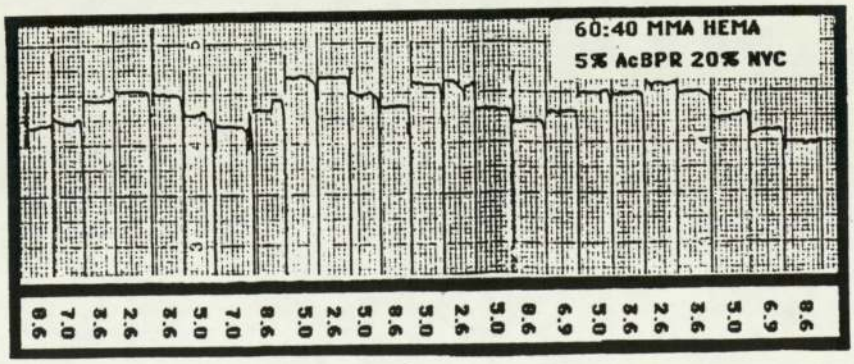


FIG 7.2 Response to Changing pH: 40:60 HEMA:MMA;5% AcBPR

Coatings Containing Varying Percentages Of NVC

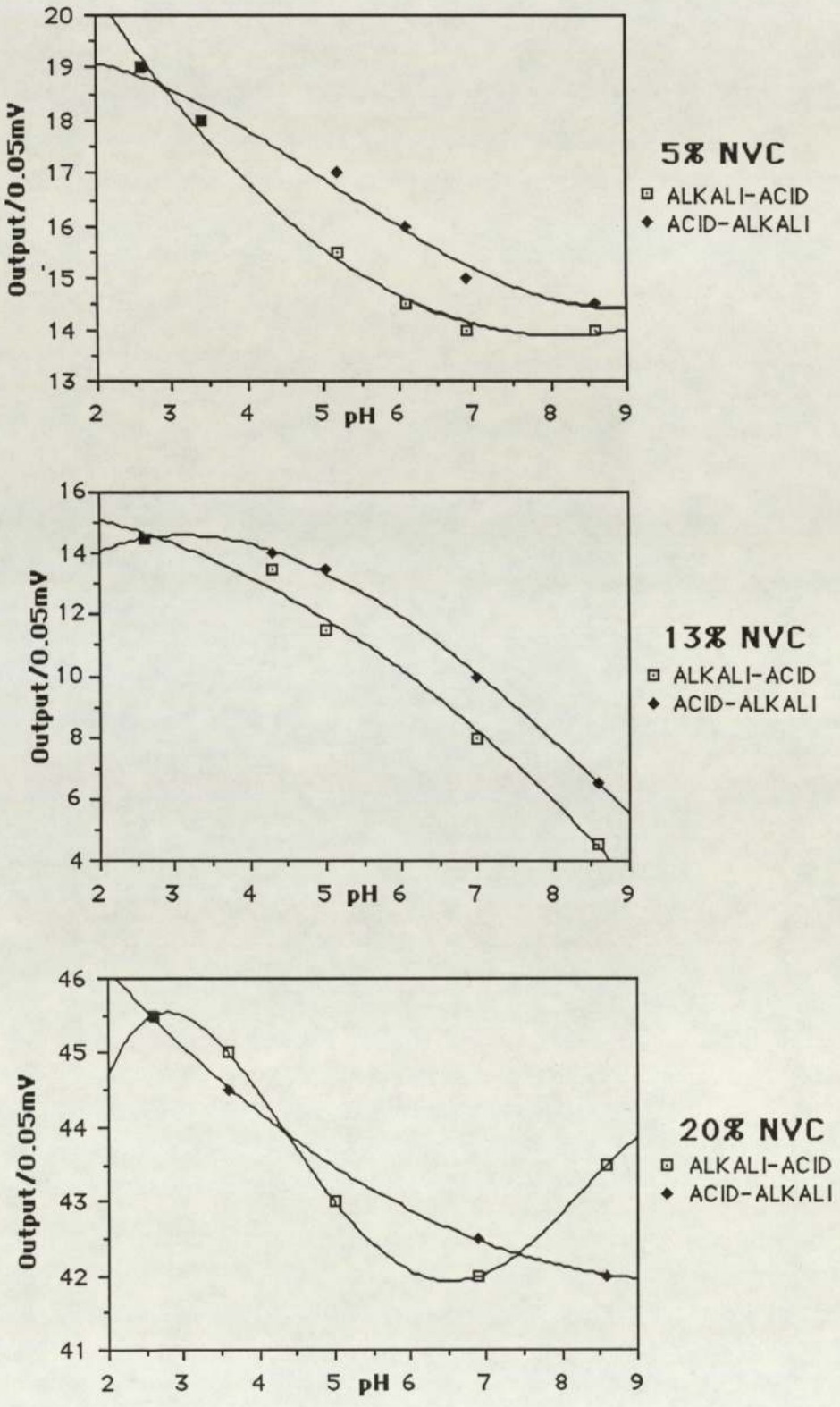


FIG 7.3 Response to Changing pH: 25:75 HEMA:MMA:5% AcBPR Coatings Containing Varying Percentages Of NVC Chart Recordings.

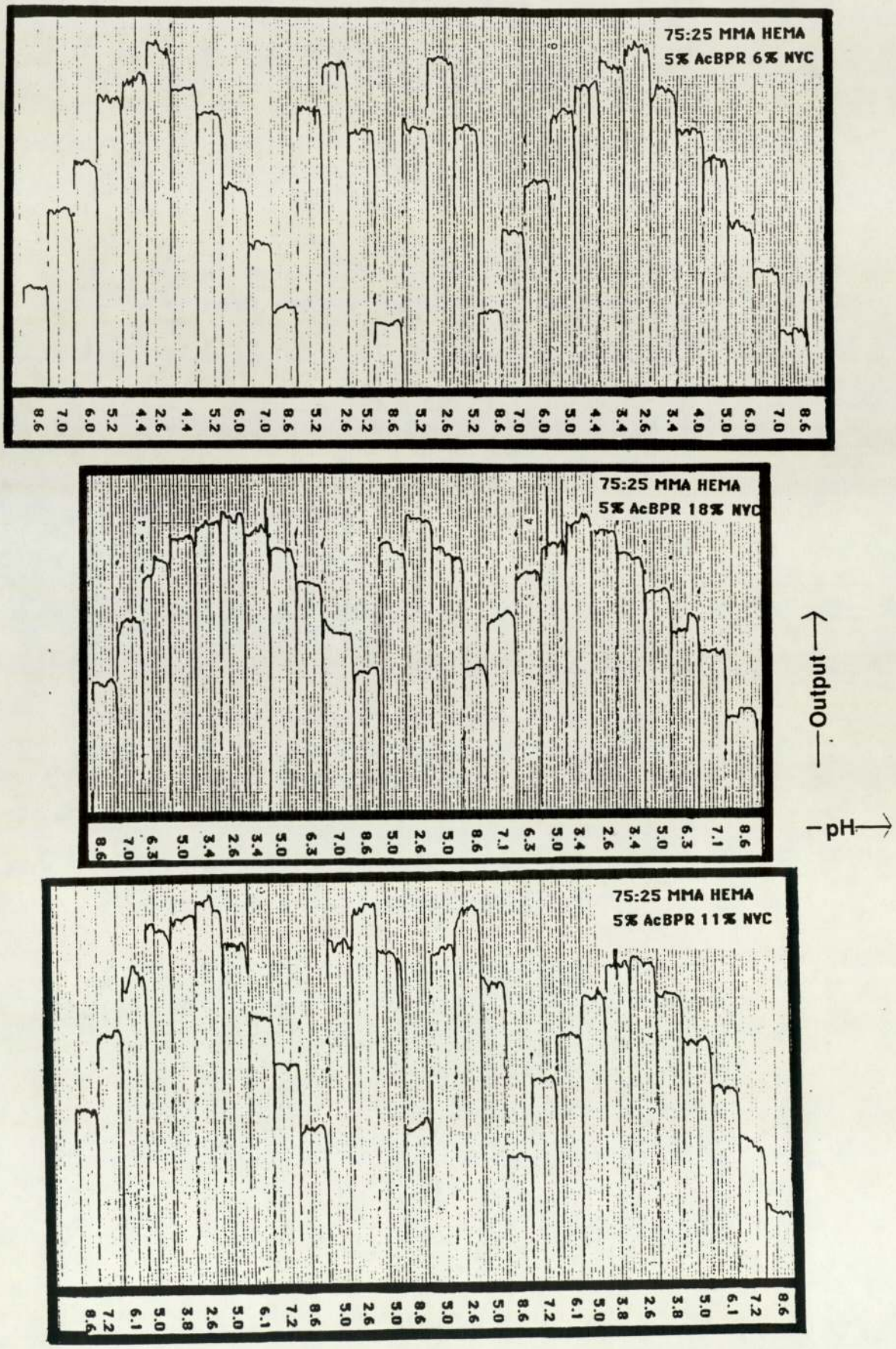


FIG 7.4 Response to Changing pH: 25:75 HEMA:MMA;5% AcBPR

Coatings Containing Varying Percentages Of NVC

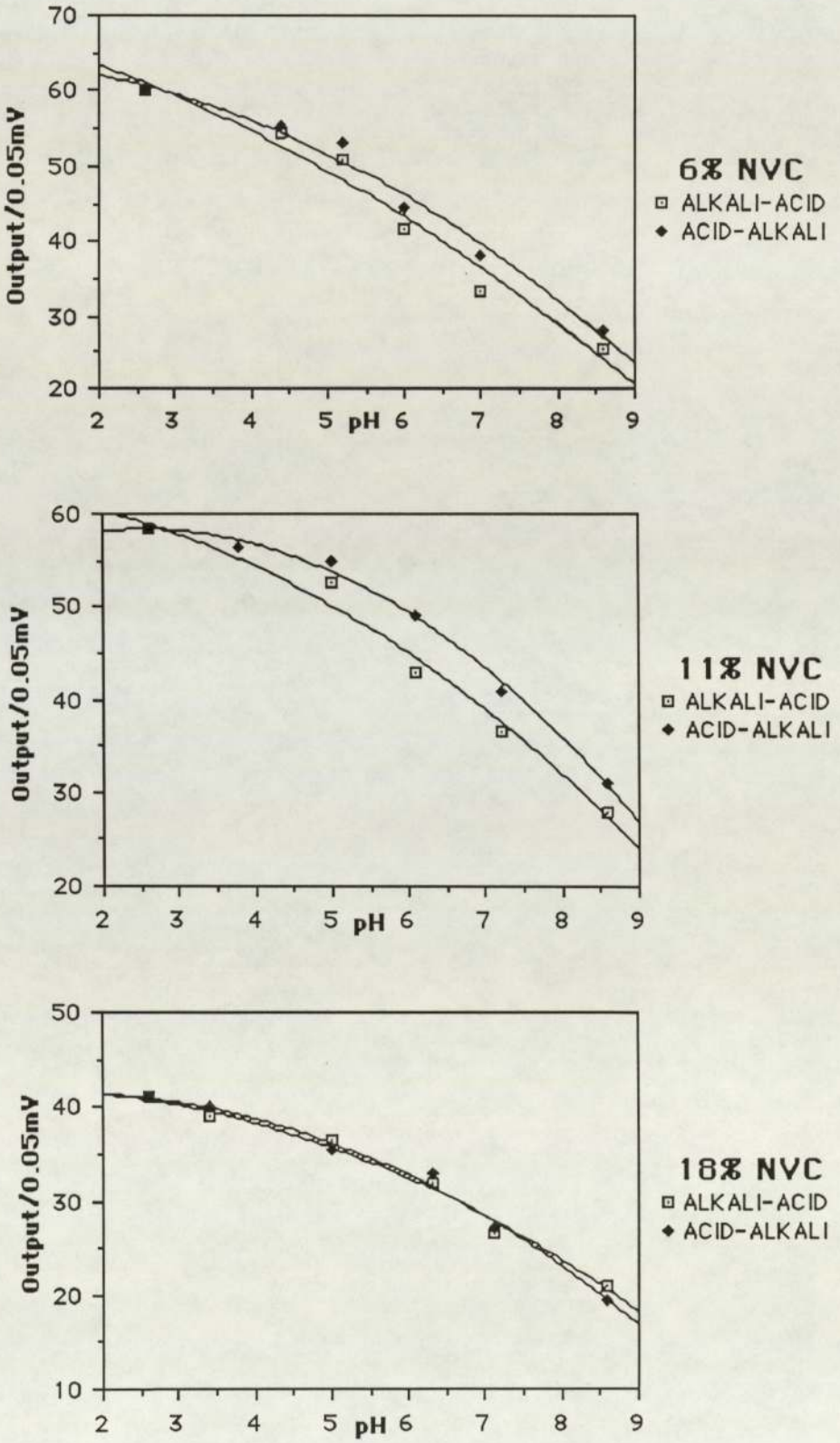
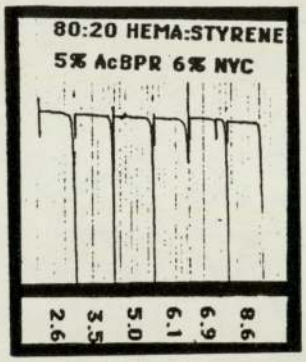
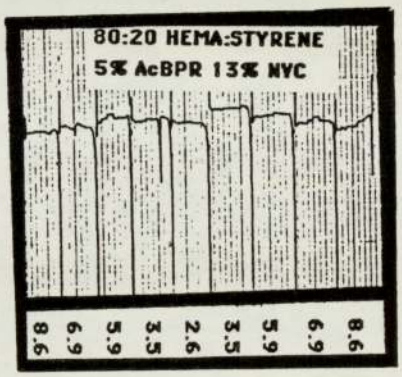
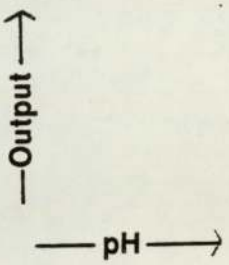


FIG 7.5 Response to Changing pH: 80:20 HEMA:Styrene:3% AcBPR Coatings Containing Varying Percentages Of NVC Chart Recordings.







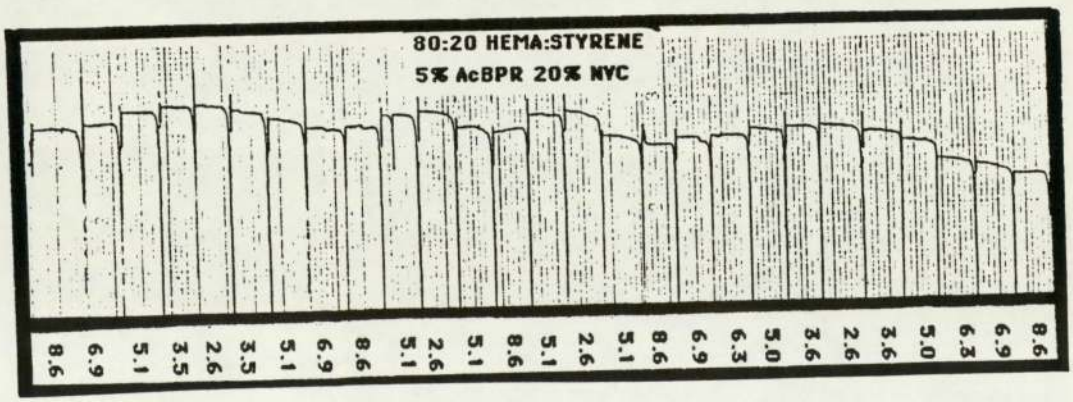


FIG 7.6 Response to Changing pH: MMA:5% AcBPR

Coatings Containing 5% NVC

Chart Recordings.

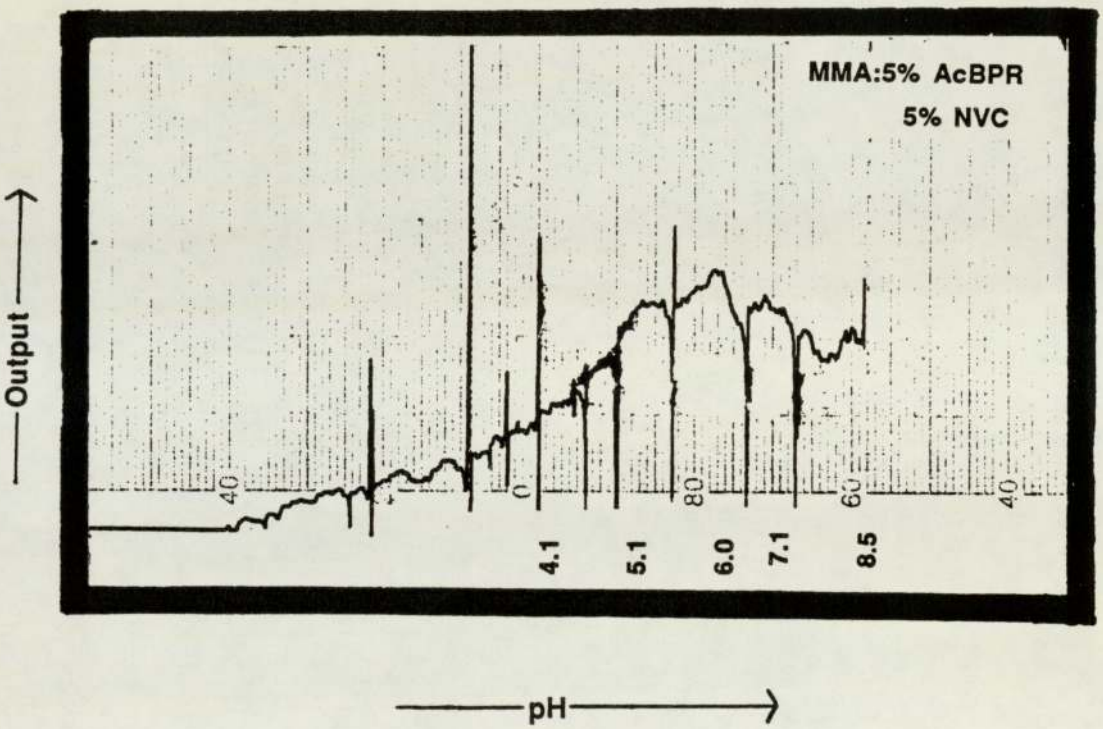


FIG 7.7 Effect On Response Gradient Of Percentage NVC

For MMA:HEMA:AcBPR Coatings

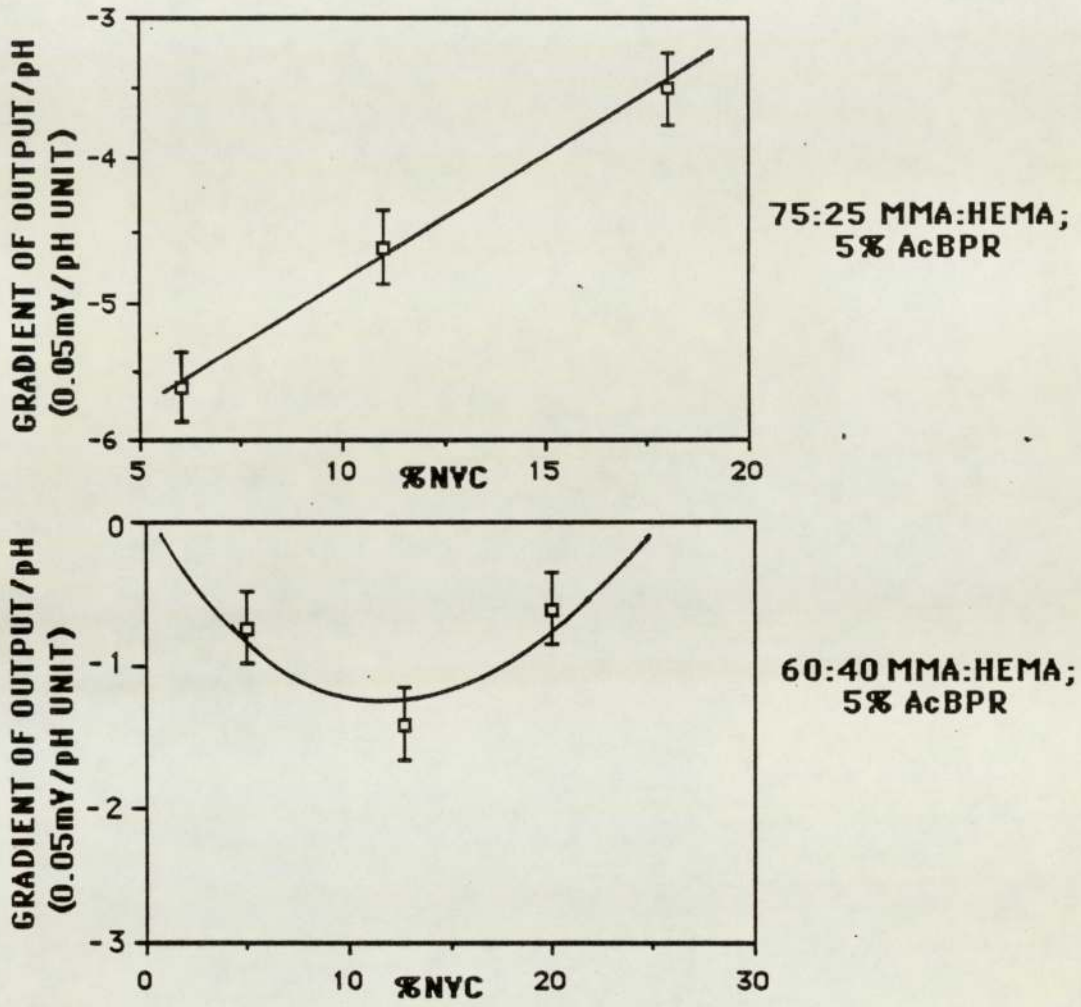
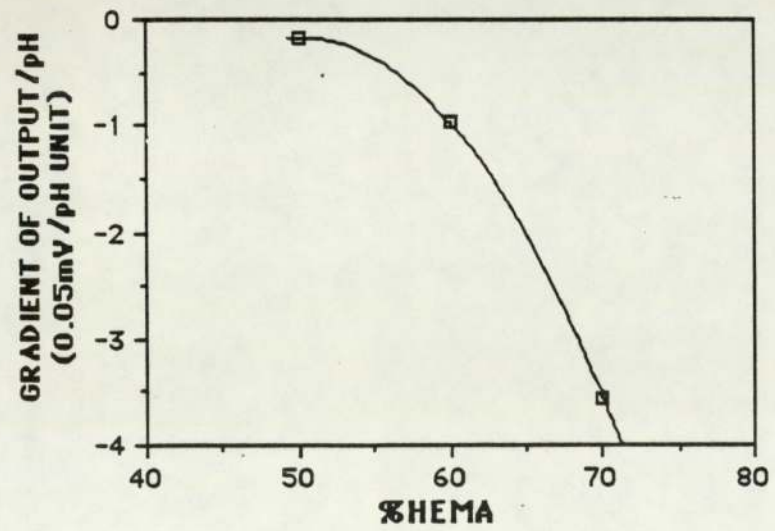


FIG 7.8 Effect On Response Gradient Of Ratio HEMA:MMA:5%NVC



CHAPTER 8

THE PROTOTYPE PROBE: A PHOTOGRAPHIC STUDY OF SENSOR COATINGS.

8.1 PREPARATION OF MEMBRANES FOR MICROSCOPY

In order to study the structure, or the physical nature of the coatings, photographs of films of the coating solutions, cast on a layer of polymethylmethacrylate upon glass microscope slides, and photopolymerised under U.V., were taken, on the Leitz Dialux 20 fluorescence microscope. Since NVC is fluorescent it is possible to view its dispersion within the polymer film. Fluorescence photographs were taken of the coatings where specific areas, rather than a general background fluorescence was apparent.

8.2 DISCUSSION OF PHOTOGRAPHS

In the membranes where no reagent is present, of 50:50 HEMA:MMA 5% NVC FIG 8.1c, large pores are visible and fluorescense increases around edges of these FIG 8.1d. In HEMA 20% and 12% NVC FIG 8.2c,d, undissolved crystals of NVC are clearly visible. Otherwise, the HEMA:MMA;NVC and the HEMA;NVC membranes tend to be fairly homogeneous in nature FIGs 8.1-8.2.

The photographs show that the membranes which contain reagent (FIGs 8.3-8.5) are generally inhomogeneous and that the polymer system is multi-phased. This effect is most apparent in the 75:25 MMA:HEMA; 5% AcBPR systems where a "crazed" or "coring" effect is present FIG 8.4. These networks generally seem to be macroporous with holes of the order of 0.02mm diameter, in the case of 80:20 HEMA:STYRENE;3% AcBPR (FIGs 8.5) and 40:60 HEMA:MMA;5% AcBPR with 5 and 20% NVC, the pore size increases with NVC content. The fluorescence photograph shows an increase in fluorescence around the pores, but this could possibly be an edge effect (FIG 8.3e).

For 40:60 and 25:75 HEMA:MMA;5% AcBPR, the islands appear to

increase in size with increasing NVC and so also, does the definition of the pores. The fluorescence picture shows that within these islands, surrounded by reagent, there is an inhomogeneous mixture of NVC and at 13% NVC in the case of 40:60 HEMA:MMA there are undissolved crystals of NVC FIG 8.3d. This may in some way explain why there is an optimum value of percentage NVC, rather than a general decrease in response range with increasing NVC content in FIG 7.7.

The inhomogeneity and macroporosity of the low water content membranes, containing AcBPR might explain the rapid response of the membranes to changing pH. The lack of response of the 80:20 HEMA:MMA;3% AcBPR, may well be due to its optical properties rather than its physical properties: The refractive index may be slightly higher than, or very close to, that of MMA and thus internal reflection, along the fibre, either does not occur or the range of response is minimal.

8.3 CONCLUSIONS

The inhomogeneity and macroporosity of the membrane is obviously an important factor in determining its rapid and reversible response characteristics. The possible presence of a higher water content phase may account for seeing the effects of changing refractive index at such apparently low water contents. However an increase in optical sensitivity as the refractive index of the coating approaches that of poly-MMA may also be the cause.

At this point we have established that the changing refractive index of these low water content hydrogel coatings is as a result of their inhomogeneity and macroporosity. In this respect, since a macroporous system should allow the transport of large species into the membrane, we may be able to use this system in the detection of larger organic or ionic species. Thus the following chapter studies the prototype sensor response in a range of environments.

FIGS 8.1 a HEMA:MMA COATINGS CONTAINING 5% NVC

SCALE 1cm = 20μm

FIG 8.1a 70:30 HEMA:MMA:5%NVC

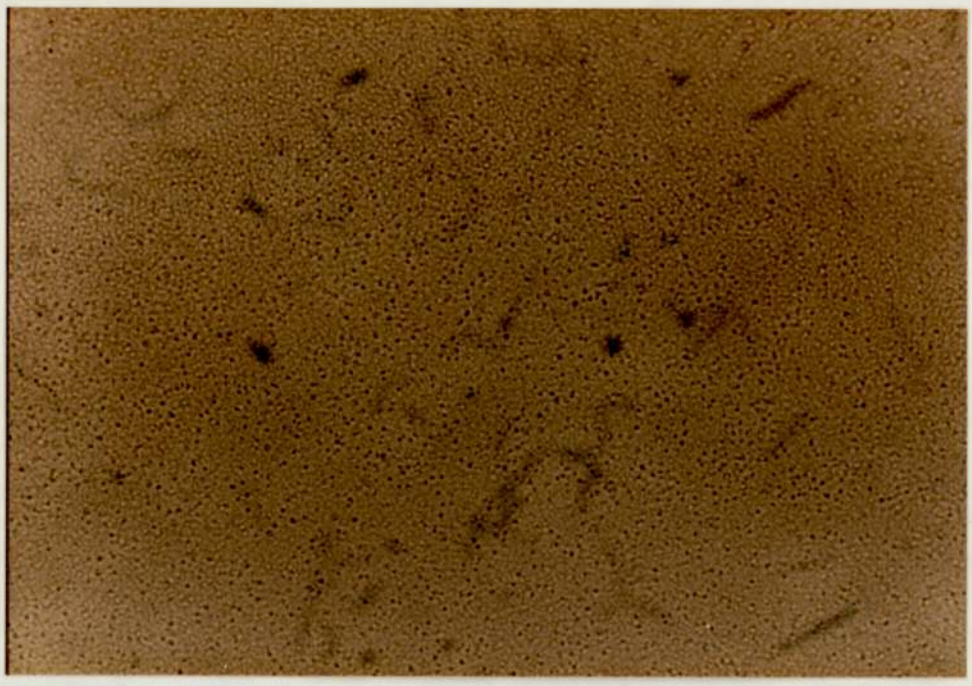


FIG 8.1b 60:40 HEMA:MMA:5% NVC

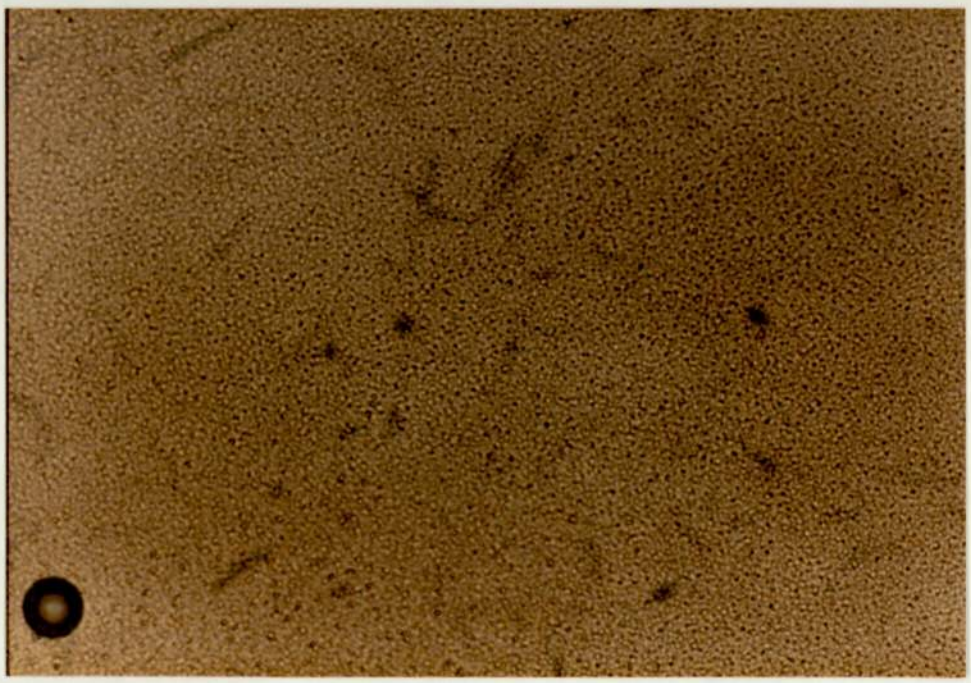


FIG 8.1c 50:50 HEMA:MMA;5%NVC

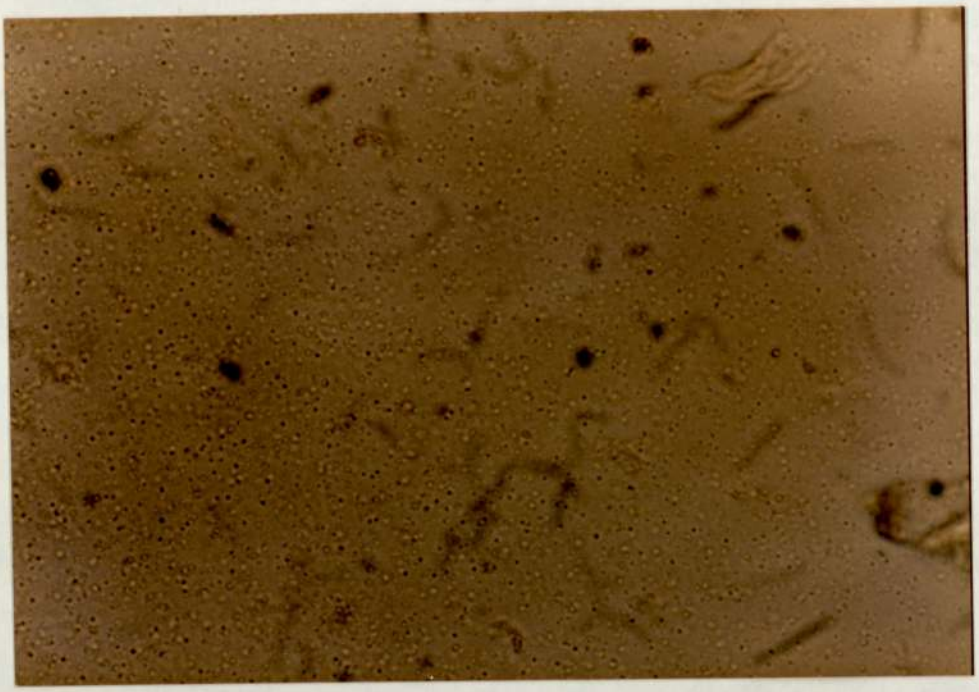


FIG 8.1d 50:50 HEMA:MMA;5% NVC
(FLUORESCENCE)

FIGS 8.2 a HEMA COATINGS CONTAINING VARYING AMOUNTS OF NVC

SCALE 1cm = 20μm

FIG 8.2a HEMA 5%NVC

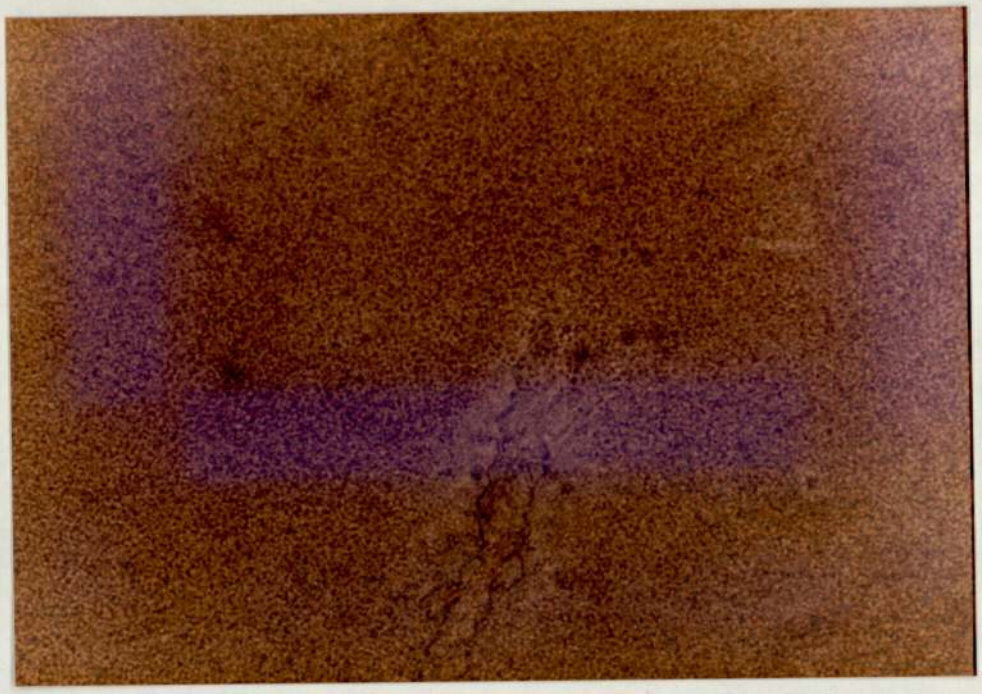


FIG 8.2b HEMA 12% NVC

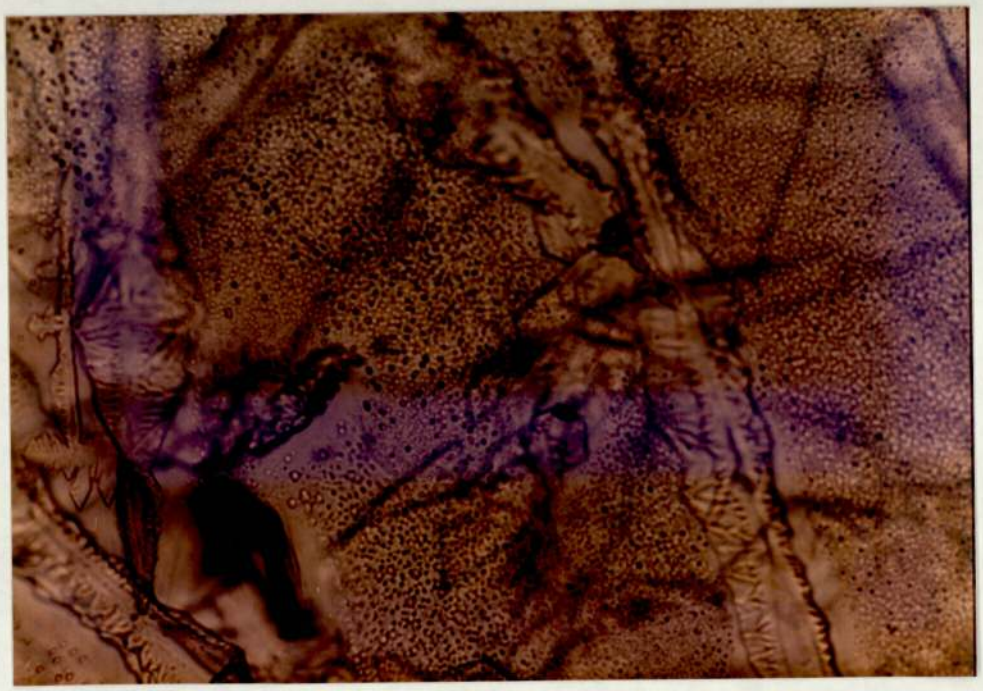


FIG 8.2c HEMA 20% NVC

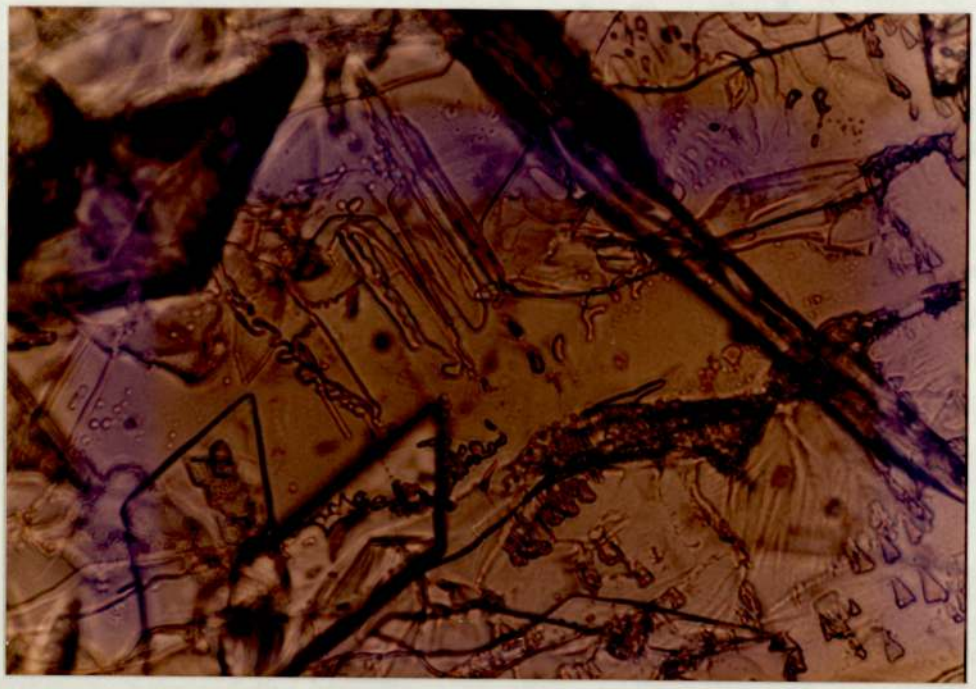


FIG 8.2d HEMA 20% NVC
(FLUORESCENCE)

FIGS 8.3a 40:60 HEMA:MMA;5% AcBPR COATINGS WITH VARYING AMOUNTS OF NVC

SCALE 1cm = 20μm

FIG 8.3a 40:60 HEMA:MMA;5% AcBPR;5% NVC

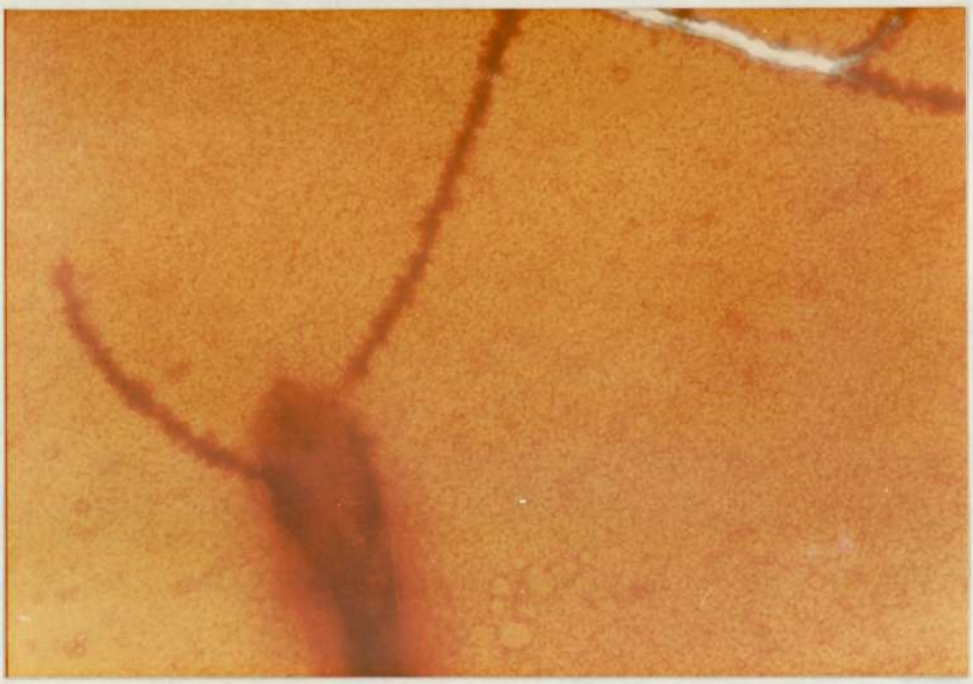


FIG 8.3b 40:60 HEMA:MMA;5% AcBPR;13% NVC

FIG 8.3c 40:60 HEMA:MMA;5% AcBPR;20% NVC

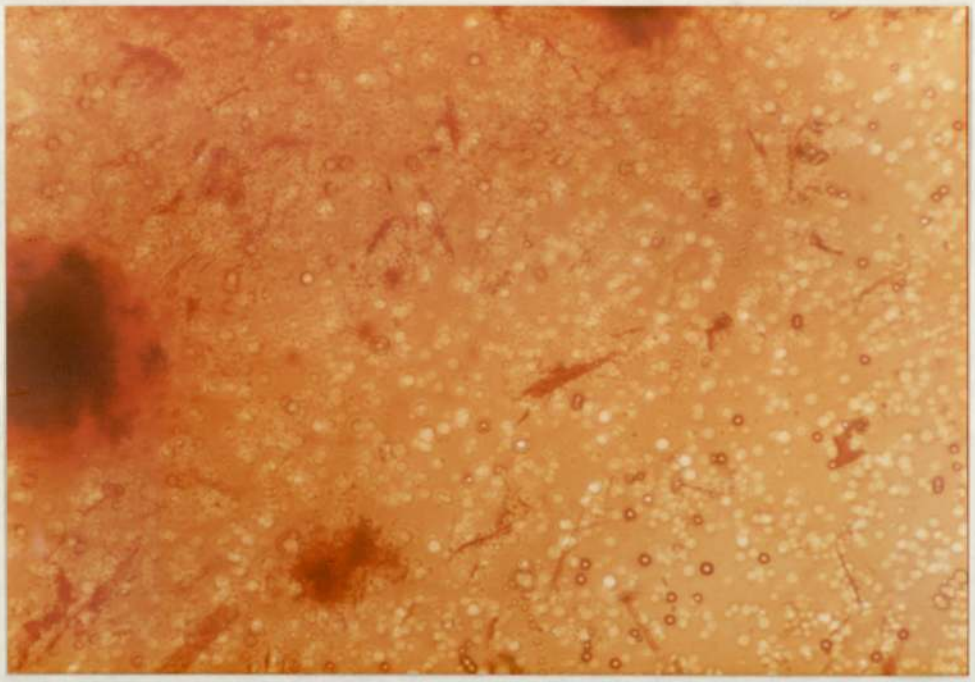


FIG 8.3d 40:60 HEMA:MMA;5% AcBPR;13% NVC
(FLUORESCENCE)

FIG 8.3e 40:60 HEMA:MMA;5% AcBPR;20% NVC (FLUORESCENCE)

a

FIGS 8.4 25:75 HEMA:MMA;5% AcBPR COATINGS CONTAINING VARYING AMOUNTS OF NVC

SCALE 1cm = 35μm

FIG 8.4a 25:75 HEMA:MMA;5% AcBPR;6% NVC

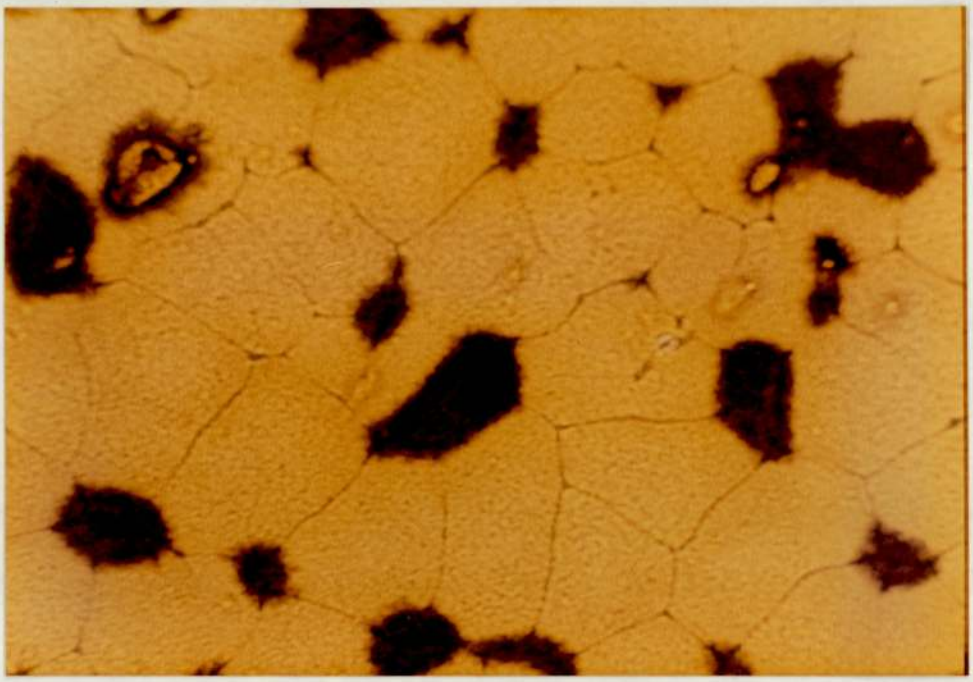


FIG 8.4b 25:75 HEMA:MMA;5%AcBPR;11% NVC

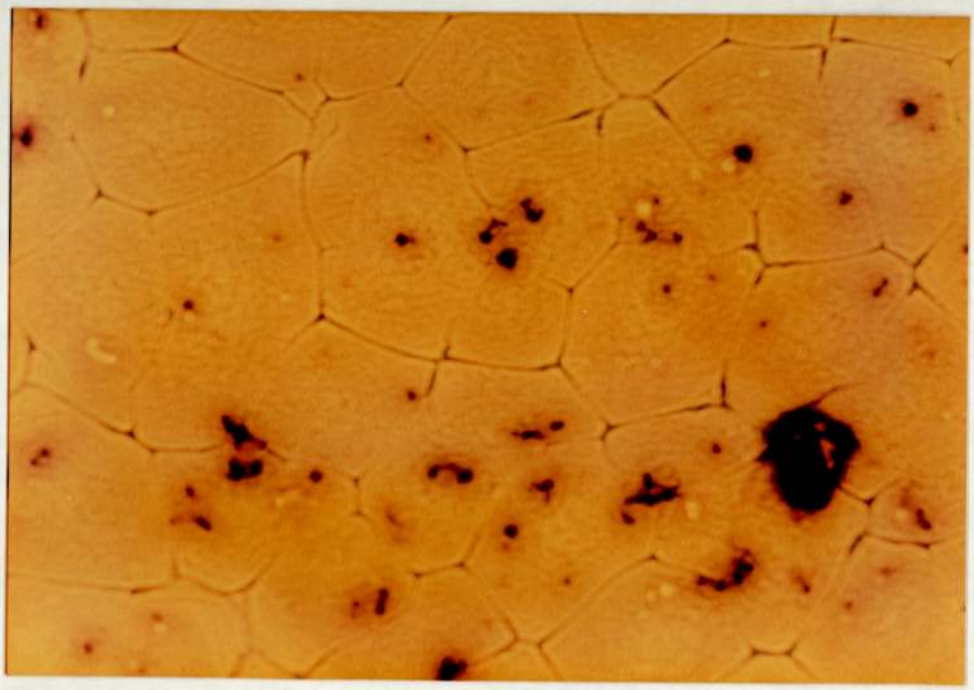


FIG 8.4c 25:75 HEMA:MMA;5% AcBPR;18% NVC

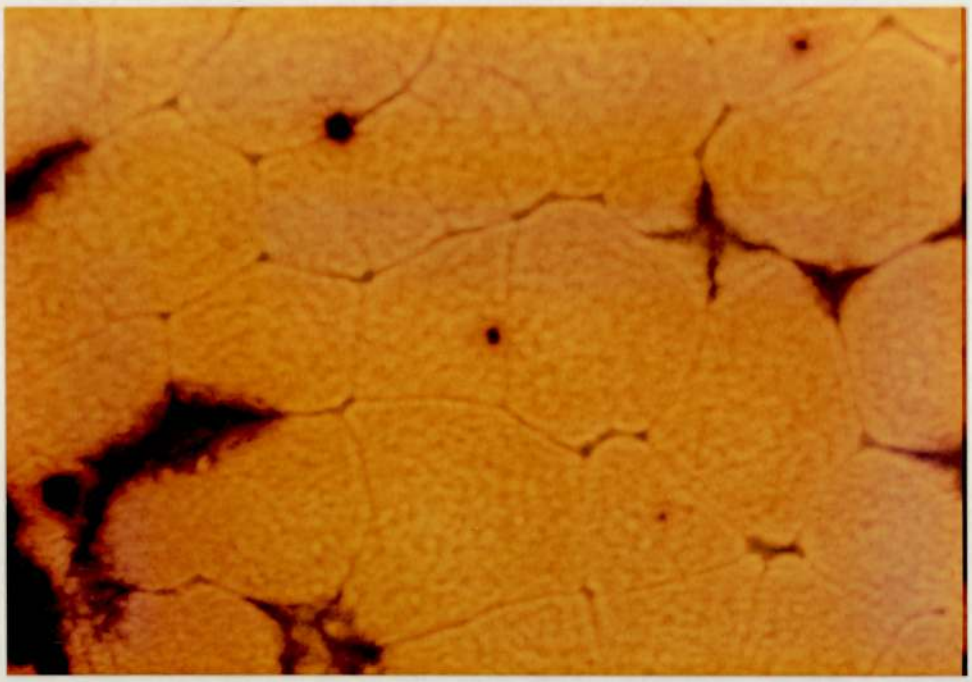


FIG 8.4d 25:75 HEMA:MMA;5% AcBPR;18% NVC
(FLUORESCENCE)

FIGS 8.5a 80:20 HEMA:STYRENE;5% AcBPR COATINGS CONTAINING VARYING AMOUNTS OF NVC

SCALE 1cm = 20μm

FIG 8.5a 80:20 HEMA:STYRENE;5%AcBPR;6% NVC

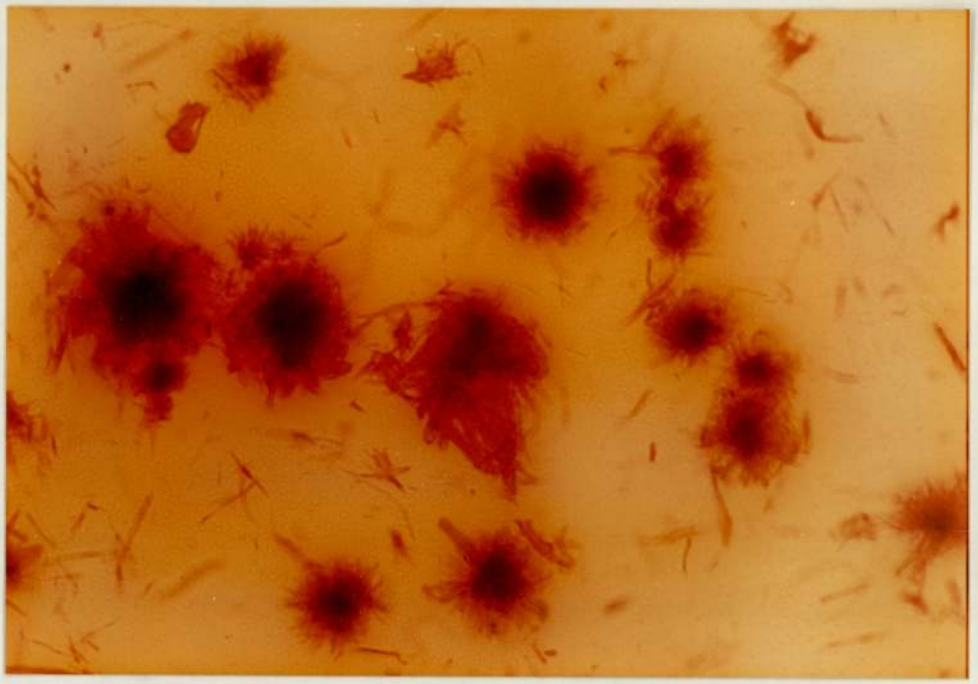


FIG8.5b 80:20 HEMA:STYRENE;5% AcBPR;12% NVC

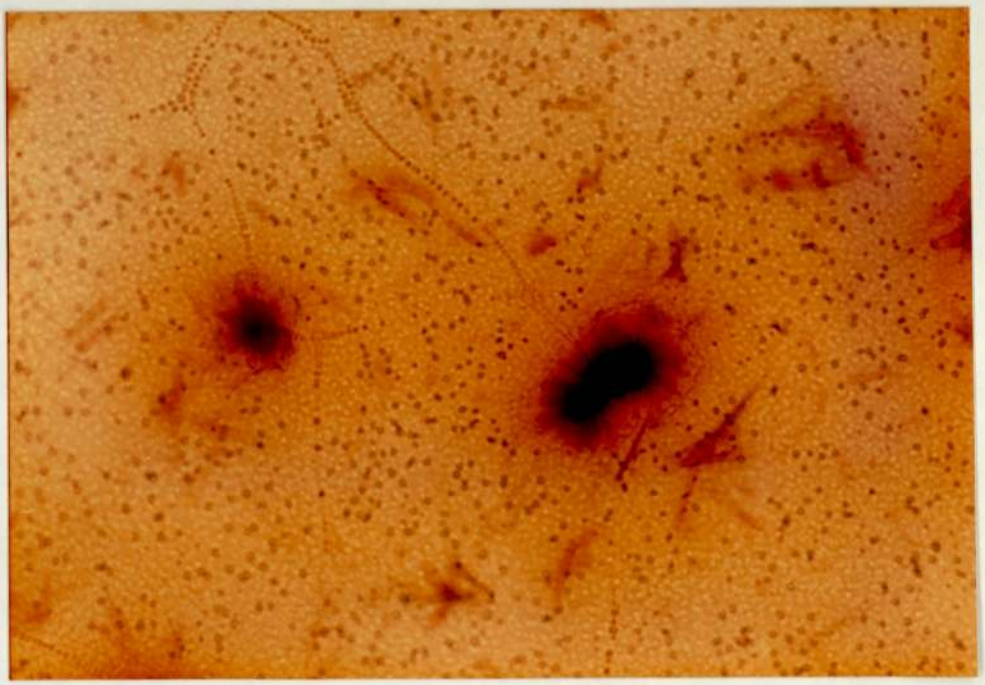
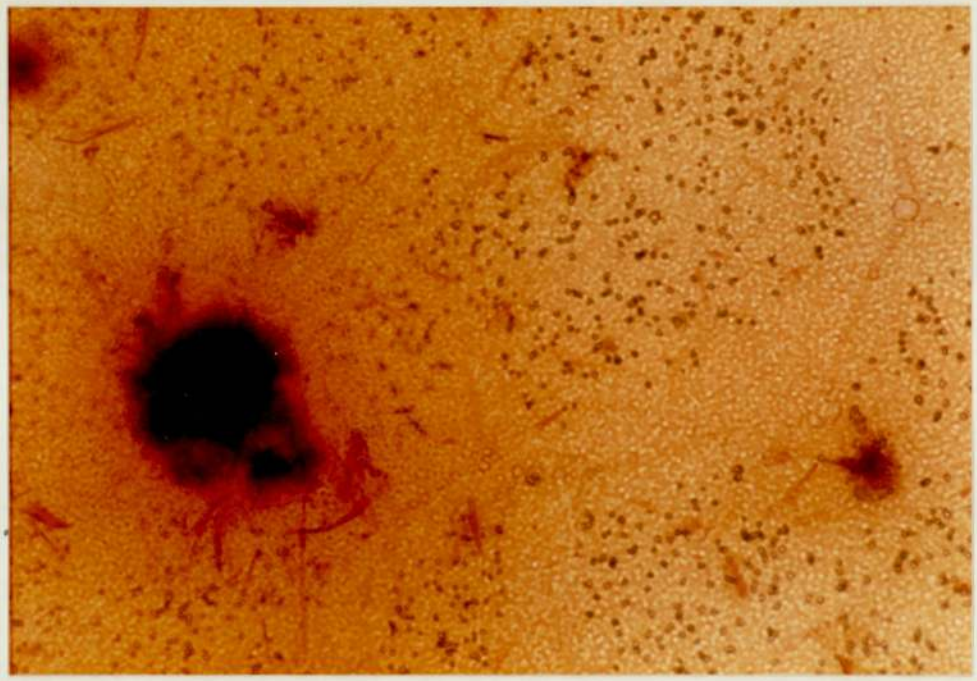


FIG8.5c 80:20 HEMA:STYRENE;5% AcBPR;20% NVC



CHAPTER 9

THE PROTOTYPE PROBE: RESPONSE TO VARIOUS CHANGING ENVIRONMENTS.

9.1 THE RESPONSE OF THE SENSOR TO CHANGING ACTIVITY OF VARIOUS CATIONS AND ANIONS

9.1.1 Transport Of Ions Through The Hydrogel Membrane

The macroporosity of the hydrogel matrices studied in chapter 7 indicates that these low water content systems might lend themselves to the detection of tonicity changes, via refractive index, in alternative environments. Since the coating which showed the most promise in terms of its reversible and rapid response was 75:25 MMA:HEMA;5% AcBPR;20%NVC, this was the coating chosen for further studies.

In order to study the effects of ionic species on the reagent modified hydrogel coatings, it is necessary to study the effects of varying both cations and anions, on the sensor response. If the sensor is responding to a change in the water content of the coating and therefore refractive index, the water structuring ability of the ions will play a part in determining the sensor output. In addition, the pH of the test solution will also have an effect. In fact, this response may well override any effects brought about by the water structuring ability of the constituent ions of the test solution and it is therefore important to note the pH changes, with changing salt concentration.

9.1.2 Experimental

In order to test the response of the sensor to different cations and anions, standard solutions of K_2SO_4, K_2HPO_4, KOH, KSCN, KCl, HCl, NaCl, $CaCl_2$, were made and each was successively diluted to provide a range of concentrations. The sensor was constructed as in section 7.1.3 with the coating solution containing the linear polymer 75:25 MMA:HEMA ;5% AcBPR; 20% NVC. The pH of each solution was

measured before testing the response and the output was plotted against both pH and pX, where X is the cation of the salt. The results of the plots are shown in FIGS 9.1 and 9.2.

9.1.3 Discussion Of Results

As the results obtained do not directly reflect a response to changing pH, these can be considered to be distinct responses to a changing cation or anion in the test solution. Neither the direction or magnitude of the response is dependent on pH change. All of the potassium salts show an increase in response with increasing potassium activity, as is the case for sodium chloride. However, the responses for calcium chloride and hydrochloric acid, show the reverse effect.

In order to draw conclusions from the results individually, we must first decide how to interpret them generally. Therefore, the major implications of the direction of the response (a positive or negative gradient) are indicated below.

1) A decrease in output corresponds to an increase in refractive index. The refractive index of the hydrogel must therefore be approaching that of poly-MMA, i.e the membrane is dehydrating.

2) A decrease in response with increasing concentration of the cation, i.e a decrease in px^+ indicates that the salt has a hydrating effect on the membrane. This corresponds to a positive slope.

3) A negative slope therefore represents a salt which has a dehydrating effect with increased concentration.

FIG 9.1 RESPONSE OF SENSOR TO A SERIES OF CHLORIDES.

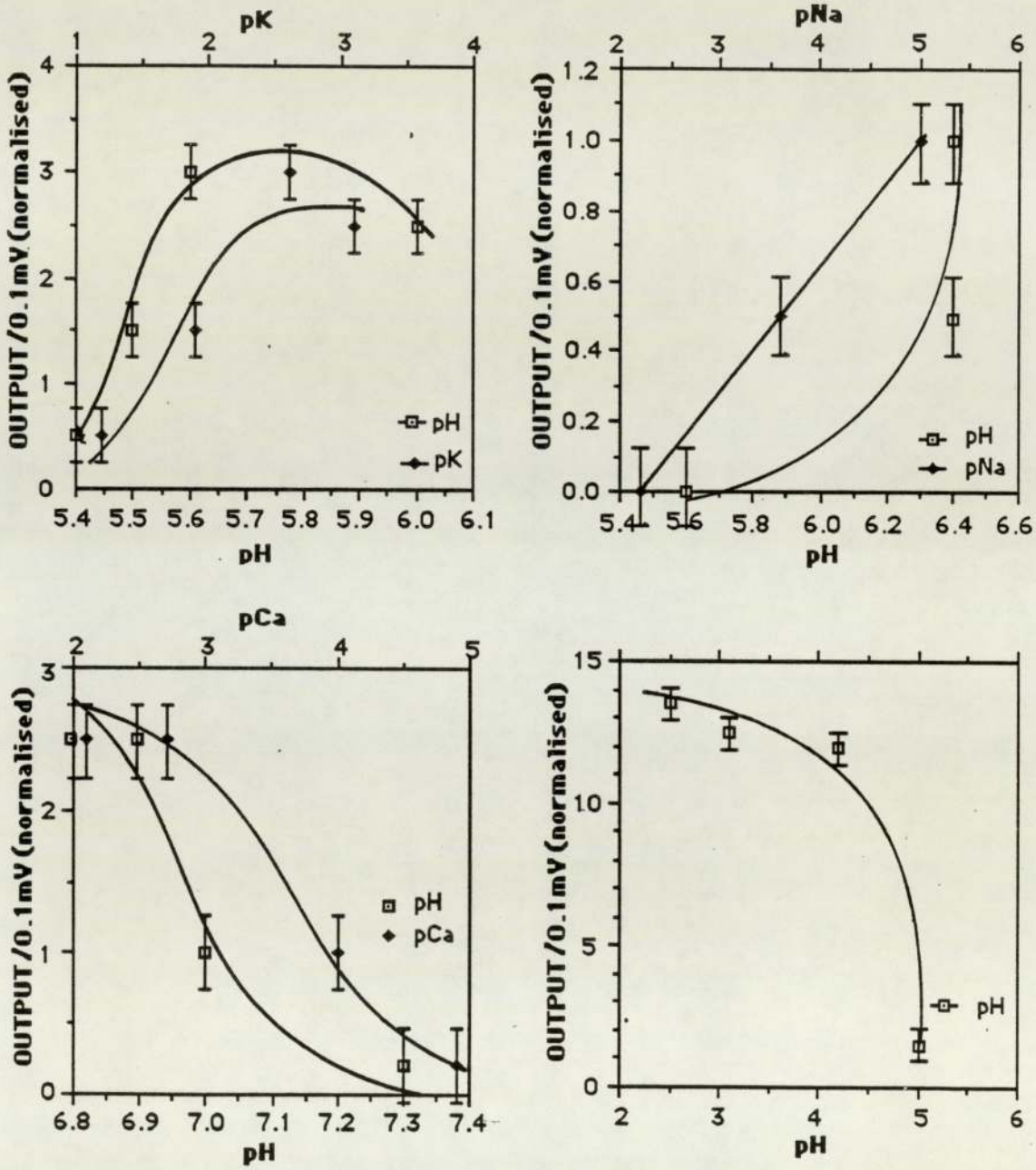
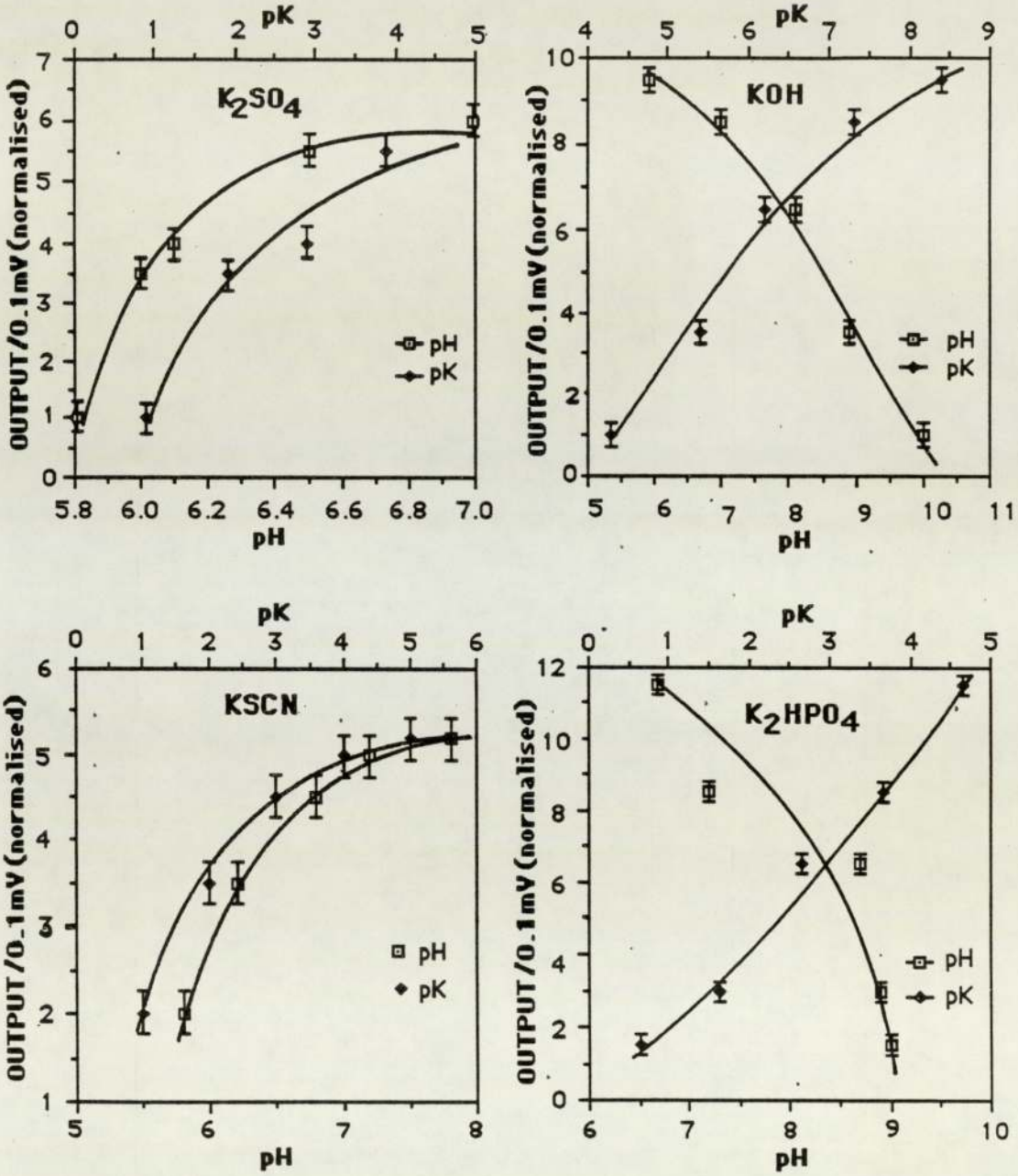


FIG 9.2 RESPONSE OF SENSOR TO A SERIES OF POTASSIUM SALTS



Next, considering the series of chlorides studied and represented by FIG 9.1: the general observations made are:

1) Sodium and potassium chloride both have a similar effect in that an increased concentration results in hydration of the membrane.

2) The opposite however, is true for calcium chloride and hydrochloric acid which have a dehydrating effect.

3) The responses which are consistent with previous observations from earlier experiments with buffers are those of calcium chloride and hydrochloric acid, ie. An increase in output with decreasing pH.

4) If we look at the approximate gradients of the outputs, which are:

KCl	10mV/pH
NaCl	1mV/pH
$CaCl_2$	-4mV/pH
HCl	-8mV/pH

In terms of increasing water structuring ability, we would place these ions in the order: K^+, Na^+, Ca^{2+}, and H^+ being a special case, since the presence of H^+ in water results in the formation of a hydronium ion (H_3O^+) and the transport mechanism through the membrane is therefore different. If we consider the gradients in terms of their positive magnitude, then there is some relation between the results and the water structuring ability of the cations.

Considering the responses obtained from the four potassium salts tested:

1) The results for potassium hydroxide and dipotassium hydrogen phosphate indicate that there is a consistency in the direction of response with pH change and this is again comparable with the changes witnessed with both

hydrochloric acid and buffer solutions (i.e. an increase in response with a decrease in pH and thus an increasing dehydration of the membrane). However this is not the case for potassium sulphate and potassium thiocyanate.

2) The approximate gradients of the responses were calculated to be:

K_2SO_4	7mV/pH
KOH	2mV/pH
KSCN	2mV/pH
K_2HPO_4	2mV/pH

Although these are of approximately the same gradient, the largest effect can be seen from the sulphate anion, which is known to have a high water structuring and therefore a dehydrating effect on the membrane.

9.1.4 Conclusions

It is therefore reasonable to conclude that there is a combination of dehydrating and hydrating effects which contribute to the overall response obtained for each individual salt. However, the response obtained is unique for each salt and can therefore be assumed to be a legitimate response to the species present.

In order to study the transport mechanisms more thoroughly for these salts, we need a means of obtaining results which are directly related to the presence of ions in the matrix and not on its changing optical characteristics. The coated wire electrode was considered to be a viable technique for studying both this phenomenon and the effect that the presence of the reagent has on transport properties in the matrix.

9.2 COATED WIRE ELECTRODE STUDIES.

9.2.1 Introduction

From the work carried out on the optical fibre sensor developed in this

project, it has become apparent that the response of the sensor is based on a refractive index change in the hydrogel coating. However, the question arises as to why potassium and sodium chloride behave differently to calcium chloride and hydrochloric acid in this respect. In addition, it would be a significant step in starting to understand the operating principals of this device, if we could study the diffusion of ions into the sensor membrane, with disregard to the effect that their presence has on the optical characteristics of the membrane as a sensor coating.

The coated wire electrode provides a means of testing the change in potential across a membrane/solution interface, the construction and theory of which are described in both chapters one and two. Since the reagent is a charged species possessing both hydroxy and sulphonic acid groups, the protonation and deprotonation of, or the interaction of cations with, these groups would result in a change in EMF across the membrane. A study of a series of chlorides on the coated wire may thus provide us with further insight, regarding the dehydrating and hydrating effects imposed on the hydrogel coating by different ions and thus on the optical sensor response.

9.2.2 Construction Of The CWE

A coated wire electrode was constructed using conventional techniques, described in chapter two and the coating solution of 75:25 MMA:HEMA;5% AcBPR;20% NVC, which was then photopolymerised under UV for two hours. Initial attempts to obtain a response to pH, from buffer solutions of dipotassium hydrogen phosphate and citric acid, proved to be impractical since an equilibrium response to each test solution could not be obtained after a period of 3-4 hours and in fact, the response time of these CWE's appeared to be 12 hours or more.

Therefore, the tip of a clean platinum wire was covered with a bead of

epoxy resin, in order to seal off the bare end. The wire and dry epoxy bead were then coated with a thin layer of solution, which was slightly thicker than that used on the fibre optic sensors (<1μ), and left under UV for 1 hour to polymerise.

9.2.3 Test Solutions And Results Plotted

A range of test solutions were made up for $CaCl_2$ KCl, NaCl, and HCl, with concentrations ranging between 10^{-1} and 10^{-5}M. The responses of the electrode to these solutions was obtained, taking the least dilute sample first for each range of salt solutions, and was plotted against time. The equilibrium response in mV was then plotted against -log[cation], for each set of test solutions as in FIGS 9.3-9.7. It is important to note that the order in which these solutions were tested is as stated above, i.e $CaCl_2$, KCl, NaCl, HCl.

FIG 9.3 CWE RESPONSE: Output Against Time For Changing Concentration Of Calcium Chloride

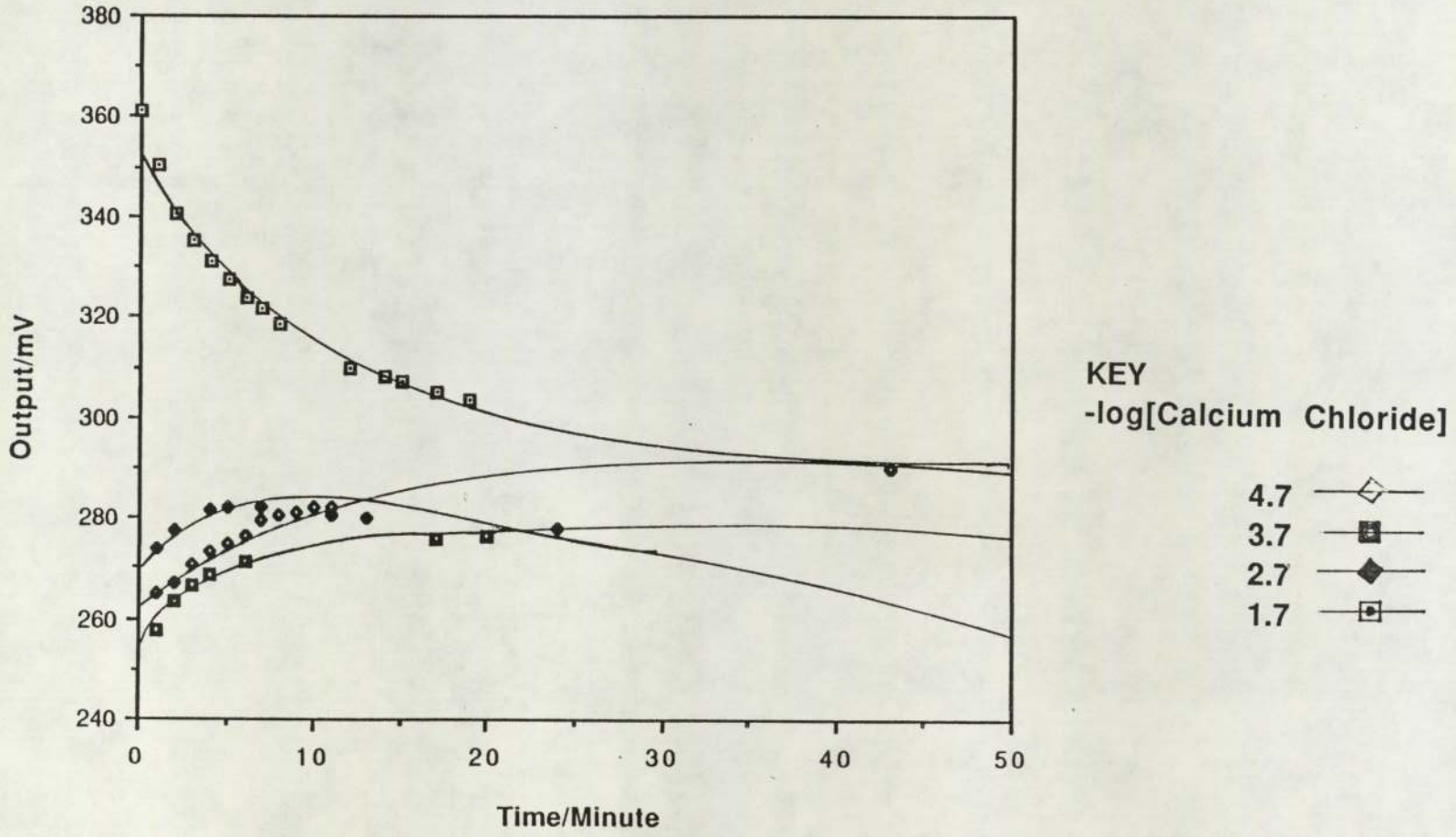


FIG 9.4 CWE RESPONSE: Output Against Time For Changing Concentration Of Potassium Chloride

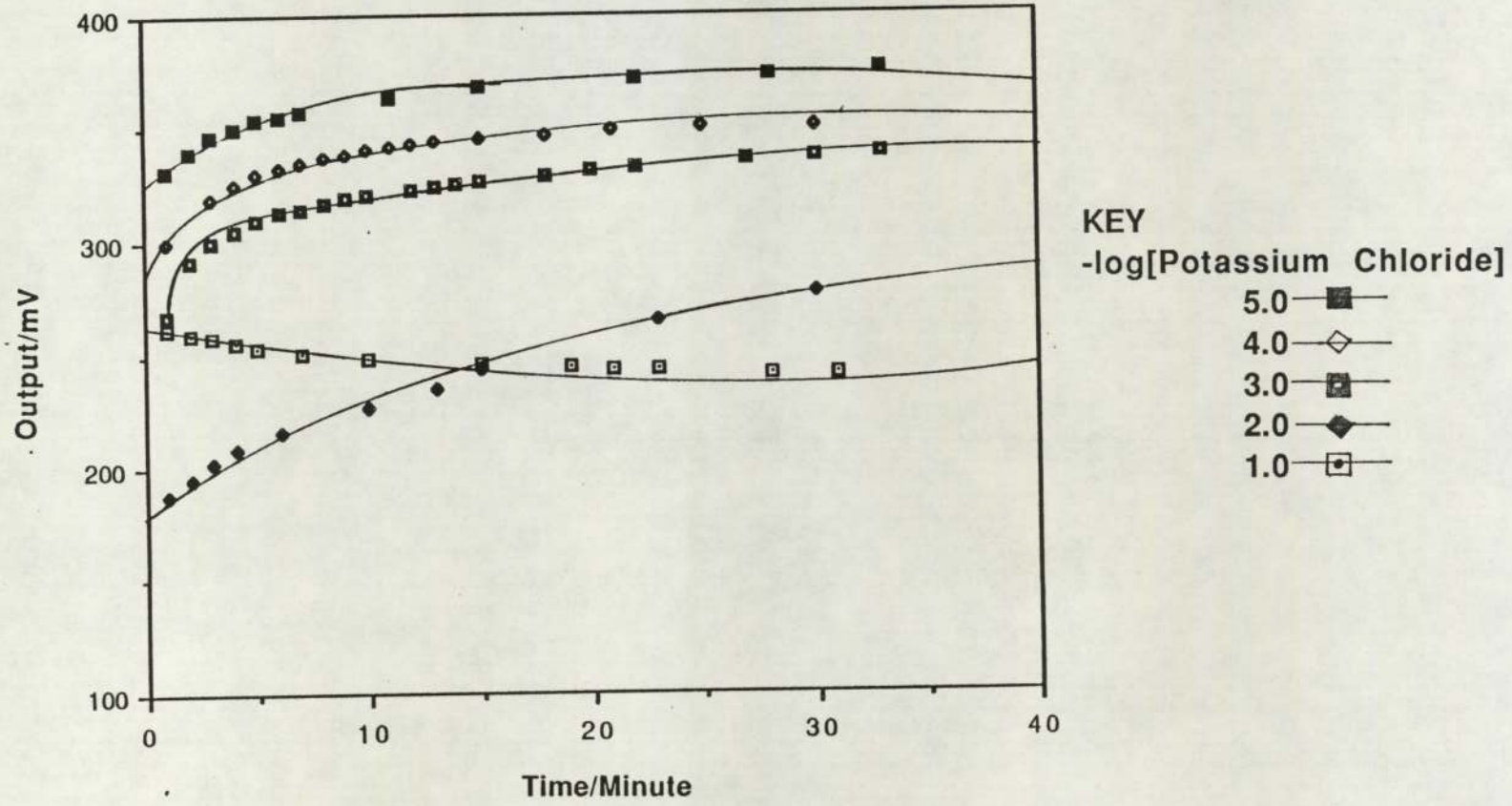


FIG 9.5 CWE RESPONSE: Output Against Time For Changing Concentration Of Sodium Chloride

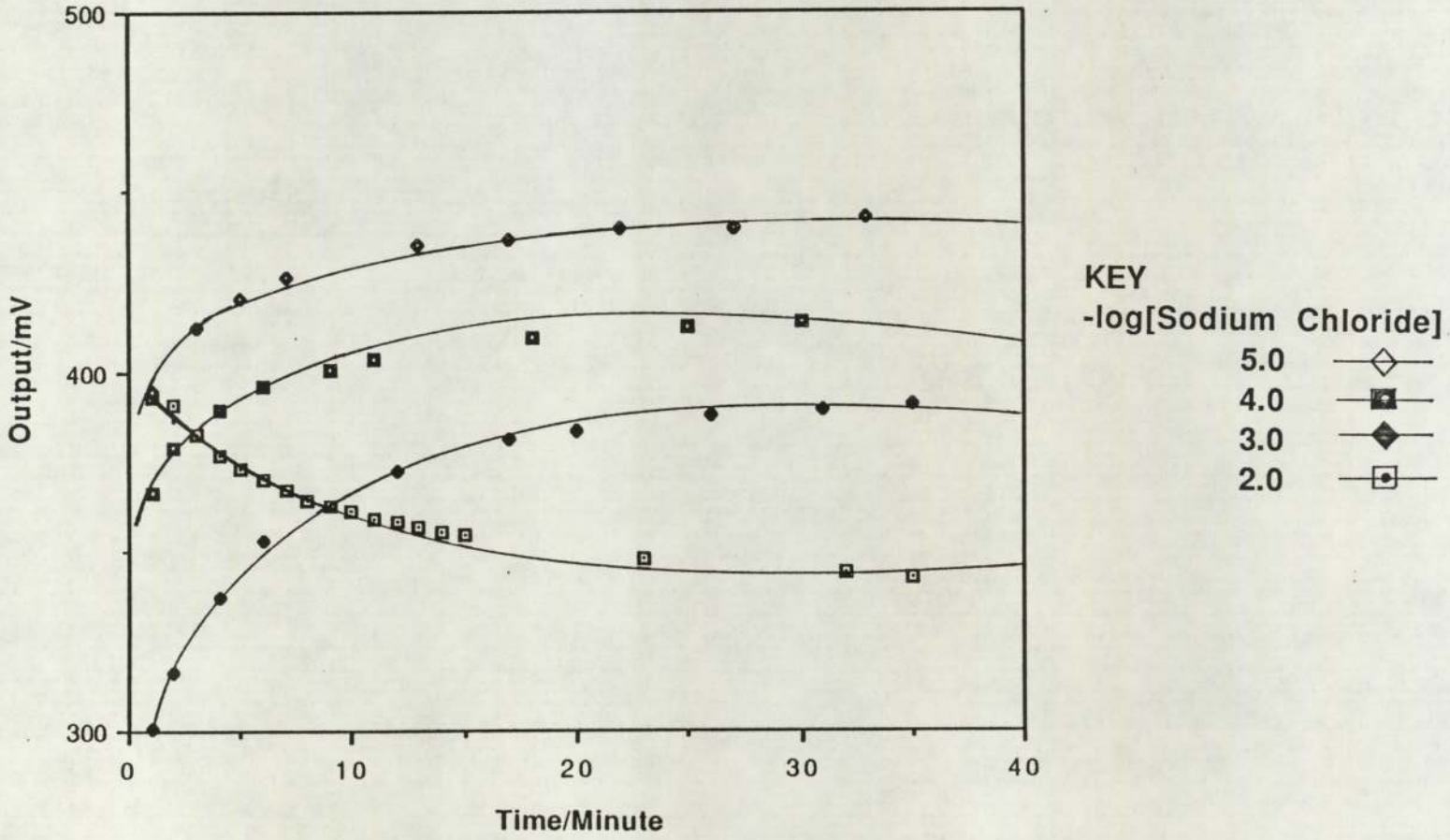


FIG 9.6 CWE RESPONSE: Output Against Time For Changing Concentration Of Hydrochloric Acid

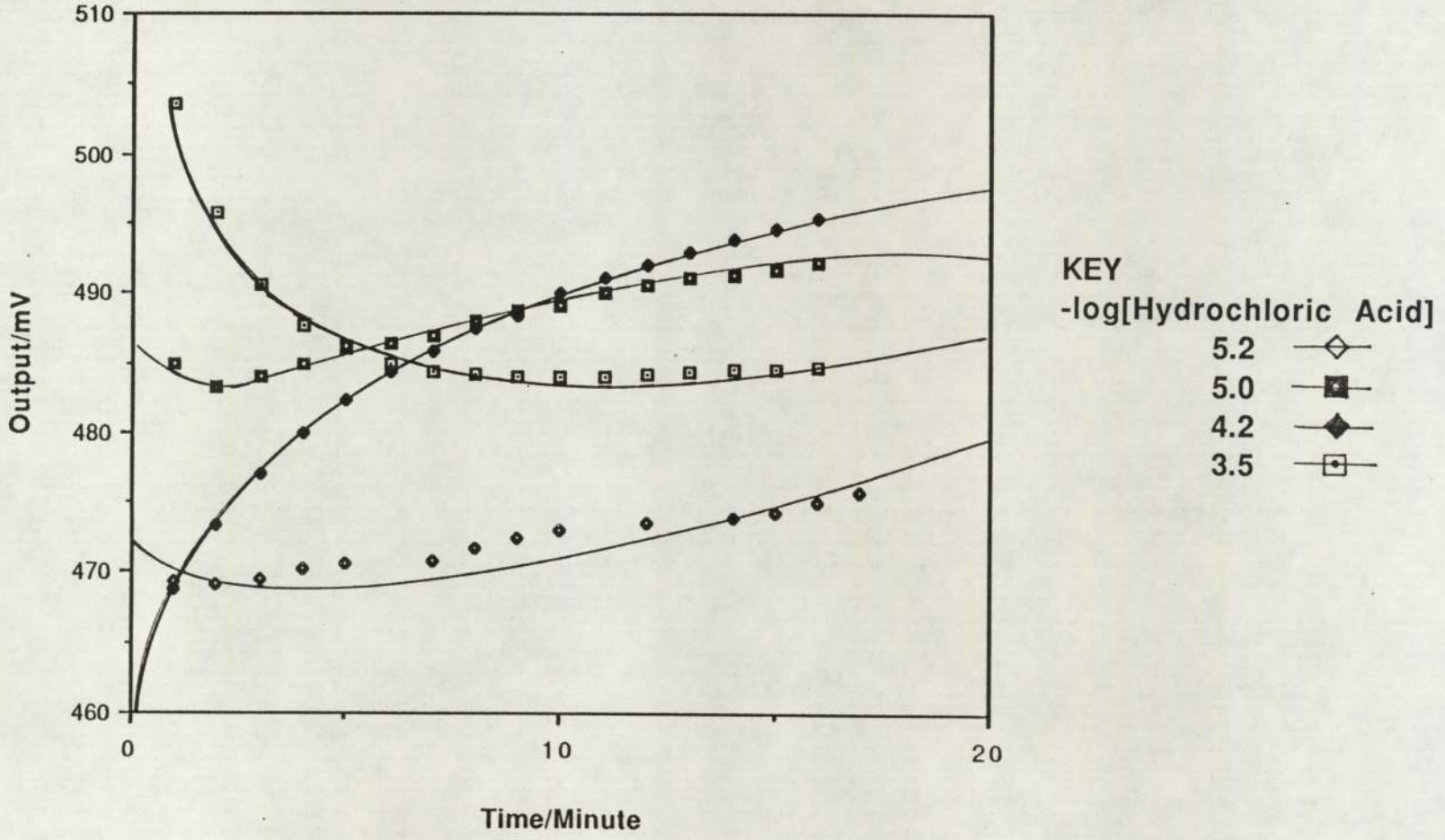
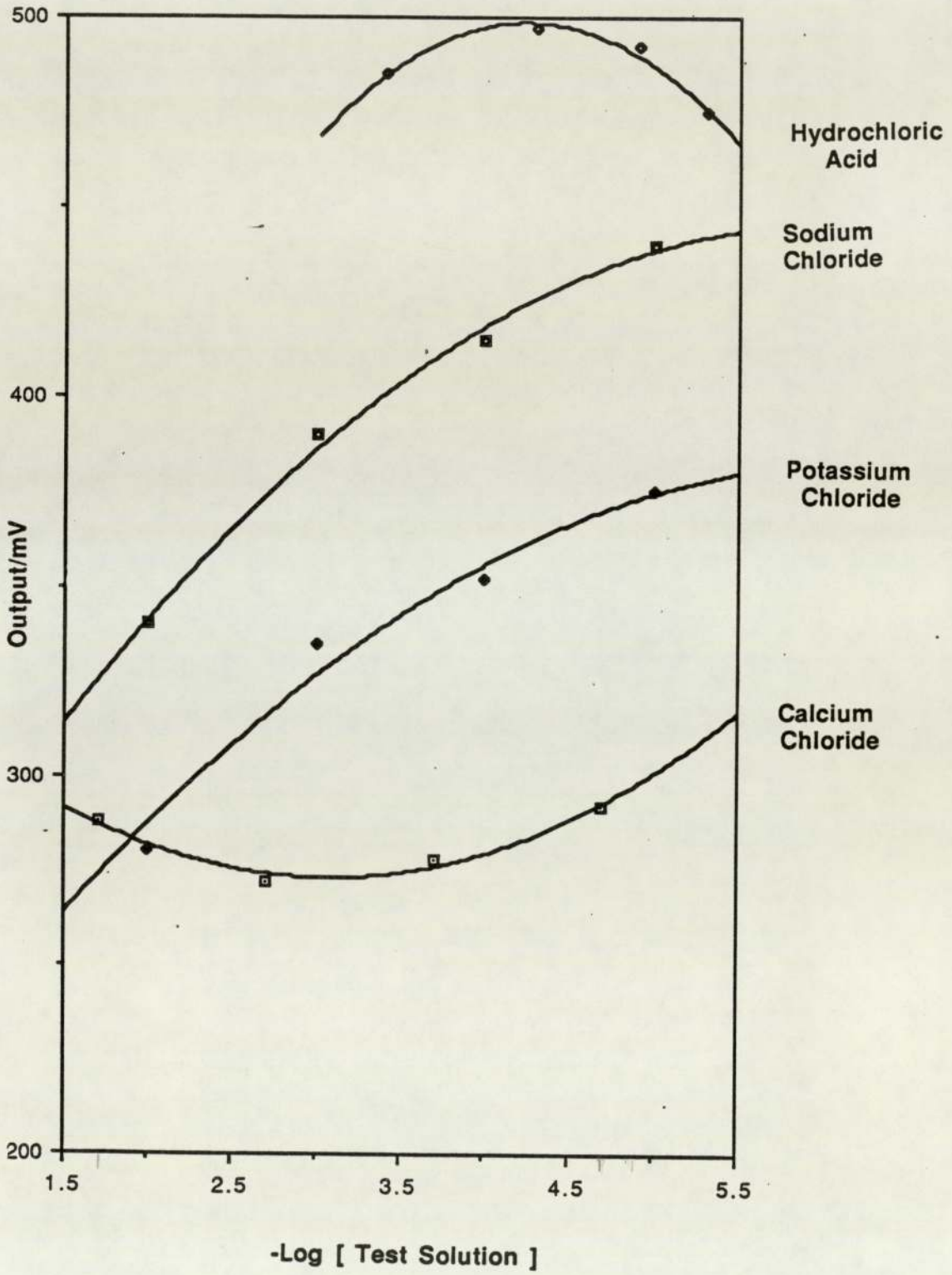


FIG 9.7 -Log [Test Solution] Against Equilibrium Output Of CWE



9.2.4 Discussion of CWE Response Curves

The curves shown in FIGS 9.3-9.6 indicate that there is a genuine response to the above cations. Although the response to calcium appears to be relatively small compared to potassium and sodium, it was a legitimate response to changing concentration and not fluctuation or error in the reading. This proposal is supported by the large response to calcium noted in the fibre optic sensors, implying that calcium may be diffusing through this very low water content membrane (<10%).

One would expect both potassium and sodium to behave in a similar manner, since they are both monovalent cations, of similar hydrodynamic radii. Both of these ions could interact with the reagent molecule by forming its sodium or potassium salt via reaction with the sulphonic acid group. The incorporation of the reagent AcBPR was noted to respond to calcium, in chapter 5, and this may be by the formation of a chelate across either two sulphonic acid or two phenol groups. However, the CWE results imply that either the calcium is not altering the EMF across the membrane and/or that it is not actually diffusing into the membrane. However the results obtained in the fibre optic studies contradict these, since the magnitude, although not the direction, of membrane response to calcium, is similar to that of potassium. An explanation for this may lie in the relative membrane thickness: The effects that were observed for the CWE may be a response only at the surface of the coating, especially in view of the fact that the response appears to be far more rapid and much smaller than that of potassium and sodium. In the optical fibre sensors, the coating is very thin and is infact of the order of magnitude of a wavelength of light (<1μ). Therefore, the effect of calcium at the surface of the

coating may affect the membrane properties sufficiently to produce a response from the evanescent wave, this being due to the penetration depth of the evanescent wave or the physical depth of penetration of the effect of calcium on membrane water content.

At infinite dilution of any species we would expect the response curves to converge. However, the results do not indicate that this effect is inevitable. The fact that the output increases for each set of solutions in the order that they were tested (calcium, potassium, sodium, hydrochloric acid), suggests that there may be some salt remaining in the membrane from the previous test solution.

If potassium and sodium were acting in the manner which we have hypothesised (by forming the sodium or potassium salt of the reagent molecule) then we would expect an increase in concentration of either cation to reduce the number of SO_3^-sites. These would be replaced by sites which would be more hydrophilic and thus the water content of the membrane would be increased. In the fibre optic sensors this would result in a response of positive gradient. Calcium however, would act to bridge two sites, possibly only at the surface, thus effectively pulling them together and reducing membrane water content, resulting in a response of negative gradient. In both cases, one would also expect the pH of the test solution to affect the number of available sites and therefore the response of the sensor.

9.3 CONCLUSIONS

The effect of decreasing pH and increasing calcium concentration on the fibre optic sensor is to dehydrate the hydrogel coating with increased concentration. A possible explanation for this effect may be that either two sulphonic acid groups or two phenol groups are forming a chelate with calcium. This bridging effect will act to reduce the membrane water content. This may only be occurring at the surface of the

sensor coating, hence the very small response visible in the CWE studies.

Potassium and sodium act to hydrate the membrane with increased concentration. It is likely that the sulphonic acid group on the reagent molecule is acting as an ion exchanger in the detection of potassium and sodium. The formation of the sodium or potassium salt on this group will create a more water structuring environment and hence hydrate the membrane.

9.4 FURTHER STUDIES

Further studies to this work would lie in resolving the mechanism by which transport through the matrix is occurring. ie whether or not the SO_3^- group on the reagent is acting as an ion exchanger. This could be achieved via the study of the CWE response when the membrane is conditioned between each change of test solution. The "regeneration" of the ion exchange group should result in absolute rather than relative output results for each solution. In addition, the study of alternative environments, especially divalent cations such as Mg^{2+}, may reveal more clues as to why and how calcium has an opposite effect to sodium and potassium on membrane water content and hence sensor response.

CHAPTER 10

DISCUSSION OF RESULTS AND SUGGESTIONS FOR FURTHER WORK

10.1 DISCUSSION OF RESULTS

The results of this work show that it is feasible to chemically bind a functionalised reagent into a hydrogel membrane and still retain both the sensing ability of the reagent and the rapid transport characteristics of the membrane (chapter 3). Chemical encapsulation of the indicator, and thus the elimination of leaching, was a great step forward in improving the life time and the feasibility of the device as a clinical sensor. In this respect, the cell adhesion studies (section 3.5.3) carried out on thermally crosslinked HEMA:AcBPR membranes, suggested that the presence of the reagent, in this case did not affect the cytotoxicity of the membrane. However, it is obvious that further biocompatibility studies, dependent on the intended use of any clinical sensor, would have to be carried out. In this work, this is especially true, in view of the fact that the more successful devices were produced via photopolymerisation and thus, the incorporation of NVC. The production of linear polymers, via solution polymerisation techniques and subsequent photopolymerisation has proved to be an efficient and successful technique in applying hydrogel coatings to fibre optics.

The configuration of the optical fibre, chosen to utilise the evanescent wave component of the source radiation, allowed thin coatings of hydrogels to be applied to the fibre optic. In fact, another benefit of utilising this configuration was that it improved the reproducibility of the device. This is mainly due to the fact that the evanescent wave only penetrates the outer coating to a depth of a fraction of a wavelength. Therefore, as long as the coating thickness is greater than this penetration depth, its specific thickness is unimportant. The chemical etching process used to strip the fluoropolymer coating from the fibre optic core (section

4.2.3) also contributes to the overall reproducibility of production of the device.

The choice of poly-MMA fibre optics, made because of their compatible refractive index, specifically with poly-MMA containing hydrogels, has proved to be a suitable one. In addition, the construction of a relatively simple and inexpensive test bed (chapter 4), combined with the low water content hydrogel coating and poly-MMA fibre, provided a system which was both sensitive and stable, with a reversible and rapid response (chapter7). This is due to the inhomogeneity and macroporosity of these low water content hydrogel coatings, discovered in the photographic study (chapter8).

The nature of the sensor response is not directly colourimetric since the changing refractive index of the coating results in a more prominent effect than that of the colour change. The presence of the colourimetric reagent however, has proved to be of importance in the type of response associated with each sensed species, most likely due to the action of the sulphonic acid group, present on the reagent, as a cation exchanger. In fact we would not expect to obtain any response if the reagent were not present in the membrane, at such low water contents (<10%), and further studies to this work, within these laboratories have shown this to be the case. In this respect, the way in which the reagent molecule is functionalised is evidently important in determining the transport characteristics of the resulting fibre optic coating.

The constituent monomers of the linear polymer present in the sensor coating solution and the manner in which it is applied to the fibre optic, ie by photopolymerisation, as well as providing a means of producing a thin uniform film, has also been shown to be an important factor in determining the ultimate sensing characteristics. The presence of NVC in the HEMA:MMA membranes, containing the

reagent AcBPR, results in the formation of a heterogeneous and possibly macroporous coating, which may also be facilitating the transport of ions to the fibre interface. In addition, the percentage of NVC added to the coating solution also determines both the range and the stability or degree of "noise" in the sensor output.

Although this work had originally set out to develop a colourimetric system, an alternative system, based on the changing refractive index of a hydrogel with tonicity and pH, emerged. The experimental chapters have at least begun to interpret the effects of both sensor coating composition and the composition of the test solution on the resulting response of the developed optical device. Further investigation and therefore, understanding of the operating principals of these systems, could lead to the development of either more selective sensors, of a similar nature or of true colourimetric and multi-functional devices.

The fact that the sensor responds primarily to water content change is an advantage in one respect, since the sensitivity range is then far greater and more linear than that associated with a colourimetric response. Although this system does not provide the specificity of a colourimetric device, permselectivity can be controlled by the incorporation of a purposely chosen, exterior hydrogel coating, since complete hydrogel membranes possess the properties of being permselective[152]. The "cut off " point and therefore the permselectivity of hydrogel membranes is dictated by their monomer composition. Hydrogel coatings used in this work (section 6.2) have also demonstrated a "cut off "point, at which they will not permit transport of relatively large species.

In summary, the initial aims of this work were to investigate the use of hydrogels as reagent support matrices for fibre optic sensors. It was shown that a reagent molecule can be chemically incorporated into a hydrogel matrix, whilst still

retaining its sensing ability. A simple but sensitive and stable test bed was constructed and the fibre optic and flow through system were easily prepared, assembled and incorporated into the test bed. By the optimisation of linear polymer components in the optical fibre coating, a device with reversible and rapid response to a variety of test environments can be obtained.

10.2 FURTHER WORK

Primarily, further work, already being carried out within the group, involves a more detailed study of the sensor responses to different test solutions and thus the variation in cation and anion concentration. This includes the use of coating solutions of 75:25 MMA:HEMA; 20%NVC membranes, with and without 5% AcBPR. As was commented above, the absence of reagent provides a sensor with no useful response, to date.

It would be valuable to study the sensing characteristics, of both the optical sensor developed in this project and a coated wire electrode, described in chapter 9, to a wider variety of both cations and anions, in order to assess both the transport and sensing mechanisms more fully. In this respect, the responses of barium, magnesium and lithium chloride, would provide additional information regarding the sensor behaviour towards both divalent and an additional monovalent cation. As an extension to this study, the importance of the type of anions present in the salt to the sensor response should also be examined.

The selective diffusion of ions through homogeneous hydrogel membranes can be determined by the water content of the membrane. Since the characteristics of the sensor are evidently not selective, a study of the response curves produced when selective layers are placed over the reagent containing layer, would explore the feasibility of producing more selective devices, utilising the techniques developed in

this work.

In the previous chapter of this work, it was suggested that the sulphonic acid group on the dye was acting as an ion exchanger. Thus, the response of the coated wire electrode could be studied in more detail, determining the effects of "regenerating" these groups after the testing of each sample solution. In addition to supporting this hypothesis, this course of action would assist in determining whether output values on the coated wire have any absolute significance or whether they are relative to the initial state of the reagent. The interpretation of this study could then be directly related to the optical sensor results and will be an important factor in assessing the reversibility of the sensor coatings and hence the reproducibility of results.

Conversely, in order to study the effects of cations and anions on the water content of the sensor coatings and thus the contribution made by this factor to optical response, direct measurement of water content changes in complete membranes can be carried out as in section 6.4.

Although the optical sensor response is due to a changing refractive index of the hydrogel matrix and this offers the advantages of being able to detect a single species over a wide concentration range, a colourimetric reagent may offer the potential of multiple sensing. The development of alternative sensor coatings may result in a sensor which is of a truly colourimetric nature. In order to restrict the refractive index change, the water content of the hydrogel matrix would need to be low and this may present problems regarding ion transport through the matrix. However, thin coatings of the hydrogel may result in detectable colourimetric responses to ions present at the membrane surface, within an acceptable response time. In addition, the immobilisation of reagent molecules similar to

bromopyrogallol red could be achieved by utilising the sulphonic acid group. This would limit the effect of this highly water structuring group and thus possibly aid the production of a colourimetric device. Similarly, a study of alternative reagents which do not contain water structuring groups may be profitable.

In this respect, the development of more sophisticated electronics in order to detect changes in the absorbtion spectra of the source radiation at different wavelengths, corresponding to the detection of different species, by either a single reagent or a combination of more specific reagents, would be useful.

In general, we need a deeper understanding of the present system by further electrochemical and optical study of alternative membrane coatings and test solutions, in order to develop more selective and/or multi-functional sensing devices.

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