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DEVELOPMENT OF NOVEL SENSORS FOR ANIONS OF ENVIRONMENTAL INTEREST

T. LE GOFF

Ph. D. 2000

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DEVELOPMENT OF NOVEL SENSORS FOR ANIONS OF ENVIRONMENTAL INTEREST

By

Thierry Le Goff

A thesis submitted to the University of Plymouth
in partial fulfilment for the degree of

DOCTOR OF PHILOSOPHY

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DEVELOPMENT OF NOVEL SENSORS FOR ANIONS OF ENVIRONMENTAL INTEREST

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ABSTRACT

A range of ion-selective electrodes (ISEs) for the determination of nitrate has been produced based upon rubbery membranes having covalently bound betaine salt sensor molecules. The best performing electrode contained N,N,N-triallyl leucine betaine (6.5 % m/m) covalently bound to polystyrene-*block*-polybutadiene-*block*-polystyrene (SBS) (43.5% m/m), with 2-nitrophenyloctyl ether (2-NPOE) as solvent mediator (40 % m/m) and dicumyl peroxide (DCP) as free radical initiator (10% m/m). The Nernstian slope was -59.1 mV per decade over a linear range of 1×10^{-1}-5×10^{-6} mol dm^{-3} nitrate, a limit of detection of 0.34 µmol dm^{-3} nitrate and a selectivity coefficient for nitrate against chloride ($k^{pot}_{NO_3^-, Cl^-}$) of 3.4×10^{-3}. The speed of response was less than 1 minute over the linear Nernstian range. The lifetime in the laboratory exceeded 5 months with no potentiometric drift over the linear Nernstian range. Temperature dependency (0-25°C), pH range (2-12) and a selection of interfering anions (F^-, Cl^-, Br^-, I^-, SCN^-, ClO_4^-, HCO_3^-, NO_2^-, SO_4^{2-}, phthalate) were studied.

A field evaluation by continuous immersion in both agricultural drainage weirs and a river were undertaken. The nitrate results obtained with the ISEs compared very favourably (R^2=0.99) with those obtained with a segmented-flow instrument in a concentration range 0.47-16 ppm nitrate-N. The electrodes performed continuously for over 5 months in run-off water from a field and over 2 months in river water. The ISEs did not require re-calibration and no deterioration in performance or fouling of the membrane surface was observed.

A preliminary investigation of a phosphate ionophore based upon a heterocyclic macrocycle was also undertaken. This work, based on previous literature, resulted in a dibasic phosphate electrode having a linear Nernstian range from 3×10^{-3} to 1×10^{-6} mol dm^{-3}, a slope of -27 mV per activity decade and a limit of detection of 1×10^{-6} mol dm^{-3} HPO_4^{2-}.

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Relevant scientific seminars and conferences were attended at which work was presented; external institutions were visited for consultation purposes, and material prepared for publication.

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- Applications of electrochemistry in sensor development. Department of Chemistry-University of Southampton and Solartron, February 1997

- 5th & 6th Bill Carruthers Symposium. University of Exeter, April 1997 & 1998

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  Poster presented: "Development of nitrate-selective electrode for *in-situ* measurements".

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  Oral presentation: "*In-situ* nitrate monitoring using ion-selective electrode".

Signed..........

Date......14/04/00...

CHAPTER 1

INTRODUCTION

1.1 Phosphate and nitrate - Cause for concern?

1.1.1 Phosphorus in soil and water

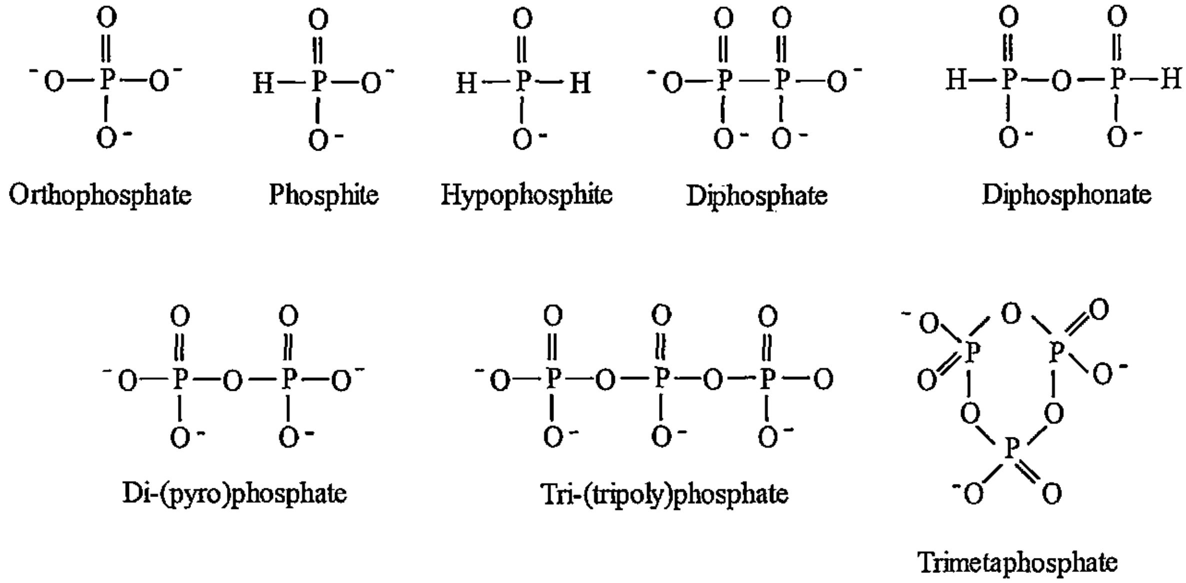
Phosphorus is an essential life supporting nutrient and occurs in both organic and inorganic forms in soils and in water systems.

The majority of organic phosphorus compounds can contain either a P-C bond or a P-O-C bond. They occur naturally e.g inositol hexaphosphates ($(C_6H_6O_6)(PO_3)_6$) in soil, or may be formed from orthophosphate in biological treatment process. They can also come from fertilizers, herbicides, insecticides and fungicides. Organic phosphorus compounds may be necessary for plant growth and are found mostly in seeds as mixed calcium-magnesium-potassium salts, where they are believed to be as a store of phosphate and trace metals (Haygarth and Jarvis, 1999).

The inorganic forms of phosphorus are mainly iron and aluminium phosphates in acid soils and calcium phosphates in alkali soils. They are extremely insoluble in water and levels of soluble phosphorus in soil solution of surface soils are in the range from 0.01 to 0.1 mg l^{-1} P. Inorganic phosphorus exhibits nine oxidation states from +5 to -3. Oxoacids, as described in figure 1.1, such as orthophosphate, P(+5), phosphite, P(+3), hypophosphite, P(+1) and diphosphate, P(+4) are known as well as derivatives. Diphosphonate is known to be a phosphorylation agent for biological substances

(Fujiwara, 1994). Pyrophosphates occured in soil and are involved in biological cycling, polyphosphates are also known to occur in soils and could be of microbial origin (Haygarth and Jarvis, 1999) and can also come from detergents e.g sodium tripolyphosphate (STPP) (Clark *et al.*, 1992).

Figure 1.1 **Phosphorus acid species**



Orthophosphate

Phosphite

Hypophosphite

Diphosphate

Diphosphonate

Di-(pyro)phosphate

Tri-(tripoly)phosphate

Trimetaphosphate

In excess concentrations, in a freshwater environment, phosphate may have a negative effect on aquatic ecology and water quality. Phosphate levels in fresh water systems have increased in the past 50 years, for example the level of orthophosphate has increased in the river Frome (Dorset) by 21% from 1965-1972 levels (Heathwaite *et al.*, 1996) and by 15% from 1980 to 1986 in the Slapton Catchment in South West England (Burt *et al.*, 1996). Four different sources of phosphorus can be distinguished:

- constituents of industrial disharges e.g. commercial cleaning solutions
- livestock manure
- agriculture (P-fertilizers)

- effluents from sewage treatment works
- soil particles

Domestic sources of P have been estimated as contributing 53% of total P released to water in the UK (Lund and Moss, 1990). However, sources of P can vary from a country to an other as shown in table 1.1.

Table 1.1 **Estimated percentage contributions to the total load of phosphorus for selected countries in Europe from Lund and Moss, 1990**

Countries	Point sources %			Diffuse sources %		Weight total P load (kt yr^{-1})
	Domestic	Industrial	Stock Unit	Agriculture	Natural	
UK	53	5	20	16	6	68
Denmark	21	2	58	13	6	15.6
Ireland	20	2	36	30	12	17
Norway	33	3	10	10	44	7.5
Europe	37	4	30	17	14	28.1

In Japan, an average of 1.8g P person^{-1} day^{-1} is released in domestic wastewaters (Goda, 1986). An estimation has been made in the USA where the detergents account for about 46% of total P content of domestic effluents (Alhajjar *et al.*, 1989). The composition of final effluents from domestic septic tanks has been investigated (Whelan and Titamni, 1982). This study showed that the major contaminants were N and P. Total P levels were around 17 mg l^{-1}, almost all present as dissolved orthophosphate. In the USA, reported

total P levels were between 11 and 31 mg l^{-1} and 85% was as orthophosphate (Reneau *et al.*, 1989).

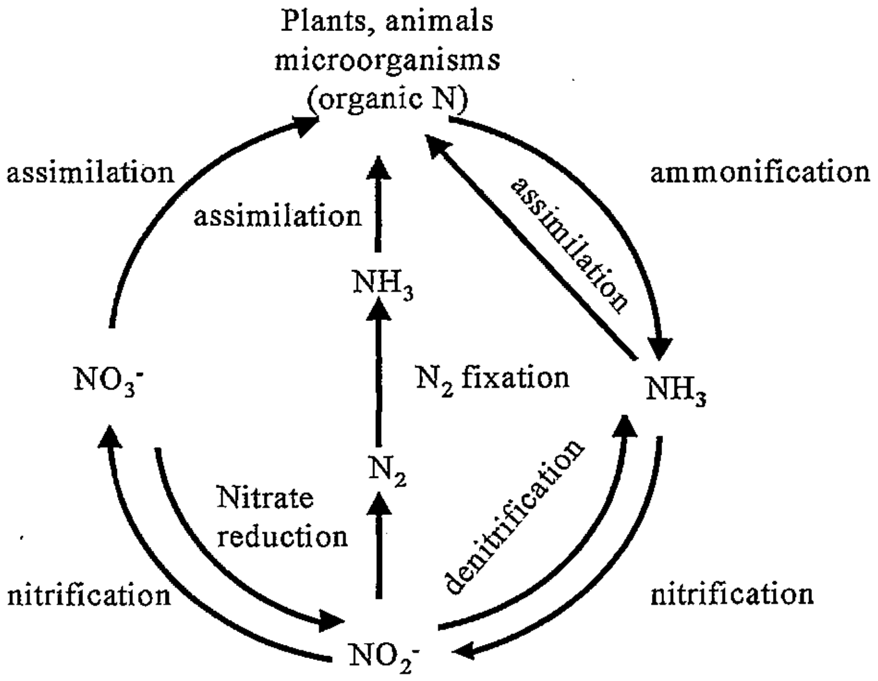
Traditionally, soil phosphorus (P) has been considered as insoluble in water and past agronomic studies considered P leaching as insignificant (Gardwood and Tyson, 1973; Marrs *et al.*, 1991). For this reason, farmers were encouraged to use phosphatic fertilizers thereby transferring small amounts of phosphate from agricultural lands to water systems. This transfer depends on rainfall and is not strongly influenced by the quantity of P applied to the land. However, it has been recently shown that transfer of P as small as 10 µg l^{-1} (2-3 kg ha^{-1} yr^{-1} P) from agricultural land can contribute to eutrophication (Foy and Withers, 1995; Haygarth, 1997).

1.1.2 The nitrogen cycle in freshwater systems

Nitrogen (N) is a very important element for life. Inorganic-N in the environment occurs in different forms i.e ammonia (NH_3), nitrite (NO_2^-), nitrate (NO_3^-) and gaseous nitrogen (N_2). This latter form can be fixed by conversion to ammonia by the action of bacterial genus e.g genus *Rhizobium* present in the roots of certain plants. Generally, nitrate is the main source of N in the soil for plants. Ammonia (NH_3) in soils can be formed by the breakdown of organic-N from plants by microbial action. Under conditions of good aeration and favourable temperatures, different organisms (chemoautotrophic bacteria) oxidise the ammonia first to nitrite (NO_2^-) and then to nitrate (NO_3^-), a process called nitrification. The oxidation from nitrite to nitrate is generally faster than that from ammonia to nitrite, so that no nitrite accumulates. However, the opposite phenomenon can be observed under anaerobic or microaerobic conditions which converts nitrate to nitrogen oxides and gaseous nitrogen (denitrification) and ammonia (nitrate respiration).

In fresh water systems, nitrogen is measured as NH_4-N, NO_3-N and NO_2-N in mg l^{-1}. The nitrogen cycle is presented in figure 1.2.

Figure 1.2 Nitrogen cycle


Plants, animals
microorganisms
(organic N)
assimilation
assimilation
assimilation
ammonification
NH_3
N_2 fixation
NO_3^-
NH_3
N_2
Nitrate
reduction
denitrification
nitrification
nitrification
NO_2^-

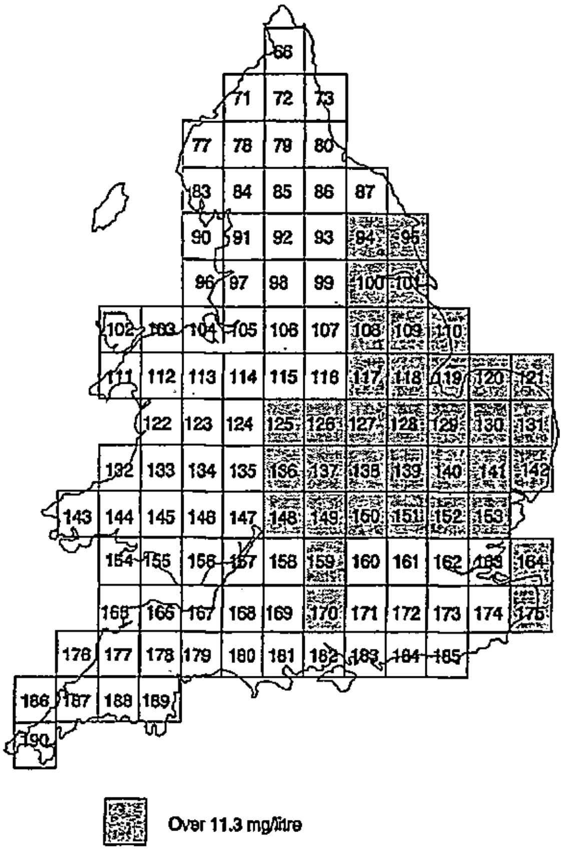
1.1.3 Nitrate in fresh water systems

Nitrate concentrations in fresh water systems are increasing (Burt *et al.*, 1996). This rise is attributed to intensive agricultural practices with the use of N-fertilizers but also from industrial and domestic wastewaters.

Domestic waste water is not a negligible source of N in the environment. In Japan, the average daily diet of Man contained 13.3 g N and about 85% is released in domestic wastewaters (Ukita *et al.*, 1986), almost all as dissolved ammonia (Reneau *et al.*, 1989). However, the excessive use of N-fertilizers remains one of the main source of nitrate in water systems. For example in the UK, the annual usage of N-fertilizers has increased

from about 400 kt, in 1960, to 1.3 Mt, in 1980 (Gasser, 1982). However, in the mid-1980's, the use of N-fertilizers reached a plateau in the UK (Parkinson, 1993). The problem of using excessive amounts of N-fertilizers comes from the fact that nitrate is not totally bound to the soil and is very soluble in water. Therefore it can leach into water systems. Nitrate leaching is one of the loss processes of concern for both economic reasons and its impact on water quality. The European Community (EC) Nitrate Directive (CEC, 1991) requires that the concentration of nitrate-N in drinking water should not exceed 11.3 mg l^{-1}. Figure 1.3 shows that in many areas in UK this limit is exceeded for groundwater (Owen, 1992).

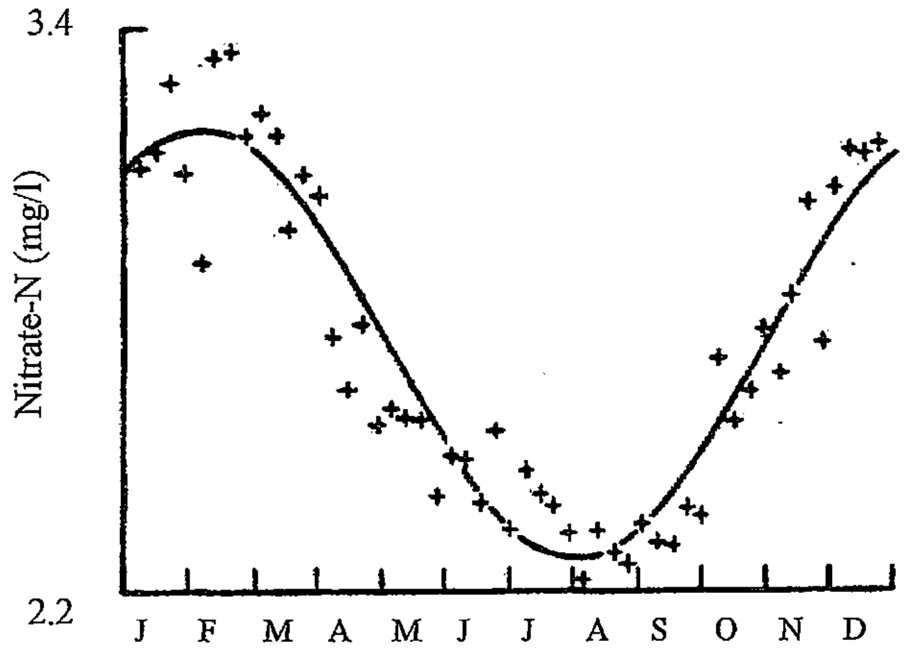
Figure 1.3 Nitrate concentrations in groundwater (from Owen, 1992)



One of the major concerns of the environmental policies of national governments within the EC is the optimisation of land use and farming intensity. Nitrate leaching depends on different factors such as season, type of soils, land management including drainage and amount of N-fertilizers used, plant cover and rainfall (Scholefield *et al.*, 1993; Scholefield and Stone, 1995). Nitrate leaching follows a seasonal trend with minimum losses during the growing season due to plant uptakes and lack of drainage and significant losses during winter.

Nitrate levels in river waters also tend to follow a seasonal trend as shown in figure 1.4.

Figure 1.4 Means of weekly nitrate concentrations in river Frome (Dorset) from 1965 to 1975 plotted against time of the year (from Casey and Clarke, 1979)



1.1.4 Eutrophication

Eutrophication of lakes, rivers, reservoirs and estuaries is probably the most visible effect of an excess of nutrients in fresh water systems. This phenomenon occurs when inorganic

N and P are added to a water systems. The nutrients cause a rapid growth of algae and other aquatic plants which may be toxic to shellfish and fish, e.g. *aphanizomenon flos-aquae* blue-green alga. When this excess of vegetation dies the oxygen available is dramatically reduced, killing life in the water system and obviously making the water non-drinkable (Hutchinson, 1969).

1.1.5 Effects in Man

Nitrate itself is not very toxic but its conversion to other products, after ingestion can be the cause of diseases. Infantile methaemoglobinaemia or 'Blue-Baby Syndrome' is a well known disorder caused by the reduction of nitrate to nitrite (in the mouth or elsewhere in the body where the pH is relatively high) and conversion of oxyhaemoglobin to methaemoglobin restricting the oxygen uptake (European Chemical Industry Ecology and Toxicology, 1988). If the conversion reaches 45-65%, death can occur. However, adults have a different enzyme-reduction system in the gut reducing the chance of suffering this disorder. Around 2000 cases of 'Blue-Baby Syndrome' were reported by the World Health Organisation (WHO) between 1945 and 1986. The last case of methaemoglobinaemia in Britain was reported in 1972 (Heathwaite *et al.*, 1993).

Under basic conditions, nitrosamines may also be formed. In 1973, Hill *et al.* showed that the formation of nitrosamines increased in patients suffering from bladder infections and achlorohydria (Hill *et al.*, 1973). Nitrosamines were found to be carcinogenic in certain animals. However, there is no evidence of their carcinogenicity in man (WHO, 1996).

1.2 Monitoring nitrate and phosphate in water systems

1.2.1 Determination of nitrate

The determination of nitrate in natural waters is not straigthforward, due to the presence of interferents. Some techniques also suffer from their limited linear range of application. The standard methods, and others used for the determination nitrate in natural waters are summarised in table 1.2 (Callaway, 1995). Most of the techniques presented for the determination of nitrate are only suitable for laboratory use. Therefore field samples have to be collected and analysed as soon as possible. Storage can affect the nitrate concentration in the samples due to biological activity. For short term storage, it is recommended to filter (0.45µm) the sample and to keep it refrigerated (4°C for 24 hours), for longer storage H_2SO_4 may be used to preserve the sample but nitrate and nitrite will be determined as a single species. Chloroform may also be used.

In the water industry air segmented continuous flow analysers are commonly used for the simultaneous determination of nitrate, nitrite, phosphate, silicate and ammonia. They require technical staff, chemicals and regular re-calibration. Between 20 to 80 samples can be analysed every hour. The principle of the method to determine nitrate is based on the conversion of nitrate to nitrite using a copper-cadmium reducing column. The nitrite thereby generated then diazotises sulphanilamide which subsequently couples with N-(-1-naphthyl)-ethylenediamine to form an azo dye with an absorbance at 520 nm which is measured with a spectrophotometer or a colorimeter.

Table 1.2 Examples of methods used for the determination of nitrate in natural waters

Method	Technique	Sample	Interferents	Linear range ($mg\ NO_3^-\text{-}N\ l^{-1}$)	Reference
A. Direct UV spectrophotometry		Uncontaminated natural waters	Turbidity, organic matter, NO_2^-, Cr^{6+}	0.03-3	Armstrong, 1963
B. Potentiometry	Nitrate-selective electrode	Fresh waters	Chloride, bicarbonate	0.14 to 1400	Zuther *et al.*, 1994 and Sutton *et al.*, 1999
C. Copper Cadmium reduction	Colorimetry or spectrophotomety	Seawater, drinking and waste waters	Turbidity, particulate matter	0.01 to 1.0 (manual) 0.5 to 10 (automated)	Nydahl, 1976 and Stainton, 1974
D. Chemiluminescence		Seawater			Garside, 1972
E. Titanous chloride reduction	NH_3 gas sensing electrode	Natural waters	NH_3, nitrite	0.01 to 10	Braunstein *et al.*, 1980
F. Ion chromatography		Drinking waters		0.07 to 1.8	Papadoyannis *et al.*, 1999
G. Hydrazine reduction	Colorimetry or spectrophotomety	Seawater, drinking and waste waters	Turbidity, particulate matter	0.01 to 10	Kamphake *at al.*, 1967 and Kempers *et al.*, 1988
H. Polarography		Drinking and river water		0.07-0.7	Noufi *et al.*, 1990

1.2.2 Determination of phosphate

The sampling method and storage time of phosphate samples is critical to obtaining good accuracy. Analysis should be carried out immediately after sampling or should be filtered (0.45 µm) and stored at sub-zero temperatures for long-term storage. Depending on the forms of phosphate to be determined, some additives can be used to preserve the sample. For total P, hydrochloric acid can be added. For the determination of different forms of phosphate, preservation by $HgCl_2$ is recommended (Callaway, 1995). Orthophosphate is mainly determined using colorimetric methods and requires non-turbid and filtered samples. These methods and a few others are summarised in table 1.3.

Air segmented continuous flow analysers (autoanalysers) are also commonly used in the water industry for the determination of orthophosphate using the 'molybdenum blue method'. The principle of this method is the reaction between orthophosphate, ammonium molybdate and potassium antimonyl tartrate in acidic medium forming an antimony-phosphomolybdate complex. On reduction with ascorbic acid (Murphy and Riley, 1962) a blue colour is formed which is measured using spectrophotometry (880 nm). The development of the blue colour depends on the temperature with an optimum at 60°C (Pai *et al.*, 1990). This method can be used to measure orthophosphate levels down to 1 µg PO_4^{3-}-P l^{-1}. However, arsenate (0-1.0 mg AsO_4-As l^{-1}), silicate (0-50 mg SiO_4-Si l^{-1}), fluoride (0-200 mg F l^{-1}) and nitrite (1 mg NO_2^--N l^{-1}) interfere with the phosphate determination (Blomqvist *et al.*, 1993).

Table 1.3 Examples of methods used for the determination of orthophosphate in natural waters

Method	Technique	Sample	Interferents	Linear range ($mg\ PO_4^{3-}$-$P\ l^{-1}$)	Reference
A. Vanadomolybdo-phosphoric acid	Spectrophotometry (yellow complex)	Seawater, drinking and river waters	As, Si, NO_2 and F	1-20	Abbot *et al.*, 1963
B. Stannous chloride; Molybdenum blue method	Spectrophotometry	Seawater, drinking and river waters	As, Si, NO_2 and F	0.007-0.1	Callaway, 1995
C. Ascorbic acid; Molybdenum blue method	Spectrophotometry	Seawater, drinking and river waters	As, Si, NO_2 and F	0.01-6 (manual) 0.001-0.1 (automated)	Edwards *et al.*, 1965 Henriksen, 1966
D. Potentiometry	Biosensor	Natural waters		0.03-0.3	Conrath *et al.*, 1995
E. Ion-chromatography		Saline waters		0.17-6.7	Galceran *et al.*, 1993
F. Reaction of molybdophosphate and cationic water-soluble porphyrin	Fluorimetry	Seawater, drinking and river waters	Mg, Ca (> 0.1 mol dm^{-3})	0.003-0.1	Tabata and Harada, 1992

1.2.3 Conclusion

The methods commonly used to determine either nitrate or phosphate require sampling, technical staff, chemicals and regular re-calibration. For environmental work, the technique to be used should be capable of continuous or 'spot' on site monitoring (the former to be operator independent for a reasonable period), non-polluting i.e no waste involved, simple to use by a variety of personnel (i.e non-chemists). An ion-selective electrode best fits those requirements.

1.3 Ion-selective electrodes (ISEs)

1.3.1 Theory

1.3.1.1 Response mechanism

ISEs are a type of chemical sensor. They are categorised as potentiometric sensors and can be sub-divided into two main groups: solid state and liquid membranes.

The theory of ISEs is well established especially owing to the pioneering work of Eisenman's group (Eisenman *et al.*, 1957, 1969) and others (Morf, 1975, 1976; Boles and Buck, 1973; Nicolsky, 1937).

The most important part of an ISE is the ion-selective membrane. In the case of polymeric membranes, a water insoluble viscous liquid is placed between the sample and the inner filling solution as shown in figure 1.5. The liquid membrane is generally composed of 66% m/m plasticizer, 33% m/m polyvinyl chloride (PVC) and 1% m/m sensor molecule (Craggs *et al.*, 1974).

The electrochemical cell for a potentiometric sensor is composed of:

- A sensing electrode consisting of the liquid membrane, an inner reference electrode (Ag/AgCl) and the inner filling solution (a mixture of chloride and analyte salts).
- An external reference electrode

A typical electrode arrangement is illustrated in figure 1.5.

Figure 1.5 **Electrode arrangement**

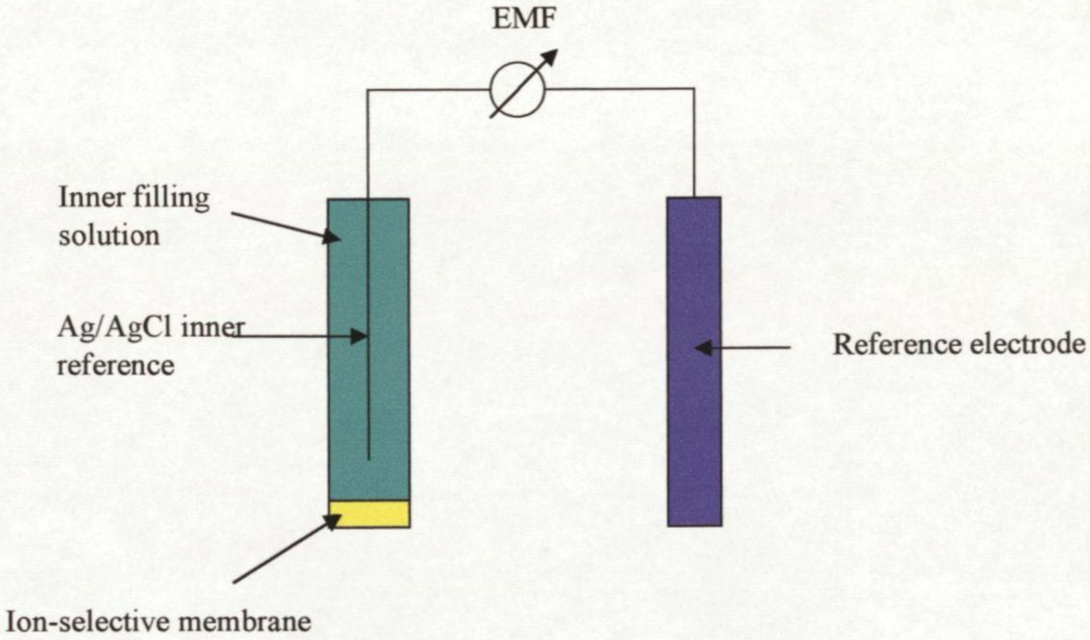

EMF
Inner filling solution
Ag/AgCl inner reference
Reference electrode
Ion-selective membrane

Figure 1.5 can also be represented as:

$$\overbrace{\underbrace{\text{Hg, Hg}_2\text{Cl}_2 \mid \text{Satd. KCl}}_{\text{Calomel electrode}} \parallel \text{Outer filling solution}}^{\text{Double junction reference electrode}} \parallel \text{Sample} \mid \overbrace{\text{Liquid ion-selective membrane} \mid \text{Internal filling solution} \mid \text{AgCl} \mid \text{Ag}}^{\text{Sensing electrode}}$$

ISEs develop an electrical potential when they are placed in a solution containing the ion of interest. The potential arising at the interface between the ion-selective membrane and the sample is related to the activity of the analyte (a_J) as opposed to its concentration [J]. [J] is related to a_J by equation 1.1:

$$a_J = \gamma_J [J] \tag{1.1}$$

where γ_J is the activity coefficient.

γ_J can be determined since it is related to a quantity termed the ionic strength (I), defined below, by the Debye-Hückel limiting law which can be written:

$$\log \gamma_J = -Az^2 \sqrt{I} \tag{1.2}$$

where A is a constant depending on relative permitivity, temperature and solvent and has a value of 0.509/ (mol kg)$^{-0.5}$ for an aqueous solution; z is the ionic charge and I, the ionic strength of the solution is given by:

$$I = 0.5 \sum_i [J]_i z_i^2 \tag{1.3}$$

the limiting law only applies if:

- The dissociation of the electrolyte is complete
- The ionic interactions are qualitatively described by the Coulomb's law for point charges
- The cause of the solution non-ideality is due only to coulombic interactions between the ions

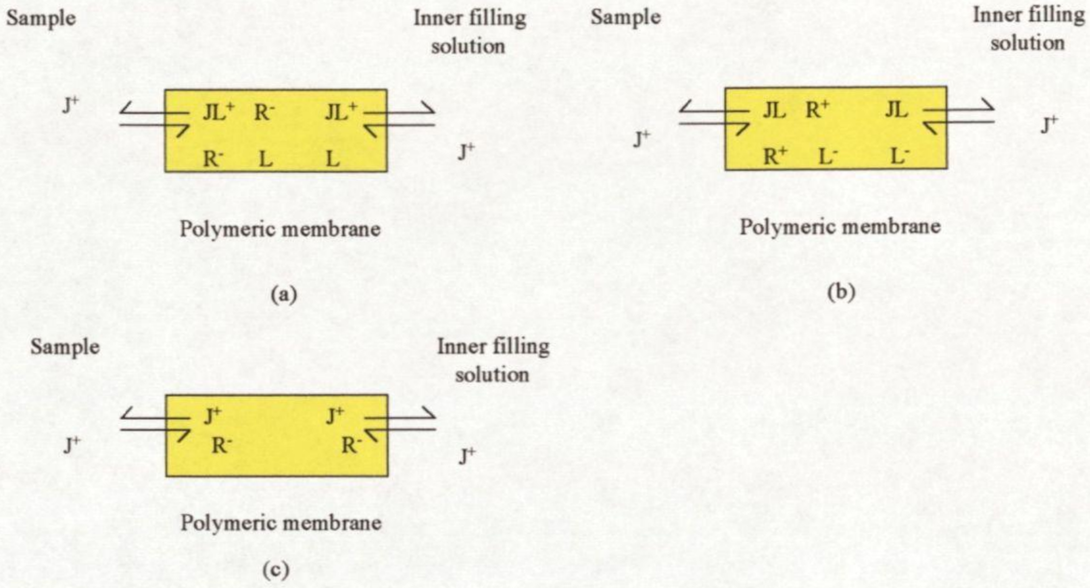
It can be seen that if I→0 then log $\gamma_J \to 0$ and therefore $\gamma_J \to 1$ and activities approach concentrations.

Accordingly to equation 1.3 if [J] increases, the ionic strength (I) of the solution increases and thus γ_J decreases. Using equation 1.2, the relationship between a_J and [J] is non-linear.

For this reason, the use of an Ionic Strength Adjustment Buffer (ISAB) is recommended to keep the ionic strength to a constant value.

The response mechanism of an ISE depends upon the sensor used. Three cases have to be differentiated, neutral carrier-based ISE (a), charged carrier-based ISE (b) and ion-exchanger-based ISE (c) as shown in figure 1.6.

Figure 1.6 Equilibria between membranes, sample and inner filling solution in the case of a cation-selective membrane (from Bakker *et al.*, 1997)


Sample
Inner filling solution
J^+
JL^+ R^- JL^+
R^- L L
J^+
Polymeric membrane
Sample
Inner filling solution
J^+
JL R^+ JL
R^+ L^- L^-
J^+
Polymeric membrane
Sample
Inner filling solution
J^+
J^+ R^-
J^+ R^-
J^+
Polymeric membrane

(a) (b) (c)

(a) L is an electrically neutral carrier, R^- are anionic sites and J^+ is the cationic analyte; (b) L^- is the charged carrier and R^+ are cationic sites; (c) R^- is a cation-exchanger.

The EMF measured across the cell (E_{cell}) is the sum of the individual potential contributions such as liquid junction potentials, membrane potential (E_m), electrode potential of the reference electrode and electrode potential of the sensing electrode. If we

make the assumption that apart from E_m all the other contributors of the cell potential remain constant (E_{cst}) then the observed potential is directly related to the membrane potential.

$$E_{cell} = E_{cst} + E_m \qquad (1.4)$$

E_m can be divided into three separate potential contributions: the phase boundary potentials at the sample-membrane interface and membrane-inner filling solution interface and the diffusion potential. For a very long time it was assumed that electrode potential was due to ion transport through a membrane (Donnan, 1911; Štefanac and Simon, 1966). Recently, however, several workers have shown that the diffusion potential can be considered negligible (Pungor, 1992, 1997; Bakker *et al.*, 1994a). Also the potential arising at the interface between the membrane and the inner filling solution is sample independent. Therefore, the membrane potential is established at the interface between the membrane and the sample, in the first nanometers of the membrane (Bakker *et al.*, 1997; Pungor, 1998).

$$E_m = E_{PB} + E_{cst} \qquad (1.5)$$

E_{PB} is the difference between the electrical potentials for the aqueous phase (sample) and the organic phase (membrane). The relationship between electrical (ϕ(aq)), electrochemical ($\tilde{\mu}$(aq)) and chemical (μ(aq)) potentials for the aqueous phase can be expressed as shown in equation 1.6 (Guggenheim, 1930):

$$\tilde{\mu}(aq) = \mu(aq) + zF\phi(aq) \qquad (1.6)$$

And μ(aq) can be expressed as in equation 1.7:

$$\mu(aq) = \mu^{o}_{(aq)} + RT\ln a_J(aq) \qquad (1.7)$$

By combining equation 1.6 and 1.7, equation 1.8 can be obtained

$$\tilde{\mu}(aq) = \mu^o_{(aq)} + RT\ln a_J(aq) + zF\phi(aq) \tag{1.8}$$

And if we do the same for the organic phase, equation 1.9 can be obtained:

$$\tilde{\mu}(org) = \mu^o_{(org)} + RT\ln a_J(org) + zF\phi(org) \tag{1.9}$$

where μ° is the standard chemical potential, J is the ion, z is the ionic charge, F is the Faraday constant, T is the absolute temperature and R is the gas constant.

In equilibrium, the electrochemical potentials, $\tilde{\mu}$, of the aqueous phase ($\tilde{\mu}$(aq)) and the organic phase ($\tilde{\mu}$(org)) are equal for all the ions present, and therefore $\tilde{\mu}$(aq)= $\tilde{\mu}$(org) (Štulik, 1994). The phase boundary potential (E_{PB}) is the difference between the electrical potential for the organic and aqueous phase, as shown in equation 1.10:

$$E_{PB} = \Delta\phi = \phi_{(org)} - \phi_{(aq)} \tag{1.10}$$

From equations 1.8 and 1.9, E_{PB} can be expressed as in equation 1.11:

$$E_{PB} = \frac{\mu^o_{(aq)} - \mu^o_{(org)}}{zF} + \frac{RT}{zF}\ln\frac{a_J(aq)}{a_J(org)} \tag{1.11}$$

Using equations 1.5 and 1.11 the membrane potential (E_m) can be related to the activity of the analyte in the organic and aqueous phase as in equation 1.12:

$$E_m = E_{cst} + \frac{\mu^o_{(aq)} - \mu^o_{(org)}}{zF} + \frac{RT}{zF}\ln\frac{a_J(aq)}{a_J(org)} \tag{1.12}$$

The activity of the ion in the organic phase (a_I(org)) is a function of the concentration of the sensor molecule and is a sample independent parameter (Bakker *et al.*, 1997). Therefore, E_m, can be expressed in the well-known Nernst equation 1.13:

$$E_m = E^o + \frac{RT}{zF}\ln a_J(aq) \tag{1.13}$$

where E° is defined as the standard electrode potential regrouping all the sample independent potential contributions. It was mentioned previously that the use of an ISAB will keep the activity coefficient (γ) to a constant value, therefore by combining equations 1.1, 1.4, 1.13 the cell potential (E_{cell}) can be related to the logarithm of the concentration of the analyte as in equation 1.16.

$$E_m = E^o + \frac{RT}{zF}\ln\left(\gamma[J]_{(aq)}\right) \qquad (1.14)$$

$$\therefore E_m = E^o + \frac{RT}{zF}\ln\gamma + \frac{RT}{zF}\ln\left([J]_{(aq)}\right) \qquad (1.15)$$

$$\therefore E_{cell} = E^o + \frac{2.303RT}{zF}\log\left([J]_{(aq)}\right) \qquad (1.16)$$

Therefore, by substitution the theoretical Nernstian slope for a singly charged anion is -59.12 mV per decade.

1.3.1.2 Selectivity

No electrode has total specificity for one ion in the presence of all other ions. The selectivity is one of the most important characteristics of a sensor molecule. Different types of interferences may be encountered in practice. Ions with the same charge sign as the analyte may be sensed by the electrode.

Ross (Ross, 1967) studied the effect of foreign cations on his calcium-selective electrode potentials and showed that for all ions investigated (H^+, Na^+, K^+, NH_4^+, Mg^{2+} and Ba^{2+}) the potentials fitted the empirical equation 1.17 known as the Nicolsky-Eisenman equation (Nicolsky, 1937):

$$E_{cell} = E^\circ + \frac{RT}{z_I F}\ln\left[a_I(IJ) + k_{IJ}^{pot} a_J(IJ)^{\frac{z_I}{z_J}}\right] \qquad (1.17)$$

Where z_I and z_J are the charge and sign of the primary and secondary ion, $a_I(IJ)$ is the activity of the primary ion (I) in the mixed solution (IJ), $a_J(IJ)$ is the activity of the interfering ion (J) in the mixed solution (IJ) and k^{pot}_{IJ} is the selectivity coefficient (potentiometric). The k^{pot}_{IJ} value is a measure of the degree of selectivity shown by the electrode for the analyte, I, in the presence of an interferent, J. The Nicolsky-Eisenman equation is used to determine k^{pot}_{IJ}. Different experimental methods are available to determine k^{pot}_{IJ} using the Nicolsky-Eisenman equation.

The value for the selectivity coefficient for a primary ion (I) versus an interfering ion (J) depends on the method used to determine it and comparisons between different k^{pot}_{IJ} is difficult unless the same method is used. However selectivity coefficients are useful parameters for ISEs. There are three main methods for their determination.

The first method named the Separate Solution Method (SSM) (IUPAC, 1979) consists in measuring and comparing the cell potential (E_I) of a solution containing only the primary ion (I) with that (E_J) of a solution containing only the interfering ion (J). The method has the advantage of being rapid and convenient. This is especially useful when studying a large number of interferent ions, but the measured data are often not representative for a real mixed sample solution. The selectivity coefficient k^{pot}_{IJ} may be calculated from the Nernst equation and Nicolsky-Eisenman equation as described in equation 1.17 as follows:

$$E_I = E^o + \frac{RT}{z_I F}\ln a_I(I) \tag{1.18}$$

$$E_J = E^o + \frac{RT}{z_I F}\ln\left[k_{IJ}^{pot} a_J(J)^{\frac{z_I}{z_J}}\right] \tag{1.19}$$

$$E_J - E_I = \frac{2.303RT}{z_I F}\left(\log\left[k_{IJ}^{pot} a_J(J)^{\frac{z_I}{z_J}}\right] - \log a_I(I)\right) \qquad (1.20)$$

When $a_J(J)=a_I(I)$, equation 1.20 can be reduced to 1.21

$$\frac{E_J - E_I}{\dfrac{2.303RT}{z_I F}} = \log k_{IJ}^{pot} + \left(\frac{z_I}{z_J} - 1\right)\log a_I(I) \qquad (1.21)$$

Rearranging to

$$\log k_{IJ}^{pot} = \frac{E_J - E_I}{\dfrac{2.303RT}{z_I F}} + \left(1 - \frac{z_I}{z_J}\right)\log a_I(I) \qquad (1.22)$$

The second method called the Fixed Interference Method (FIM) overcomes the disadvantage with the separate solutions method and was used for all selectivity coefficient determinations in this work unless stated otherwise. This was performed according to IUPAC recommandations (Guilbault, 1976). Solutions were prepared with a constant level of interfering ion (generally 1.0 x 10^{-2} mol dm^{-3}) and varying the level of the primary ion. The determination of k^{pot}_{IJ} follows from the graph illustrated in figure 1.7.

The second one is based on the assumption that in the curve region (BC) both ions are contributing equally to the observed electrode potential. Therefore, equation 1.24 can be written:

$$a_I(IJ) = k_{IJ}^{pot} a_J(IJ)^{\frac{z_I}{z_J}} \tag{1.24}$$

and by combining equation 1.17 and 1.24, equation 1.25 is found:

$$E_{cell} = E^{\circ} + \frac{RT}{z_I F} \ln[2a_I(IJ)] \tag{1.25}$$

Now the difference between the electrode potential in a solution containing the primary ion only, I, (equation 1.13) and that in a solution of primary ion with a background of interefering ion , J, (equation 1.17) can be written as in equations 1.26 and 1.27.

$$\Delta E = \frac{2.303RT}{z_I F} \left(\log[2a_I(IJ)] - \log[a_I(I)]\right) \tag{1.26}$$

If $a_I(IJ)=a_I(I)$ equation 1.26 can be reduced to 1.27:

$$\Delta E = \frac{2.303RT}{z_I F} \log 2 = \frac{18}{z_I} \tag{1.27}$$

Thus, the point at which the calibration curve for the primary ion (singly charged) in the presence of a constant background of interfering ion differs from the extrapolation of the Nernstian slope (AB) by 18 mV, the potentiometric selectivity coefficient can be calculated as expressed in equation 1.23:

$$k_{IJ}^{pot} = \frac{a_I(IJ)}{a_J(IJ)^{\frac{z_I}{z_J}}} \tag{1.23}$$

The third method is called Matched Potential Method (MPM) and has been developed by Gadzekpo and Christian (Gadzekpo and Christian, 1984; Christian, 1994).

ionic equilibria at the membrane/sample interface, and this is applicable to primary and secondary ions of different charges. The new formalism is based on equation 1.28.

$$a_I(I) = a_I(IJ) + \left(k_{IJ}^{pot}\right)^{\frac{z_J}{z_I}} a_I(IJ)^{1-\frac{z_J}{z_I}} a_J(IJ) \tag{1.28}$$

Using this formalism and equation 1.13 the selectivity coefficient can be determined as described in equation 1.29.

$$k_{IJ}^{pot}(corrected) = \frac{a_I(IJ)}{a_J(IJ)^{\frac{z_I}{z_J}}} \left(\frac{p_{IJ}}{100}\right)^{\frac{z_I}{z_J}} \tag{1.29}$$

Where p_{IJ} is maximum tolerable relative error in the measurement of $a_I(IJ)$ under the presence of the interfering ion (J). This error has to be smaller than 10% and can be calculated as shown in equation 1.30.

$$p_{IJ} = (k_{IJ}^{pot})^{\frac{z_J}{z_I}} \frac{a_J(IJ)}{a_I(IJ)^{\frac{z_J}{z_I}}} * 100 \tag{1.30}$$

1.3.2 Classification of ISEs

ISEs can be classified according to the membrane material and the electrode arrangement.

1.3.2.1 Type of membranes

According to IUPAC recommendations ISE membranes are classified as follows (Guilbault *et al.*, 1976):

- Liquid membranes

  Classical ion-exchanger

    - Charged carrier
    - Neutral carrier
- Solid membranes
    - Homogeneous: Glass membrane, crystal membrane
    - Heterogeneous: Crystalline substance in inert matrix
- Membrane in special electrodes
    - Gas-sensing electrode
    - Enzyme
    - Microbial electrode
    - Tissue electrode
    - Optode

It is not proposed to consider all the membrane types in detail but to focus essentially on liquid membranes containing ion-exchangers, neutral or charged carriers for the determination of nitrate and phosphate ions. The origins of ISEs go back to the beginning of the century with the development of the first pH-glass electrodes (Haber and Klemensiewicz, 1909). Eisenman extended the applications of glass electrodes by studying the effect of the glass composition on selectivity for sodium and potassium ions (Eisenman *et al.*, 1957).

In 1964 Moore and Pressman discovered that the antibiotic valinomycin exhibited a selectivity for alkali cations in rat liver michondria (Moore and Pressman, 1964). The first neutral carrier membrane reported (Štefanac *et al.*, 1966) utilised this selectivity to develop an electrode for the determination of potassium. Pressman suggested that such chemicals inducing alkali-ion permeability should be termed ionophores (Pressman *et al.*, 1967). The first ISE based upon an ion-exchanger was for calcium (Ross, 1967), and was based upon calcium didecyl phosphate in di-n-octylphenyl phosphonate. At the time organic liquid membranes required only to be hydrophobic, non-volatile and to interact reversibly with the analyte. Commercial versions of these were bulky and difficult to assemble.

The most significant advance in liquid membrane electrodes was probably the development of the PVC membrane (Moody *et al.,* 1970). This membrane was based upon the same calcium sensor used previously by Ross. Further details about the origin of ISEs, can be found in the literature (Oggenfus *et al.*, 1986; Frant, 1997)

1.3.2.2 Electrode arrangement

The second classification of membrane electrodes is based on the size and shape of the electrode body as shown in figure 1.9. Each arrangement depends on the applications e.g. on-line monitoring was performed using flow-through ISEs, thin films ISEs were used for intracortical studies and continuous intravascular measurements were carried out with catheter ISEs.

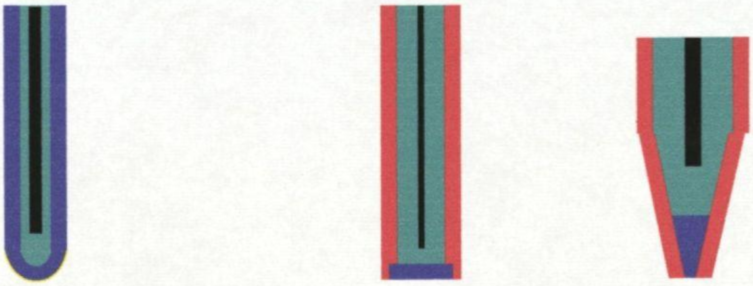
The coated wire electrode (CWE) was first developed by Cattrall (Cattrall *et al.*, 1971). In the first CWE, the polymeric membrane was directly cast to the surface of a platinum wire. The great advantage of CWEs is their ability to be fabricated into any configuration for inclusion in a flow-cell. However, they are in general inferior with respect to drift and reproducibility than conventional ISE (Cattrall, 1997).

In 1970, Bergvelt introduced the first Ion-Selective Field Effect Transistor (ISFET) (Miyahara and Simon, 1991). ISFETs are composed of a p-type semiconductor in which there are two regions of n-type semiconductors termed the source (S) and drain (D). Virtually any sensor can be used in ISFET and their combination with liquid membranes was first proposed by Moss (Moss *et al.*, 1975). The principle of action is based upon the magnitude of current flow between S and D which is directly related to the membrane potential.

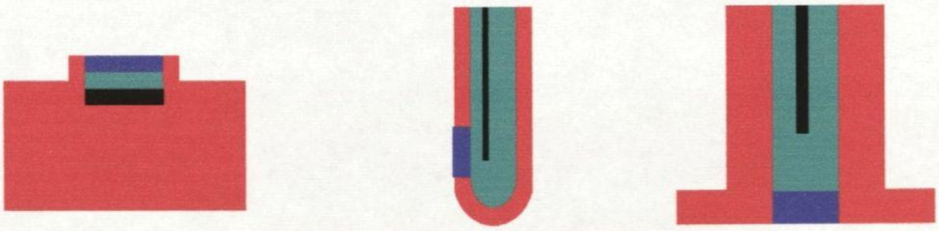
Ion-selective microelectrodes contain an ion-selective membrane in the tip of a glass micro-pipette. The diameter of the tip is generally 1µm. The main advantage of these devices is to offer non-destructive method of measuring intracellular ions (Zhen *et al.*, 1992).

Macroelectrodes contain an ion-selective membrane inserted into the tip of a commercially available electrode body as illustrated in the experimental section 2.2.3.1.1. The diameter of these ISEs is generally 0.5 cm. They are used for a wide range of applications. Many components necessary for the fabrication of liquid membranes are commercially available (Fluka Selectophore, 1996).

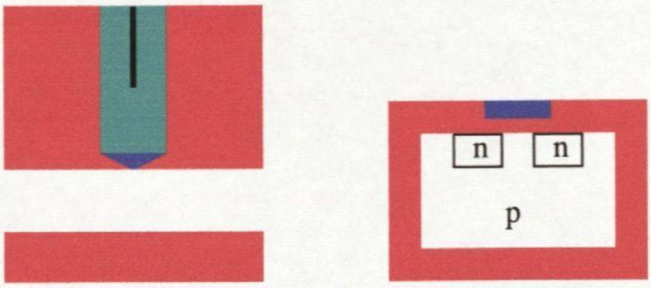
Figure 1.9 **Electrode arrangement**



Coated wire electrode Macroelectrode Microelectrode



Thin film electrode Catheter electrode Surface electrode



Flow-through electrode Ion-selective field effect transistor

🟦 Ion-selective membrane, 🟥 Insulating material, 🟩 Inner filling solution, ⬛ Ag/AgCl inner reference.

1.4 Survey of existing phosphate-ISEs

1.4.1 Modified electrodes

Vermes developed a triple charged phosphate sensor based on silver phosphate-modified electrodes (Vermes and Grabner, 1990). This electrode was suitable for the potentiometric determination of phosphate ions and for the determination of phosphate by a standard addition method. The electrode was fabricated by electrochemical deposition of silver on platinum and subsequent anodic dissolution of deposited Ag in alkali phosphate solutions using a conventional three-electrode cell and a potentiostat with a programmable function generator. These Ag_3PO_4- modified electrodes exhibited a Nernstian response to tribasic-phosphate over a range from 3 x 10^{-5} to 3 x 10^{-9} mol dm^{-3} PO_4^{3-}. The pH range was 7-10 and the slope was -19.5 ± 0.8 mV per decade. The sensitivity of these electrodes was good but chloride ions interfered strongly with the phosphate response with a reported potentiometric selectivity coefficient of 1.0 x 10^7. The electrode stability was another limiting factor with a reported lifetime of 50 hours.

1.4.2 Metallic cobalt wires

The use of phosphate-selective coated-wire electrodes based on cobalt phtalocyanine (CoPc-based CWE) was investigated by Liu (Liu *et al.*, 1990). Various metal phtalocyanines were investigated but CoPc gave the best results. The phosphate-selective membrane was composed of 1% m/m CoPc, 69% m/m dioctyl sebacate and 30% m/m PVC. The resulting electrode responded to $H_2PO_4^-$ activity with a sub-Nernstian slope of -43 ± 2 mV per activity decade over a pH range from 4 to 7. The limit of detection was about 7 µmol dm^{-3} with a linear range between 10^{-1} and 10^{-5} mol dm^{-3} $H_2PO_4^-$. The electrode was not very selective against interferents. The selectivity coefficients for many anions were reported and showed that chloride (J) was a strong interfering anion (k^{pot}_{IJ} = 1.4 x 10^{-1}).

Chen developed an $H_2PO_4^-$-ISE simply based on a metallic cobalt wire (Chen *et al.*, 1997). At pH 5, a linear response with a sub-Nernstian slope of -38.0 ± 0.5 mV per decade was obtained in the range 1.0 x 10^{-5}- 5.0 x 10^{-3} mol dm^{-3}, and a limit of detection of 1.0 x 10^{-6} mol dm^{-3}. The electrode did not exhibit a good selectivity against interferents with a k^{pot}_{IJ} of 1.4 x 10^{-2} for both nitrate and chloride. The speed of response was fast (about 20 seconds). Details on the long term stability were not reported. However, Engblom also used a cobalt wire to determine singly charged phosphate in a soil extract (Engblom, 1999) and reported that after only 24 hours storage in solution a crust was formed at the surface of the metallic wire affecting the speed of response. In order to get reproducible results the cobalt wire had to be polished almost everyday which would be a limiting factor for in-situ measurement.

Xiao also used a monobasic phosphate-ISE based on a cobalt matrix (Xiao *et al.*, 1995). However, the electrode was prepared by coating a cobalt rod with PTFE. The surface of one end was polished and the other end was welded to a copper wire. The potentiometric response showed a linear activity range from 10^{-2} to 10^{-5} mol dm^{-3} and a limit of detection of 5.0 x 10^{-5} mol dm^{-3}. The Nernstian slope was -55.0 mV per decade. The selectivity coefficients for $H_2PO_4^-$ against interferents (J) were 2.0 x 10^{-3} (chloride), 8.0 x 10^{-4} (nitrate), 1.0 x 10^{-3} (sulfate), 1.0 x 10^{-3} (acetate), 1.0 x 10^{-2} (bromide) and 4.0 x 10^{-3} (arsenate). These results represented an improvement on those obtained by both Chen and

Englblom. Xiao also postulated that the cobalt oxide layer covering the electrode surface was at the origin of the potentiometric response toward $H_2PO_4^-$.

1.4.3 Hydroxyapatite powders

Petrucelli developed a sensor for the monobasic form of phosphate based on hydroxyapatite powder (Petrucelli *et al.*, 1996). The hydroxyapatite powder was prepared by precipitation from a calcium hydroxide suspension and phosphoric acid solution. Two electrode arrangements were investigated.

The first electrode arrangement was prepared by placing at the bottom of a glass tube a mixture of graphite and epoxy polymer. A layer of hydroxyapatite was then incorporated onto the surface of the polymer and finally a layer of colloidal silver on the other side of the polymer. This electrode exhibited (at pH 5) a narrow linear Nernstian range between between 6.0 x 10^{-5} and 2.0 x 10^{-4} with a slope of -55.0 ± 1.2 mV per decade and a limit of detection of 4.0 x 10^{-5} mol dm^{-3} monobasic phosphate.

The second electrode arrangement was based on hydroxyapatite sticks. These were fabricated using a mixture of hydroxyapatite powder and 0.2% m/v poly(vinyl alcohol) placed in a glass tube, dried and sintered at 1000°C. The resulting stick was placed in a glass tube and coated with a conducting polymer. This electrode exhibited a wider linear range between 5.0 x 10^{-5} and 5.0 x 10^{-2} and a limit of detection of 2.5 x 10^{-5} mol dm^{-3} monobasic phosphate but the slope was sub-Nernstian (-33.8 ± 0.6 mV) at a pH of 5. The selectivity coefficients for phosphate against interferents (J) were respectively 2.2 x 10^{-4} (sulfate), 1.6 x 10^{-4} (citrate), 2.2 x 10^{-3} (acetate), 1.2 x 10^{-3} (chloride), 3.9 x 10^{-5} (nitrate) and 4.0 x 10^{-5} (perchlorate).

1.4.4 Biosensors

Biosensors have also been used for the determination of phosphate.

Kubo developed a phosphate sensor based on an immobilised enzyme and an oxygen electrode (Kubo *et al.*, 1983). The enzymatic reaction was based on the oxidation of pyruvate in the presence of phosphate and oxygen as illustrated below:

$$\text{pyruvate} + \text{Phosphate} + H_2O + O_2 \xrightarrow{\text{Pyruvate oxidase}} \text{Acetylphosphate} + H_2O_2 + CO_2$$

The consumption of dissolved oxygen due to the enzymatic reaction was monitored with an oxygen electrode as a current decrease. The linear range was between 12 and 80 μmol dm^{-3}, the speed of response was 7 minutes. The electrode response was not stable and after only 7 days the response decreased to 50% of the initial value.

Wollenberger also used an enzymatic system to determine phosphate (Wollenberger *et al.*, 1992). This system was based on the sequentially acting enzymes nucleoside phosphorylase (NP) and xanthine oxidase (XOD). The detection of phosphate was based on the reactions:

$$\text{Inosine} + \text{Pi} \xrightarrow{\text{NP}} \text{ribose-1-phosphate} + \text{hypoxanthine}$$

$$\text{Hypoxanthine} + 2\,O_2 \xrightarrow{\text{XOD}} \text{uric acid} + 2\,H_2O_2$$

The two enzymes have been membrane immobilised and fixed on a Clark-type oxygen electrode. The depletion of oxygen served as a measure for phosphate concentration between 0.5 and 100 μmol dm^{-3}. The reproducibility of this electrode was good, the RSD

was 5% for ten replicates at 5µmol dm^{-3}. The lifetime was about 8 days with no loss of sensitivity.

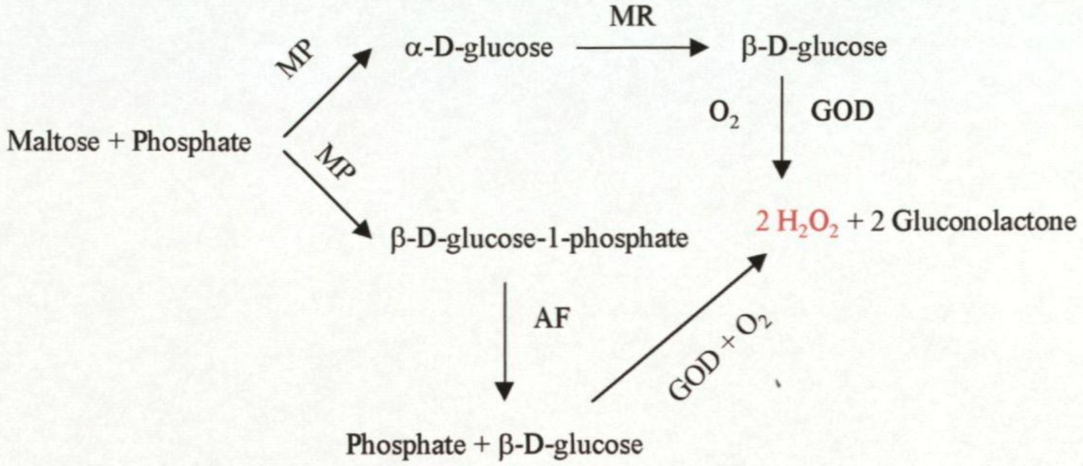
Based on the same principle phosphate was also determined using a plant tissue electrode (Campanella *et al.*, 1992). The phosphate was determined according to the following catalysed reactions:

$$\text{Glucose-6-phosphate} + H_2O \xrightarrow{\text{AF}} \text{Glucose} + H_2PO_4^-$$

$$\text{Glucose} + O_2 \xrightarrow{\text{GOD}} \text{gluconolactone} + H_2O_2$$

The two enzymes acid phosphatase (AF) and glucose oxidase (GOD) were immobilised in a potato slice tissue. The limit of detection obtained with this biosensor was 62 µmol dm^{-3} phosphate with a very narrow linear range between 1.4×10^{-3} and 8.6×10^{-5} phosphate. The speed of response was 5 minutes with a lifetime of less than 16 days with a loss of sensitivity of more than 30% after this period of time.

More recently, a highly sensitive enzyme based sensor was developed for the determination of inorganic phosphate using the same enzymatic reactions as described by Campanella *et al.* but also additional ones using maltose phosphorylase (MP) and mutarotase (MR) (Conrath *et al.*, 1995). The enzyme sequence used for the detection of inorganic phosphate is illustrated in the following enzymatic reactions:


MR
α-D-glucose
β-D-glucose
MP
MP
Maltose + Phosphate
O_2
GOD
β-D-glucose-1-phosphate
2 H_2O_2 + 2 Gluconolactone
AF
GOD + O_2
Phosphate + β-D-glucose

The four enzymes (GOD, MP, MR and AF) were immobilised on a regenerated cellulose membrane which was mounted on the tip of a platinum amperometric electrode for the detection of enzymatically formed hydrogen peroxide. The limit of detection was 1.0 x 10^{-8} mol dm^{-3} with a linear range between 0.1 and 1.0 μmol dm^{-3}. The sensitivity was good however the lifetime was not reported.

1.4.5 Liquid polymeric membranes

The first ever phosphate ISE reported in the literature was based upon a tin (IV) complex (Zarinskii *et al.*, 1980). The membrane was composed of a solution of dialkyltin phosphate in a chloroform decanol mixture. The electrode responded to the monobasic and dibasic form of phosphate over a linear range between 10^{-1} and 5 x 10^{-5} mol dm^{-3}.

Glazier reported a dibasic phosphate ISE based on derivatives of dibenzyltin dichloride (Glazier *et al.*, 1988 and 1991a,b). The electrode was fabricated with Nalgene tubing ($^{3}/_{32}$ in. i.d., $^{1}/_{32}$ in. wall) dipping in a THF solution containing 18% m/m tin compound, 33.4% m/m high molecular PVC, 12.6% m/m N,N-dimethylformamide and 36% m/m

dibutyl sebacate to form the membrane at the tip of the electrode body. The best performing tin derivative obtained was bis(p-chlorobenzyl)tin dichloride. A detection limit of 3.3 x 10^{-5} mol dm^{-3} and a linear Nernstian range from 2.2 x 10^{-4} to 1.2 x 10^{-2} mol dm^{-3} for dibasic-phosphate activity were obtained at pH 7. However the slope was super-Nernstian (slope: -33.0 ± 0.1 mV dec^{-1}). The response pattern of this electrode followed the conventional Hofmeister series. The potentiometric selectivity coefficients for phosphate against interfering ions (J) were respectively 4.9 (thiocyanide), 2.5 x 10^{-1} (iodide), 2 x 10^{-2} (nitrate), 1.6 x 10^{-2} (bromide), 3.1 x 10^{-3} (chloride) and 5 x 10^{-3} (acetate). The electrode lifetime was 28 days.

A study by Chaniotakis led to the development of a phosphate ISE based on a multidentate-tin(IV) carrier incorporated into liquid polymeric membrane (Chaniotakis *et al.*, 1993). Three tin compounds were examined and the most efficient one was tris(3-chlorodimethylstannyl-propyl) chlorostannane. The membrane composition was 32% m/m PVC, 66% m/m dioctyl sebacate and 2% m/m sensor molecule. This electrode exhibited a linear response to $H_2PO_4^-$ over a range between 10^{-2} and 10^{-4} mol dm^{-3} with a sub-Nernstian slope of - 40 mV per decade and a limit of detection of 1 x 10^{-5} mol dm^{-3}. Selectivity coefficients were not calculated and the lifetime of this electrode was very short (24 hours in total immersion).

Nishizawa used a bis-thiourea ionophore in a solvent polymeric membrane to determine HPO_4^{2-} (Nishizawa *et al.*, 1998). The best results were obtained for a membrane composed of 1% m/m phenylsubstituted bis-thiourea, 50 mol% tridodecylmethylammonium chloride (relative to the ionophore), 66% m/m 2-nitrophenyloctyl ether and 33% m/m PVC. The sensitivity of this electrode was poor; the limit of detection was (2.3 ± 0.5) x 10^{-4} mol dm^{-3}, the Nernstian slope was -29.7 ± 2 mV per decade over a very narrow linear range 10^{-2}- 2 x 10^{-3} mol dm^{-3} $H_2PO_4^{2-}$. The selectivity for dibasic phosphate ion against interfering anions was very poor. Nitrate was a very strong interferent with a reported k^{MPM}_{IJ} of 250. The electrode lifetime was about 28 days.

Antonisse used chemically modified field effect transistors (CHEMFETs) with ion-selective membranes containing uranyl salophene derivatives (Antonisse *et al.*, 1998). This electrode responded to $H_2PO_4^-$. The membrane was composed of 1% m/m sensor molecule, 20 mol% tetraoctylammonium chloride (relative to the sensor), 66% m/m dioctyl sebacate and 33% m/m PVC. This electrode yielded a high limit of detection of 6.3 x 10^{-4} mol dm^{-3} monobasic phosphate with a Nernstian slope of -56 mV per activity decade at a pH of 4.5. The selectivity coefficients for phosphate against interferents (J) were respectively 5 x 10^{-3} (sulfate), 1.2 x 10^{-2} (chloride), 5 x 10^{-2} (nitrate) and 2 x 10^{-2} (bromide).

A sensor for measuring phosphate has been reported by Carey (Carey and Riggan, 1994) comprising a cyclic polyamine ionophore for use in a dibasic phosphate-selective electrode required for dental studies. The sensor molecule (20% m/m) was trapped in PVC (45% m/m) with dibutyl sebacate (35% m/m) to give an electrode having a linear Nernstian response between 1.0 μM and 0.1 M. The limit of detection, though not reported, appeared to be slightly greater than 1.0 x 10^{-6} mol dm^{-3} HPO_4^{2-} at pH 7.2. The following selectivity coefficients, $k^{pot}_{HPO42-, J}$, were reported: Cl^-, 4.5 x 10^{-3}; NO_3^-, 1.7 x 10^{-3} and SO_4^{2-}, 1.0 x 10^{-3}. The selectivity of the electrode was then slightly improved by using dibutyl phtalate instead of dibutyl sebacate as plasticiser in the membranes (Carey and Lewis, 1996). Finally the addition of cationic and ionic highly lipophilic salts (HL) salts was investigated and showed that no significant improvements were gained by using them. The use of carboxylated PVC instead of PVC was also investigated and resulted in an increase in the linear range by two orders of magnitude from 10^{-1} to 3×10^{-9} mol dm^{-3} (Carey and Shore, 1998).

1.4.6 Conclusion

Biosensors have good sensitivity and selectivity for phosphate. However, they are costly, fragile and are usable only in laboratories. They are also pH sensitive and exhibit slow response (5-10 minutes).

Metallic cobalt wire and CWEs exhibit various results but suffer from poor reproducibility, sub-Nernstian responses, high limit of detection and limited selectivity. They are also prone to oxidation, causing the formation of crust on the surface of the wire which affects the phosphate response.

Ionophores in liquid polymeric membranes appear to be the best candidates to determine phosphate in the environment using ion-selective electrodes. Many ionophores have been investigated and the one reported by Carey exhibits the best performance in term of sensitivity and one of the best for the selectivity. However, this sensor molecule, as all the other ionophores cited here, is only trapped in PVC limiting its lifetime due to the leaching of the sensor through the membrane.

1.5 Survey of existing nitrate-ISEs

1.5.1 Background

The first nitrate-ISEs were based on ion-exchangers. The first commercial product, developed by Orion was a nitrate salt of a nonlabile transition metal complex cation such as tris(4,7-diphenyl-1,10-phenanthroline)nickel (II) (Bailey, 1980; Cattrall, 1997). This sensor was dissolved in a water immiscible solvent (p-nitrocymene). The second nitrate-exchanger was based on a quaternary ammonium salt. Beckman and Corning were the first manufacturers to use this ion-exchanger with similar results to those obtained by Orion. The first commercial versions were prone to erratic response, drift and short lifetime due to a lack of mechanical strength of the membranes and the electrical contact with the sample relied on a continuous leaking of solvent into the test solution.

However, following the work of Moody, the use of PVC for the fabrication of liquid polymeric membranes was adopted (Moody *et al.*, 1970). Davies used PVC-based membranes containing hexadecyl-tridodecylammonium nitrate, tris(4,7-diphenyl-1,10-phenanthroline)nickel (II) and 2-nitrophenyloctyl ether as plasticiser (Davies *et al.*, 1972).

The electrochemical parameters of these electrodes were similar than those originally obtained by the Orion and Corning companies but the lifetime of the electrodes was dramatically increased (Davies *et al.*, 1972).

Solvent mediators or plasticisers are an important component of liquid polymeric membranes. Their role is to soften the PVC to produce a rubbery membrane but they also play an important role in the electrode response. The first requirement for a plasticiser is to be compatible with the polymer used to prevent a two phase membrane or a greasy layer on its surface affecting the electrode response. Hulanicki investigated the influence of solvent mediators on the response of nitrate ISEs using tris(4,7-diphenyl-1,10-phenanthroline)nickel (II) nitrate as ion-exchanger in PVC-based membranes (Hulanicki *et al.*, 1978). Three plasticisers having different dielectric constant varying from 17.7 for 2-nitro-p-cymene (NC) to 23.5 for 2-nitrophenyloctyl ether (2-NPOE) and 28.3 for 2-nitrophenylphenyl ether (2-NPPE) were investigated. His studies showed that the Nernstian slope, linear range and limit of detection were similar using the different solvent mediators. However, the selectivity was better using 2-NPOE, suggesting that the dielectric constant of the medium influenced the selectivity of the electrodes. In 1984, Wegmann reported results obtained by incorporating quaternary ammonium compounds into solvent polymeric membranes with various plasticisers and composition (Wegmann *et al.*, 1984). The results showed that the ion selectivity was following the Hofmeister series in all the cases studied. All these liquid membrane electrodes studied were dependent on the distribution coefficients of the anions between the aqueous phase and organic membrane phase.

1.5.2 Quaternary ammonium salts

Nielsen developed a nitrate ISE also based on quaternary ammonium salts but the hydrophobicity of the ion-exchanger was optimised (Nielsen and Hansen, 1976). The

reason for this, was the water solubility of the original sensor leaching from the liquid polymeric membrane causing drift in the nitrate response. Nielsen found that tetra(n-dodecyl)ammonium nitrate was the best performing sensor from this standpoint. The membrane was composed of 33 % m/m PVC, 66% m/m dibutyl phtalate (DBP) and 1% quaternary ammonium salt (QAS).

In 1989, Wakida reported the first nitrate ISFET based on tridodecylmethylammonium nitrate (TDMA) and tetradecylammonium nitrate (TTMA) (Wakida *et al.*, 1989). The use of hydrophobic QAS improved the adhesion of the membrane on the insulator of the ISFET. The membrane was composed of 70% m/m ion-exchanger and 30% m/m PVC (no plasticiser). The most effective ISFET was obtained for TTMA as ion-exchanger with linear response between 1 and 10^{-5} mol dm^{-3} and a slope of -56 mV dec^{-1}. The electrode exhibited a poor selectivity with a $k^{pot}_{NO_3^-, Cl^-}$ of 1×10^{-1}.

A study by Rocher *et al.* led to the development of a nitrate ISFET (Rocher *et al.*, 1992). A differential measurement mode between a reference ISFET and the sensing ISFET was used. The insensitive layer was prepared by grafting 3-chloropropyldimethylchlorosilane on to silicon dioxide and the sensitive layer by functionalizing the grafted chlorosilane with trimethylamine. This sensor showed linear response from 10^{-1} to 10^{-3} mol dm^{-3} nitrate with a sub-Nernstian slope of -13 mV $decade^{-1}$. It was also pH dependent and had a short-term stability.

In 1993, Hara *et al.* developed a nitrate-selective coated wire electrode (CWE) based on tetraoctadecylammonium nitrate (TODAN) and investigated the influence of different

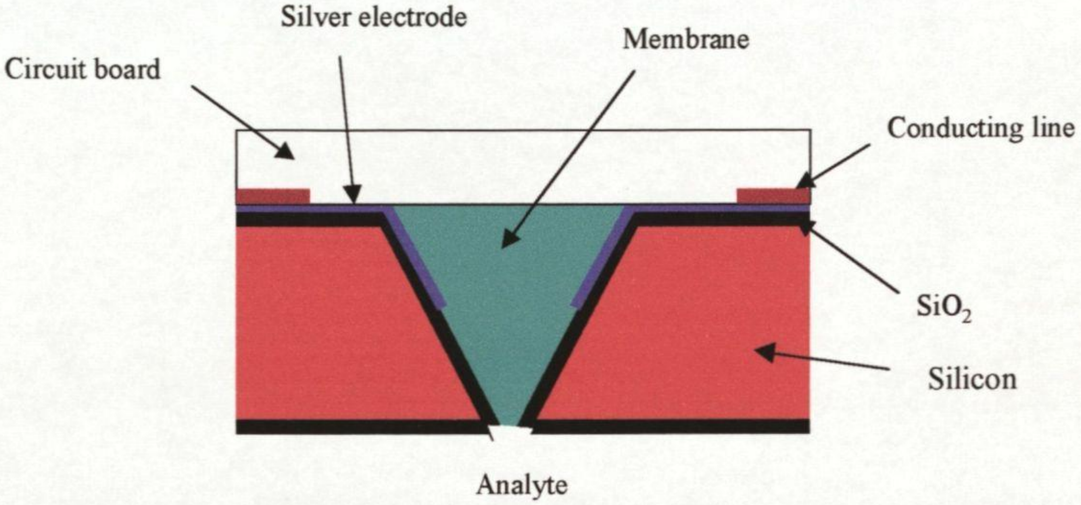
solvent mediators on its selectivity (Hara *et al.*, 1993). The membrane was composed of a mixture of stearyl alcohol (SA) as membrane matrix, TODAN as sensor and solvent mediators such as 2-NPOE or NPPE. The selectivity coefficients for various inorganic and organic interferents were measured for membranes with and without a solvent mediator. The electrode containing a solvent mediator such as 2-NPOE exhibited performance similar to that previous by evaluated quaternary ammonium salt (Zhen *et al.*, 1992). However, the membrane fabricated without a solvent mediator showed an anion-selectivity pattern not following the Hofmeister series and reducing interference caused especially by perchlorate, organic carboxylates and sulfonates.

Zuther reported a selective and long-term stable nitrate ISE (Zuther *et al.*, 1994). The electrode membrane was based on PVC, 2-NPOE and a modified quaternary ammonium salt (no details on its structure were given). The limit of detection was 1.5 ± 0.2 μmol dm^{-3} nitrate, the slope was -57.0 ± 1.0 mV per decade between a linear range from 10^{-2} and 2×10^{-5} mol dm^{-3} nitrate. The selectivity coefficients reported were 8×10^{-5} for sulfate, 3.1×10^{-4} for hydrogen carbonate and 4×10^{-3} for chloride. The lifetime of the sensor was reported to be 15 months. However, during that time, the slope was not constant, requiring regular recalibration (which would be a drawback for environmental monitoring). Moreover, the lifetime was monitored using only two sensors and after 2 months the slopes were different -55.5 mV dec^{-1} for the first one and -57.5 mV dec^{-1} for the second one and after 6 months the slopes dropped to -53 mV dec^{-1} for both sensors. This sensor was also used in a continuous flow system (Wassmus and Cammann, 1994).

Högg developed a sensor card with integrated reference for the detection of nitrate (Högg *et al.*, 1994). This sensor was based on the thick-film technology: The transducer was made of an epoxy resin substrate, two different conductive layers and an epoxy resin encapsulation; the receptor was a PVC-based nitrate-sensitive membrane composed of tridodecylammonium nitrate as ion-exchanger and 2-NPOE as plasticiser. This membrane was directly mounted on the surface of a silver layer. The reference electrode was a miniaturized Ag/AgCl-type with an encapsulated hydrogel electrolyte layer. The reproducibility of the electrode fabrication was poor because slopes as different as -52 and -58.8 mV per decade were obtained. Also the limit of detection varied from 10 to 2 μmol dm^{-3} nitrate.

Knoll developed a new method of integration of ion-sensitive membrane and silicon sensor chip (Knoll *et al.*, 1994a,b). The membrane was deposited into pyramidal containments in the sensor chip as described in figure 1.10. The most effective nitrate ISE was based on a membrane composed of 6.45% m/m tridodecylmethylammonium nitrate and 94.15 % m/m Dow Corning 730 solvent resistant sealant. The nitrate response was linear over a range between 10^{-1} and 4 x 10^{-5} mol dm^{-3} with a slope of -57 mV per decade.

Figure 1.10 Silicon chip



The limit of detection was not reported but was estimated at 5 μmol dm^{-3}. The lifetime was more than 95 days. However the sensor was not selective to nitrate over chloride with a reported k^{pot}_{IJ} of 5 x 10^{-2}.

A plasticiser-free polysiloxane membrane modified by trifluoropropyl groups was developed and investigated for the determination of nitrate using ISFET (Högg *et al*., 1996). The membrane was prepared with tetradodecylammonium bromide (3.75% m/m), fluorosiloxane polymer (94.8% m/m) and potassium tetrakis(4-chloro-phenyl)borate (1.45% m/m, 50 mol% relative to the ion-exchanger). The membrane was then deposited on the gate oxide of the ISFET. After appropriate conditioning the nitrate response was linear over a range between 10^{-2} and 3 x 10^{-4} mol dm^{-3} with a slope of -56.0 mV per decade and limit of detection was 10 μmol dm^{-3}. The selectivity coefficient for nitrate against chloride was 2 x 10^{-3}. The lifetime was more than 2 months with no drift in the

nitrate response. This nitrate-ISFET was quite selective but the linear range was very narrow and the limit of detection was too high for low nitrate determination.

The influence of the alkyl chain of quaternary ammonium salts on the selectivity was investigated by Kokovkin and Hamann (Kokovkin and Hamann, 1997). His study was based on a series of alkyl ammonium salts (R_4N^+, X^-) with R= CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11} and C_6H_{13} trapped in a PVC matrix with DBP as plasticiser. The study showed that the selectivity was improved by extending the length of the alkyl chain. The optimum selectivity was obtained for R = C_4H_9, C_5H_{11} and C_6H_{13}.

Hara and Izumiyama developed an automated continuous-flow determination system based on null-point potentiometry using a nitrate-selective electrode (Hara and Izumiyama, 1997). The membrane was composed of PVC, 2-nitrophenyldodecyl ether (NPDE), potassium tetrakis *p*-chlorophenyl borate (KTCPB) and methyltrioctadecylammonium bromide. The principle of the method was as follows: a nitrate standard solution, distilled water and ISAB were pumped into a mixing coil. Then by varying the flow rate of the sample and the distilled water, an automatic calibration graph could be achieved. Finally, the sample was pumped and the nitrate level measured. This flow-injection system allowed determination of nitrate automatically. However, fresh standard solutions were necessary to get accurate measurements and had to be changed on a daily basis. The limit of detection was around 10μmol dm^{-3} nitrate which is not sensitive enough for accurate measurements for river waters.

1.5.3 Quaternary phosphonium salts

Four different quaternary phosphonium salts, as ion-exchangers for nitrate-selective electrodes were investigated by Mitrakas (Mitrakas *et al.*, 1991). The membranes were composed of PVC, DBP and ion exchanger. The sensor molecules were tetraoctylphosphonium nitrate (TOPN) tetradodecylammonium nitrate (TDDAN), tributylhexadecylphosphonium nitrate (TBHDPN), tetradodecylphosphonium nitrate (TDDPN) and tetrahexadecylphosphonium nitrate (THDPN). The detection limit decreased in the sequence TBHDPN (1.8 x 10^{-5} mol dm^{-3}), TOPN (7 x 10^{-6} mol dm^{-3}), TDDPN (2.5 x 10^{-6} mol dm^{-3}) and THDPN (2.1 x 10^{-6} mol dm^{-3}) as the molecular weight increased. The most satisfactory electrode had a limit of detection of 2.1 x 10^{-6} mol dm^{-3} nitrate and a slope of -56.3 mV per decade over a linear range from 10^{-1} to 1.7 x 10^{-5} mol dm^{-3} nitrate. The electrode lifetime was 4 months. However, the electrode suffered from strong interferences caused especially by bromide ($k^{pot}_{NO3-, Br-}$ = 0.11) and nitrite ($k^{pot}_{NO3-,NO2-}$= 4.5 x 10^{-2}) which would be a limiting factor for *in-situ* measurements.

Rocheleau and Purdy developed a nitrate ISE based on a fixed quaternary phosphonium salt (Rocheleau and Purdy, 1992). The polymeric membrane was prepared by direct functionalization of poly(vinylbenzyl chloride) (PVBC) with trioctyl phosphine forming poly(trioctylvinylbenzylphosphonium chloride). After appropriate conditioning,, the nitrate-selective electrode exhibited a linear response from 10^{-1} to 5 x 10^{-5}, with a slope of -53.4 ± 0.5 mV per decade. The reported selectivity coefficient for nitrate against chloride was 8 x 10^{-3}. The lifetime of the electrode was claimed to be about 10 months with little deterioration of the membrane response. However, no data were given to confirm this.

1.5.4 Tris(4,7-diphenyl-1,10-phenanthroline)nickel (II) complexes

Tris(4,7-diphenyl-1,10-phenanthroline)nickel (II) complex was re-investigated as the ion-exchanger in a nitrate ISE (Lapa *et al.*, 1997). The membrane composition was as described by Hulanicki (Hulanicki *et al.*, 1978) but a different electrode arrangement was used: two semi-circular membranes were applied directly over a conductor support made of a graphite mixture with an epoxy resin at the tip of the electrode body. The two membrane potentials were added using a summing device and improved sensitivity on classical ion-selective electrodes was claimed. However, nitrite caused significant interference to the electrode, with a reported $k^{pot}_{NO3-, NO2-}$ of 1.4 x 10^{-1} using SSM (equimolar amount of both ions), which could be a problem for the nitrate monitoring of some environmental waters.

1.5.5 Special sensors

Lal developed a nitrate ISE based on nitron nitrate in 'Araldite' (Lal *et al.*, 1980). The electrode response was linear between 10^{-1} and 10^{-4} mol dm^{-3} nitrate with a slope -50 mV per decade. The lifetime of the electrode was reported to be 6 weeks with a rapid loss of sensitivity after this period of time. The selectivity of this electrode was very poor with selectivity coefficient for nitrate against chloride of 2.9 x 10^{-1} which would also limit its use.

A study by Werner showed that bis(triphenylphosphine)iminium (Aliquat 336S) and nitrate anions in nitrobenzene responded to nitrate (Werner *et al.*, 1989). The linear

Nernstian range was between 10^{-1} and 10^{-6} mol dm^{-3} nitrate activity with a very good slope of -58.1 mV per nitrate activity decade. The speed of response was about 5 minutes at low concentrations. The limit of detection was reported to be 7.9 x 10^{-7} mol dm^{-3} nitrate activity. However, this electrode suffered from considerable interference caused especially by nitrite anion and short term stability that would limit their uses in environmental waters.

Ermolenko reported a nitrate-selective sensor with a crystalline membrane (Ermolenko *et al.*, 1995). The sensor was based on a mixture of sodium diethyldithiocarbamate and sodium sulfide added to silver nitrate forming a precipitate of silver salts which were mixed and pressed to get the sensitive layer. The thin-film was completed by a solid silver contact, formed using a silver-conducting compound. After a short conditioning, it was found that the linear Nernstian response was from 10^{-1} to 10^{-5} mol dm^{-3} with a slope of -57 ± 2 mV dec^{-1}. The limit of detection was estimated to about 2μmol dm^{-3}. The reported selectivity coefficients were as follows: 2 x 10^{-2} for sulfate, 6 x 10^{-3} for phosphate and 10^{-3} for hydrogen carbonate. The sensitivity of this sensor was good, the selectivity was not as good as that reported by Zuther and the lifetime was not reported but the potential drift in a nitrate solution of 2 x 10^{-4} mol dm^{-3} was 10 mV after 3 months.

Braven reported a new kind of sensor for nitrate ISE based on a betaine; Glycine betaine hydrochloride (Braven *et al.*, 1996). The membrane composition was 5% m/m sensor, 7.5% m/m dicumyl peroxide, 41.5% m/m 2-NPOE and 46% m/m poly(acrylonitrile-butadiene) copolymer. The slope was -52 mV dec^{-1} over a linear range from 0.1 to 1.0 x 10^{-5}, the limit of detection was 1.1 x 10^{-5} nitrate, and the $k^{pot}_{NO_3^-, Cl^-}$ was 9 x 10^{-3}. The

compound was the simplest of all amino-acid betaines. It lacked any hydrophobic character. It was therefore unexpected that it would perform so well and match most nitrate-selective electrodes in sensitivity and selectivity. However, the lifetime was short because it was only trapped in the matrix.

In 1997, Sun and Fitch developed a nitrate-selective sensor based on electrochemically prepared conducting polypyrrole films (Sun and Fitch , 1997). The film was composed of 0.05 mol dm^{-3} pyrrole monomer, 0.1 mol dm^{-3} lithium nitrate and 15% DBP in acetonitrile and coated on a glassy carbon substrate by electropolymerisation. The sensor was responding to nitrate over a linear range from 10^{-1} to 7.4×10^{-5} mol dm^{-3} with a Nernstian slope of - 56.9 mV dec^{-1}. The limit of detection was 4.7×10^{-5} mol dm^{-3} nitrate and the claimed lifetime was 6 months. However, the selectivity was not reported.

An interesting study by Zhen compared nitrate ISEs made with different sensor molecules and different plasticisers (Zhen *et al.*, 1992); all had PVC as a component of the membranes. Zhen investigated methyltridodecylammonium nitrate (MTDDAN), tris(substituted, 1,10-phenanthroline)nickel (II) nitrate and bis(triphenylphosphine)iminium (Aliquat 336s). The results are summarised in Table 1.4.

Table 1.4 The main response characteristics of nitrate-selective electrodes made using three different types of nitrate sensor molecule

Electrochemical parameters .	MTDDAN	Aliquat 336s	Nickel (II) complex
Slope (mV dec^{-1})	-52 ± 1	-53 ± 1.9	-49 ± 2
Limit of detection (µmol dm^{-3})	21.5 ± 10	29.9 ± 7.7	46 ± 4
$k^{pot}_{NO3-, Cl-}$	3.5 x 10^{-2}	1.9 x 10^{-1}	1.3 x 10^{-2}

Table 1.4 showed that from the sensor molecules investigated by Zhen, the quaternary ammonium salts gave the best results. Zhen also evaluated different plasticisers, using MTTDAN, such as 2-nitrophenyloctyl ether and dibutyl phtalate showing that the most selective membrane was obtained with 2-nitrophenyloctyl ether. He also showed that no significant improvements were gained increasing the concentration of MTTDAN in the PVC-based membranes. The optimum membrane composition was 6% m/m MTTDAN, 65% m/m 2-NPOE and 29% m/m PVC. The addition 1% m/m methyltriphenyl phosphonium bromide in the membrane composition decreased chloride interference.

1.5.6 Covalent bonding

Ebdon and Ellis (Ebdon *et al.*, 1979 & 1982; Ellis *et al.*, 1980) were the first to combine a cross-linked polymeric structure and covalently attachment of an ion-exchanger for the production of a calcium-selective electrode. This was achieved by the cross-linking of poly(styrene-*block*-butadiene-*block*-styrene) (SBS) block polymer with the allyl substituents of the sensor molecule in using a free radical initiated copolymerisation. King

developed a nitrate-selective electrode using the same principle (King, 1985). The ion-exchanger was based on a quaternary ammonium salt, a proposed reaction mechanism for the covalent attachment of ion-exchanger is shown in figures 1.11 and 1.12. The reaction mechanism shows free radical attack on either the SBS polymer or quaternary ammonium salt. Immobilisation shown in figure 1.11 of the sensor molecule onto the polymer takes place in three steps. Initiation (A), radical attack on the 1,2 addition product of the polymer, propagation (B), butadiene group of polymer attaching to allyl group sensor and cross-linking (C), two polymer units cross-linked via the allyl group sensor. However, steric considerations may limit the number of polymer chains which react with the allyl groups in the quaternary ammonium salt. These SBS membranes were robust, showed a fast response and extended lifetime but suffered from poor selectivity for nitrate in the presence of interfering anions. Moody believed this was due to the lack of solvent mediator from the membrane composition (Moody *et al.*, 1988). However, it was reported that most solvent mediators used for nitrate-selective electrodes were incompatible with SBS (King, 1985; Frampton, 1992). Therefore, an alternative polymeric material, poly(acrylonitrile-butadiene) (Krynac) was selected by Frampton (Ebdon *et al.*, 1990 and 1991). The development of a hot pressing technique suitable for improved membrane preparation and large scale manufacturing was also developed. The best electrode produced contained 6.5% m/m triallyloctylammonium bromide, 7.2% m/m dicumyl peroxide and 46.6% m/m Krynac 50.75 (50% acrylonitrile content). The response to nitrate was Nernstian in the range 1 x 10^{-1} to 1 x 10^{-4} mol dm^{-3} of nitrate. The limit of detection was 4.5 x 10^{-5} and the selectivity coefficient $k^{pot}_{NO_3^-, Cl^-}$ of 5.3 x 10^{-3}. The electrode had a lifetime in excess of 665 days. In 1999, an improved nitrate-selective electrode was produced based on triallydecyl ammonium nitrate (TADAN) having a Nernstian slope of -57.7 mV $decade^{-1}$ over a nitrate range of 1.0 x 10^{-1} to 1.2 x 10^{-5} mol dm^{-3} nitrate with a limit of detection of 8.8 x 10^{-6} mol dm^{-3} nitrate (Sutton *et al.*, 1999; Scholefield *et al.*, 1999).

Figure 1.11 **Radical attack on the SBS polymer**

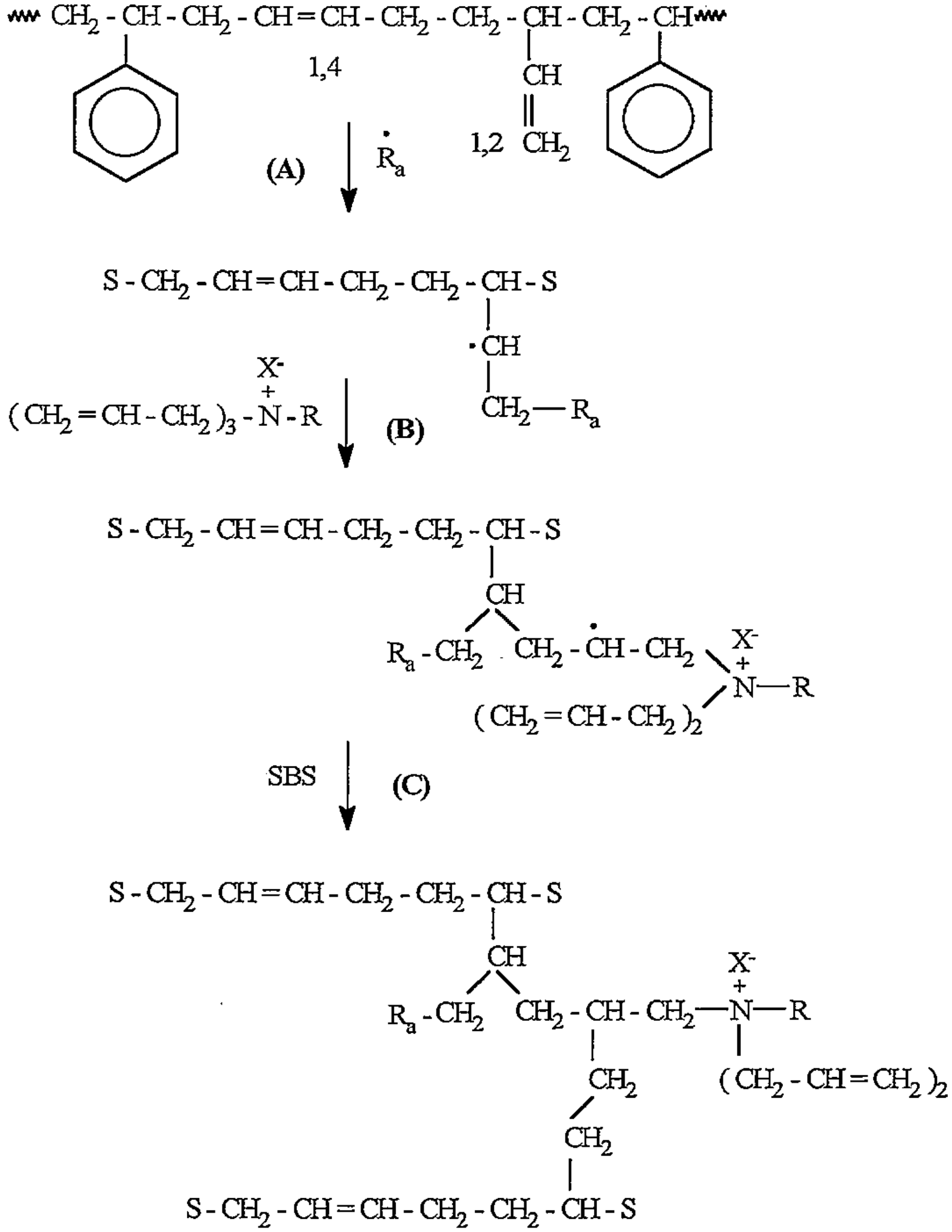
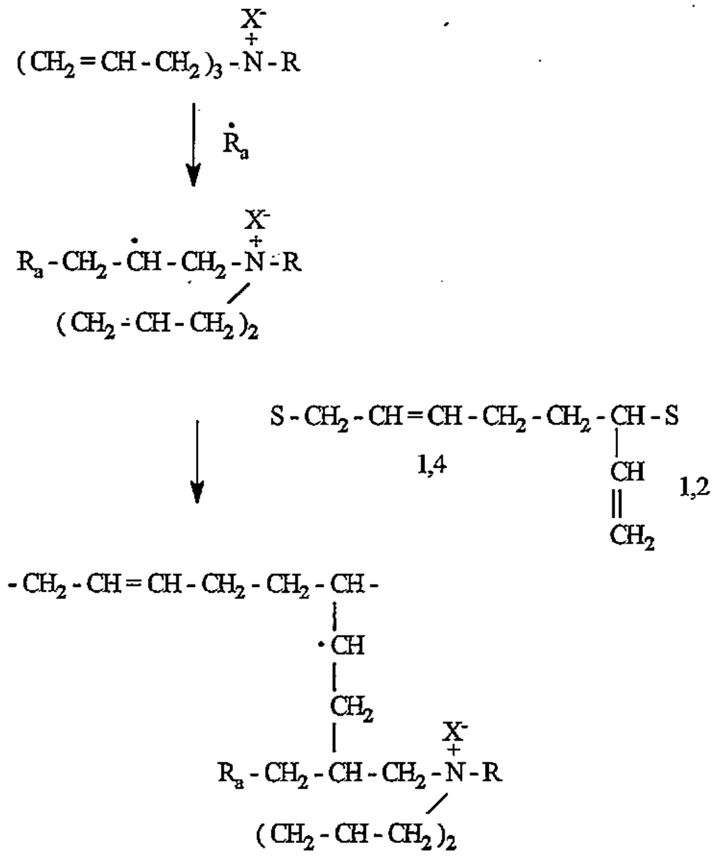

1,4
1,2
(A)
$\dot{R}_a$
(B)
(C)
SBS
S
X^-
R

Figure 1.12 Radical attack on the allyl substituent of the sensor molecule



$(CH_2=CH-CH_2)_3-\overset{+}{N}-R\ X^-$
$\dot{R}_a$
$R_a-CH_2-\dot{C}H-CH_2-\overset{+}{N}-R\ X^-$
$(CH_2=CH-CH_2)_2$
S - CH2 - CH = CH - CH2 - CH2 - CH - S
1,4
CH
CH2
1,2
- CH2 - CH = CH - CH2 - CH2 - CH -
• CH
CH2
R_a - CH2 - CH - CH2 - N - R X-
(CH2 - CH - CH2)2

1.6 Aim of the present study

Environmental legislation, water companies and health considerations indicate a pressing need for the development of on-site devices for the measurement of nitrate and phosphate in environmental and potable waters. Such electrodes are required to have a long working lifetime, a good sensitivity and selectivity, a wide linear Nernstian range, large pH range, good repeatability and reproducibility, fast response and excellent mechanical strength. Nitrate electrodes should have a linear range between 70 and 0.07 mg NO_3^--N l^{-1} (5×10^{-3} - 5×10^{-6} mol dm^{-3} NO_3^-) and the sensitivity of phosphate electrodes should be around 0.03 mg HPO_4^{2-} -P l^{-1} (1×10^{-7} mol dm^{-3} HPO_4^{2-}). Both electrodes should also be very selective especially with respect to anions likely to occur in environmental waters. At

present there are no suitable ion-selective electrodes for either nitrate or phosphate capable of being used *in situ* in environmental waters.

The first aim of this work was to attempt to develop a nitrate-selective electrode by immobilisation of a selection of α-aminoacid betaine sensor molecules (Braven *et al.*, 1996) onto a polymeric material. Optimisation of electrode performance would be determined by structure-activity relationship investigations and variation of membrane composition. After development in the laboratory the most promising electrode would be evaluated *in-situ* in environmental waters, using the criteria given previously.

The second aim was to investigate the possibility of producing a phosphate-selective electrode based on the heterocyclic macrocycle (Carey and Riggan, 1994) by modifying its structure to enable the covalent bonding of the sensor to a polymeric matrix.

CHAPTER 2

DEVELOPMENT OF A NOVEL NITRATE SENSOR FOR LIQUID POLYMERIC MEMBRANES

2.1 Materials and synthesis

2.1.1 Reagents and polymers

All the chemicals required for organic synthesis were obtained from Aldrich Ltd. (Gillingham, Dorset, UK) unless stated otherwise. Purity was routinely checked by chromatography (GC or TLC) or nuclear magnetic resonance spectroscopy. Solvents were of HPLC grade and obtained from Rathburn Chemicals Ltd. (Walkerburn, Peeblenshire, Scotland). Tetrahydrofuran (THF) was refluxed over potassium metal and freshly distilled prior to use. Dicumyl peroxide (DCP) and the solvent mediators 2-nitrophenyloctyl ether (2-NPOE), 2-nitrophenyldodecyl ether (2-NPDE), dibutyl phtalate (DBP), dibutyl sebacate (DBS) (Selectophore grade, Fluka Chemicals, Gillingham, Dorset, UK) were used as received. 2-Fluoro-2'-nitrodiphenyl ether (2-F-2'-NDPE) was synthesised according to a previous method (Sutton, 1996).

Three different polymeric materials were evaluated as matrix for nitrate-selective electrodes. The matrix was evaluated either without sensor or with sensor molecule covalently bound to it. In some cases the sensor was just entrapped.

2.1.1.1 Poly(acrylonitrile-butadiene), (Krynac)

This material is a random copolymer of acrylonitrile and butadiene. The proportion of each monomer can be adjusted and commercial grades in the range 18-50 % acrylonitrile are readily available. Krynac 50.75 (Polysar UK, Guildford, Surrey, UK) with 50 % acrylonitrile was selected in the first part of this work for its robustness (Frampton, 1992).

The polymer was purified prior to use as follows : 15 g of polymer was dissolved in 75 ml of tetrahydrofuran and reprecipitated by the addition of 200ml of cold methanol. The polymer was then collected by filtration and allowed to dry in a vacuum oven.

2.1.1.2 Polystyrene-*block*-polybutadiene-*block*-polystyrene, (SBS)

This polymer was previously used in the composition of calcium and nitrate selective electrodes (Ebdon *et al.*, 1979, 1982 and 1985; Ellis *et al.*, 1980). The first SBS used in the present study was SBS 1101 (Cariflex Styrene Butadiene Rubber, Shell Chemicals, London, UK) and contained 30 % styrene and a phenolic based anti-oxidant. The second one was obtained from Aldrich Ltd. and had a low level of anti-oxidant with the same polystyrene content.

2.1.2 Characterisation

2.1.2.1 Nuclear Magnetic Resonance spectroscopy

A Jeol EX270 MHz NMR spectrometer was used to record proton (^1H) spectra at 270 MHz, carbon-13 (^{13}C) spectra at 67.8 MHz, DEPT-135 and ^1H-^{13}C COSY spectra. Samples were prepared in a suitable deuterated solvent in 5mm NMR tubes (Aldrich, Gillingham, Dorset, UK). Samples were filtered through cellulose if necessary and care was taken to avoid high viscosity of samples to prevent signal line broadening and loss of resolution. For proton spectra, the samples contained tetramethylsilane (TMS) for referencing at 0 ppm. The residual protonated signal from deuterated chloroform ($CDCl_3$) (singlet, 7.24 ppm), deuterium oxide (D_2O) (singlet, 4.6 ppm) and deuterated dimethylsulfoxide (D_6MSO) (multiplet, 2.5 ppm) were employed as secondary references. For carbon-13 spectra, samples in $CDCl_3$ were referenced with the characteristic triplet at 77.00 ppm and samples in D_6MSO were referenced with the septet at 39.5 ppm.

2.1.2.2 Infra-Red Spectroscopy

A Bruker IFS 66 (Bruker Analytische, Karlruhe, FRG) instrument was used. All infrared spectra were recorded from 4000 cm^{-1} to 650 cm^{-1}. Liquids were analysed as thin films and solids as potassium bromide discs.

2.1.2.3 Thin Layer Chromatography

TLC analyses were carried out using Fluka 0.2 mm silica gel TLC cards with aluminium backs and containing a fluorescent indicator at 254 nm. The samples were prepared in suitable solvents at a concentration of about 10 mg ml^{-1} and 50 μl was applied to the TLC card. Two mobile phases were used (a) ethyl acetate (EA) and (b) a solvent system of 60% butanol, 20% acetic acid and 20% water (BAW). Samples were visualised using iodine vapour. Amino-acids were visualised using ninhydrin.

2.1.2.4 Gas chromatography

Gas chromatographic analysis were performed using an HRGC Carlo Erba Strumentazione gas chromatograph with a non-polar DB-5 column (BDH Chemicals Ltd.), (length 25 m, internal diameter 0.32 mm) and a flame ionisation detector.

Samples were dissolved in dichloromethane at a concentration of 0.5 mg ml^{-1} and 0.5 μl injected. The oven temperature was set at 50°C with a ramp rate of 10°C min^{-1} and a final oven temperature of 320°C held for 5 minutes.

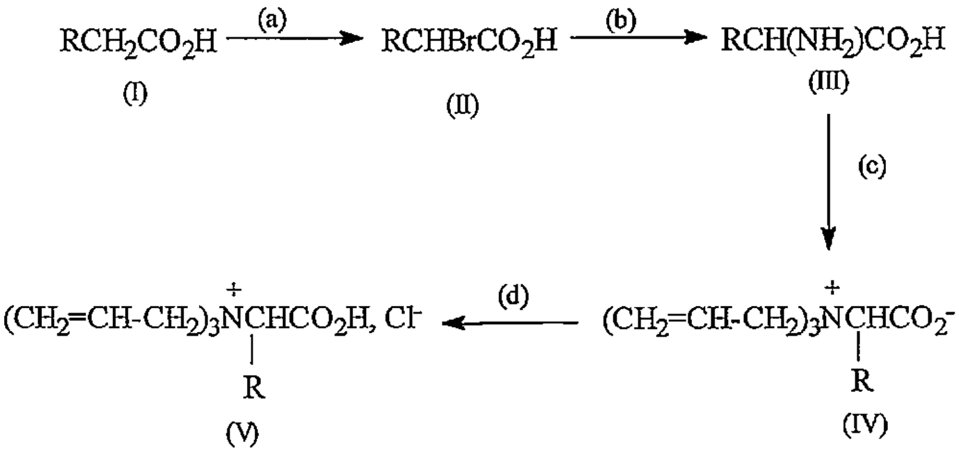
2.1.3 Betaine synthesis

Naturally occuring betaines have the structure $Me_3\overset{+}{N}CHRCO_2^-$ (Blundel *et al.*, 1986 and 1992). Liebreich reported the formation of N,N,N-trimethyl glycine betaine chloride (R=H) by heating α-chloroacetic acid and trimethylamine in alcohol (Liebreich, 1869, 1870). This route was investigated for the synthesis of N,N,N-trimethyl glycine betaine chloride or other betaines. Unfortunately this route was found to be successful only for the formation of N,N,N-trimethyl glycine betaine chloride and failed due to an elimination reaction.

2.1.3.1 Chen and Benoiton route

It was reported in the literature (Chen and Benoiton, 1976) that the synthesis of some betaines was possible using α-amino-acids and an excess of alkyl halide as shown in figure 2.1 (c). This route was finally chosen and gave good yields of pure products apart from the synthesis of N,N,N-triallyl valine betaine.

Figure 2.1 Synthetic route to form N, N, N- triallyl α-amino-acid betaine salts


RCH2CO2H
(I)
(a)
RCHBrCO2H
(II)
(b)
RCH(NH2)CO2H
(III)
(c)
(CH2=CH-CH2)3N+CHCO2-
R
(IV)
(d)
(CH2=CH-CH2)3N+CHCO2H, Cl-
R
(V)

[a, Br_2, PCl_3 (Clarke and Taylor, 1953); b, NH_3 (Marvel and Du Vigneaud, 1953); c, C_3H_5Br, MeOH,$KHCO_3$ or Na_2CO_3 (Chen and Benoiton, 1976); d, HCl (aqueous or gaseous).

The number of steps taken to synthesise any individual betaine varied according to the commercial availability of appropriate starting materials (I), (II) or (III). All synthesised compounds were examined for purity by TLC, following purification by flash chromatography on silica gel (BDH, silica gel for flash chromatography). Compounds were fully characterised by IR, NMR (^1H, ^{13}C, DEPT and correlation spectroscopy (COSY)).

All the betaine salts (V) and the ester (VI) were viscous oils at room temperature except R= H and N, N, N-triallyl taurine betaine chloride (VII). These latter were crystalline solids m.p 154°C and 205°C respectively.

The following betaine salts (V) were synthesised and used in the study :

R= H, CH_3CH_2, $CH_3(CH_2)_2$, $(CH_3)_2CH$, $CH_3(CH_2)_3$, $(CH_3)_2CHCH_2$, $CH_3(CH_2)_4$, $CH_3(CH_2)_5$, $CH_3(CH_2)_9$.

The following were also synthesised N,N,N-triallyl leucine betaine allyl ester bromide (VI), $(CH_2{=}CH\text{-}CH_2)_3\overset{+}{N}CH(CH_2CH(CH_3)_2)CO_2(CH_2\text{-}CH{=}CH_2), Br^-$, and the N,N,N-triallyl taurine chloride derivative (VII), $(CH_2{=}CH\text{-}CH_2)\overset{+}{N}CH_2\text{-}CH_2SO_3H, Cl^-$.

N,N,N-triallyl valine betaine was synthesised in two steps because the Benoiton and Chen method failed the diallyl compound was formed.

All the details of the synthesis are given in sections 2.1.3.2.8-2.1.2.4.11.

2.1.3.2 Details of the synthesis of individual compopuds

2.1.3.2.1 Synthesis of N,N,N-triallyl glycine betaine

$C_{11}H_{17}NO_2$, MW = 195.26,

$$(\underset{a}{CH_2}{=}\underset{b}{CH}\text{-}\underset{c}{CH_2})_3\overset{+}{N}\text{-}\underset{d}{CH_2}\text{-}\underset{e}{CO_2^-}$$

Glycine (2.5 g, 0.033 mol) was added to methanol (500 ml) at room temperature, followed by potassium hydrogen carbonate (25 g, 0.25 mol) and allyl bromide (35 ml, 0.39 mol). After three days stirring, the mixture was evaporated to dryness. The residue was extracted with chloroform. The chloroform extract was then filtered, dried over anhydrous sodium sulphate, and evaporated to yield N,N,N-triallyl glycine betaine as a white solid.

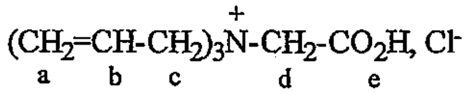
Yield : 6.5g (76%), **Rf** : 0.30 (BAW), 0 (EA).

NMR ^{1}H (D_6MSO) : δ (ppm) : 3.66 ($3H_d$, s), 4.17 ($6H_c$, d), 5.73 ($6H_a$, dd), 5.97 ($3H_b$, ddt).

NMR ^{13}C :δ (ppm) : 164.54 (C_e), 129.25 ($3C_a$), 124.15 ($3C_b$), 60.48 ($3C_c$), 58.83 (C_d).

2.1.3.2.2 Synthesis of N,N,N-triallyl glycine betaine chloride

$C_{11}H_{18}ClNO_2$, MW = 231.72,



$\underset{a}{(CH_2}=\underset{b}{CH}-\underset{c}{CH_2})_3\overset{+}{N}-\underset{d}{CH_2}-\underset{e}{CO_2H}, Cl^-$

N,N,N-Triallyl glycine betaine (3.5 g, 0.018 mol) was dissolved in water (40 ml). 2 mol dm^{-3} hydrochloric acid (10ml, 0.020mol) was added dropwise to the solution and then stirred for two hours at room temperature. The solution was filtered and evaporated to dryness. The crude product was recrystallised from hot ethanol, to give pure N,N,N-triallyl glycine betaine chloride as a white solid.

Yield : 2.90g (46.5%), **mp** : 154°C, **Rf** : 0.32 (BAW), 0 (EA).

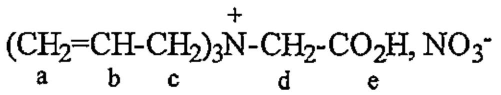
IR : $\nu_{(-OH)}$ = 3550 cm^{-1}, $\delta_{(-OH)}$ = 941 cm^{-1}, $\nu_{(-C=C)}$ = 3093, 3007 cm^{-1}, $\nu_{(-CH,\ CH2)}$ = 2932, 2850 cm^{-1}, $\delta_{(-CH\ ,\ CH2)}$ = 1475, 1457 cm^{-1}, $\nu_{(-CH,\ CH2\ in\ plane)}$ = 1412 cm^{-1}, $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 995 cm^{-1}, $\nu_{(-C=O)}$ = 1718 cm^{-1}, $\nu_{(-C-0)}$, $\delta_{(OH)}$ = 1439 cm^{-1}, $\nu_{(-C-0)}$, $\delta_{(OH)}$ = 1222 cm^{-1}, $\nu_{(-C=C-)}$ = 1639 cm^{-1}.

NMR ^{1}H (D_6MSO) : δ (ppm) : 4.41 (6H_c , d), 4.42 (H_d , s), 5.88 (6H_a , dd), 6.35 (3H_b, ddt).

NMR ^{13}C : δ (ppm) : 166.29 (C_e), 128.09 (C_a), 125.64 (C_b), 61.98 (C_c), 56.32 (C_d).

2.1.3.2.3 Synthesis of N,N,N-triallyl glycine betaine nitrate

$C_{11}H_{18}N_2O_5$, MW = 258.27,



$$(\underset{a}{CH_2}=\underset{b}{CH}-\underset{c}{CH_2})_3\overset{+}{N}-\underset{d}{CH_2}-\underset{e}{CO_2H}, NO_3^-$$

N,N,N-Triallyl glycine betaine chloride (1.02 g, 4.4 mmol) was dissolved in water (15ml) and a slight excess of silver nitrate (0.77 g, 4.53 mmol) was added to the solution. The mixture was centrifuged to separate the silver chloride precipitate and the aqueous phase which contained the product was evaporated to dryness. The crude N,N,N-triallyl glycine betaine nitrate was recrystallised from hot ethanol, to give an off-white solid.

Yield : 0.8g (67%), **mp** : 72°C, **Rf** : 0.31 (BAW), 0 (EA).

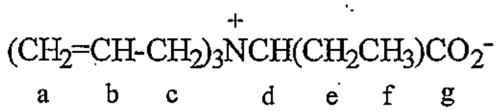
IR : $\nu_{(-OH)}$ = 3449 cm^{-1}, $\nu_{(NO3-)}$ = 1365 cm^{-1}, $\nu_{(-CH,\ CH2)}$ = 2960, 2926 cm^{-1}, $\nu_{(-C=O)}$ = 1735 cm^{-1}, $\delta_{(-CH\ ,\ CH2)}$ = 1473 cm^{-1}, $\nu_{(-CH,\ CH2\ in\ plane)}$ = 1413 cm^{-1}, $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 993 cm^{-1}, $\nu_{(-C-0)}$, $\delta_{(OH)}$ = 1455 cm^{-1}, $\nu_{(-C-0)}$, $\delta_{(OH)}$ = 1206 cm^{-1}, $\nu_{(-C=C-)}$=1640 cm^{-1}.

NMR ^{1}H (D_2O) : δ (ppm) : 3.86 (2H_d, s), 3.94 (6H_c, d), 5.54 (6H_a, dd), 5.84 (3H_b, ddt).

NMR ^{13}C : δ (ppm) : 56.37 (C_d), 61.94 ($3C_c$), 124.26 ($3C_b$), 129.90 ($3C_a$), 167.96 (C_e).

2.1.3.2.4 Synthesis of N,N,N-triallyl α-aminobutyric acid betaine

$C_{13}H_{21}O_2N$, MW = 223,



$(\underset{a}{CH_2}=\underset{b}{CH}-\underset{c}{CH_2})_3\overset{+}{N}\underset{d}{CH}(\underset{e}{CH_2}\underset{f}{CH_3})\underset{g}{CO_2^-}$

The method used to synthesise N,N,N-triallyl α-aminobutyric acid betaine is described in section 2.1.3.2.1 apart from the purification which was carried out using dry flash chromatography. Pure N,N,N-triallyl α-aminobutyric acid betaine was a viscous yellow oil.

Yield : 50 %, **Rf** : 0.40 (BAW), 0 (EA).

IR : $\nu_{(COO-)}$ = 1624.4, 1363.2 cm^{-1}, $\nu_{(-CH, CH3)}$ = 2979.4, 2854 cm^{-1}, $\nu_{(-CH, CH2)}$ = 2924.7 and 2882.2 cm^{-1} , $\delta_{(-CH, CH2)}$ = 1463.3cm^{-1}, $\delta_{(-CH, vinyl)}$ = 3086.2 cm^{-1}, $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 997.9 cm^{-1}.

NMR ^{1}H (CDCl$_3$): δ (ppm) : 0.80 ($3H_f$, t), 1.90 ($2H_e$, broad s), 3.55 ($1H_d$, dd), 4.05 ($6H_c$, dq), 5.55 ($6H_a$, dd), 5.95 ($3H_b$, ddt).

NMR ^{13}C : δ (ppm) : 10.34 (C_f), 20.58 (C_e), 62.32 ($3C_c$), 76.75 (C_d), 126.04 ($3C_b$), 128.13 ($3C_a$), 172.62 (C_g).

2.1.3.2.5 Synthesis of N,N,N-triallyl α-aminobutyric acid betaine chloride

$C_{13}H_{22}NO_2Cl$, MW = 259.5,

$(CH_2{=}CH\text{-}CH_2)_3\overset{+}{N}CH(CH_2CH_3)CO_2H,\ Cl^-$

a b c d e f g

N,N,N-Triallyl α-aminobutyric acid betaine chloride was synthesised using the method described in section 2.1.3.2.2. The pure product was a viscous yellow oil.

Yield : 90 %, **Rf** : 0.40 (BAW), 0 (EA).

IR : $\nu_{(\text{-OH})}$ = 3358 cm^{-1}, $\delta_{(\text{-OH})}$ = 1201.2, 958.2. cm^{-1}, $\nu_{(\text{-C=O})}$ = 1735.8 cm^{-1}, $\nu_{(\text{-CH, CH3})}$ = 2962.9.6, 2872.7 cm^{-1}, $\nu_{(\text{-CH, CH2})}$ = 2926.7 and 2872.7 cm^{-1}, $\delta_{(\text{-CH, CH2})}$ = 1463.5 cm^{-1}, $\nu_{(\text{-C=C-})}$ = 1641.6 cm^{-1}, $\delta_{(\text{-CH, vinyl})}$ = 3085.7 cm^{-1}, $\delta_{(\text{-CH out of plane, vinyl})}$ = 993.9 cm^{-1}.

NMR ^{1}H (D_2O): δ (ppm) : 0.74 (3H_f, t), 1.87 (2H_e, m), 3.78 (1H_d, dd), 3.95 (6H_c, dq), 5.45 (6H_a, dd), 5.91 (3H_b, ddt).

NMR ^{13}C : δ (ppm) : 9.87 (C_f), 20.58 (C_e), 62.77 (3C_c), 73.19 (C_d), 125.39 (3C_b), 128.83 (3C_a), 170.66 (C_g).

2.1.3.2.6 Synthesis of N,N,N-triallyl norvaline betaine

$C_{15}H_{21}O_2N$, MW = 235.19,

$(CH_2{=}CH\text{-}CH_2)_3\overset{+}{N}\text{-}CH(CH_2CH_2CH_3)CO_2^-$

a b c d e f g h

N,N,N-Triallyl norvaline betaine was synthesised using the method decribed in section 2.1.3.2.1. Pure N,N,N-Triallyl norvaline betaine was a white solid.

Yield : 17 %, **Rf** : 0.54 (BAW), 0 (EA), **mp** : 120°C.

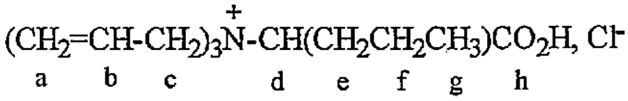
IR : $\nu_{(COO-)}$ = 1624.1.8, 1357.6 cm^{-1}, $\nu_{(-CH,\ CH3)}$ = 2965.7, 2874 cm^{-1}, $\nu_{(-CH,\ CH2)}$ = 2925.3 and 2874.4 cm^{-1}, $\delta_{(-CH,\ CH2)}$ = 1463.6cm^{-1}, $\delta_{(-CH,\ vinyl)}$ = 3084.6 cm^{-1}, $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 1000.0 cm^{-1}.

NMR ^{1}H ($CDCl_3$): δ (ppm) : 0.91 ($3H_g$, t), 1.34 (H_f, broad s), 1.63 (H_f, m) 1.99 ($2H_e$, m), 3.65 ($1H_d$, dd), 4.17 ($6H_c$, dq), 5.57 ($6H_a$, dd), 6.03 ($3H_b$, ddt).

NMR ^{13}C : δ (ppm) : 14.04 (C_g), 20.02, (C_f), 29.90 (C_e), 61.49 ($3C_c$), 76.65 (C_d), 126.03 ($3C_b$), 127.79 ($3C_a$), 167.92 (C_h).

2.1.3.2.7 Synthesis of N,N,N-triallyl norvaline betaine chloride

$C_{15}H_{22}NO_2Cl$, MW = 271.69,



$(CH_2{=}CH{-}CH_2)_3\overset{+}{N}{-}CH(CH_2CH_2CH_3)CO_2H,\ Cl^-$

N,N,N-Triallyl norvaline betaine chloride was synthesised using the method described in section 2.1.3.2.2 as a waxy white solid.

Yield : 88 %, **Rf** : 0.54 (BAW), 0 (EA).

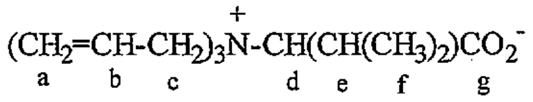
IR : $\nu_{(-OH)}$ = 3342.2 cm^{-1}, $\delta_{(-OH)}$ = 1196.1, 957.7. cm^{-1}, $\nu_{(-C=O)}$ = 1735.3 cm^{-1}, $\nu_{(-CH,\ CH3)}$ = 2969.9, 2878.5 cm^{-1}, $\nu_{(-CH,\ CH2)}$ = 2934.1 and 2878 cm^{-1}, $\delta_{(-CH,\ CH2)}$ = 1463.4 cm^{-1}, $\nu_{(-C=C-)}$ = 1650.1 cm^{-1}, $\delta_{(-CH,\ vinyl)}$ = 3097.0 cm^{-1}, $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 996.6 cm^{-1}.

NMR ^{1}H (D_2O): δ (ppm) : 0.71 ($3H_g$, t), 1.12 ($2H_f$, m), 1.84 ($2H_e$, m), 3.8 ($1H_d$, dd), 3.95 ($6H_c$, d), 5.45 ($6H_a$, dd), 5.88 ($3H_b$, ddt).

NMR ^{13}C : δ (ppm) : 13.28 (C_g), 19.27, (C_f), 28.88 (C_e), 62.82 ($3C_c$), 71.67 (C_d), 125.39 ($3C_b$), 128.92 ($3C_a$), 170.69 (C_h).

2.1.3.2.8 Attempted synthesis of N,N,N-triallyl valine betaine

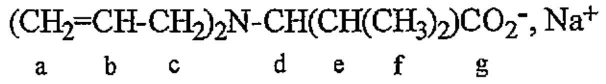
$C_{14}H_{24}NO_2Cl$, MW = 238.0,



$(\underset{a}{CH_2}=\underset{b}{CH}-\underset{c}{CH_2})_3\overset{+}{N}-\underset{d}{CH}(\underset{e}{CH}(\underset{f}{CH_3})_2)\underset{g}{CO_2^-}$

The method described in section 2.1.3.2.4 was used but failed to yield N,N,N-triallyl valine betaine. The diallyl compound being formed every time. Many different conditions were tried (time of stirring and base). The best yield of the diallyl compound was obtained after 2 days with sodium carbonate as base. Pure product was a yellow oil.

$C_{11}H_{18}O_2NNa$, MW = 218.9,



$(\underset{a}{CH_2}=\underset{b}{CH}-\underset{c}{CH_2})_2N-\underset{d}{CH}(\underset{e}{CH}(\underset{f}{CH_3})_2)\underset{g}{CO_2^-}, Na^+$

Yield : 2.5 g (45 %), **Rf** : 0.58 (BAW), 0.35 (EA).

IR spectrum : $\nu_{(COO\text{-}Na+)}$ = 1559, 1357.6 cm^{-1}, $\nu_{(\text{-}CH,\ CH3)}$ = 2960.1, 2871.6 cm^{-1}, $\nu_{(\text{-}CH,\ CH2)}$ = 2926.8 and 2871.6 cm^{-1}, $\delta_{(\text{-}CH,\ CH2)}$ = 1463.3cm^{-1}, $\delta_{(\text{-}CH,\ vinyl)}$ = 3081 cm^{-1}, $\delta_{(\text{-}CH\ out\ of\ plane,\ vinyl)}$ = 994.3 cm^{-1}.

NMR ^{1}H (D_2O): δ (ppm) : 0.8, 0.84 (6H_f, d), 1.84 (H_e, m), 2.54 (H_d, m), 3.1 (4H_c, dq), 5.04 (4H_a, dd), 5.8 (2H_b, ddt).

NMR ^{13}C : δ (ppm) : 20.09 (C_f), 20.848, (C_f), 27.53 (C_e), 53.48 (2C_c), 72.258 (C_d), 115.204 (2C_a), 138.600 (2C_b), 175.742 (C_g).

2.1.3.2.9 Synthesis of N,N-diallyl valine

$C_{11}H_{19}NO_2$, MW = 197.0,

$(CH_2{=}CH{-}CH_2)_2N{-}CH(CH(CH_3)_2)CO_2H$
a b c d e f g

N,N-Diallyl valine (as a sodium salt) (2.189 g, 10 mmol) was dissolved in water (30 ml) and treated with 2 mol dm^{-3} HCl (5 ml, 10 mmol). The mixture was allowed to stir for two hours. The solution was filtered and evaporated to dryness. The residue was extracted with chloroform. The chloroform extract was dried over anhydrous magnesium sulphate, filtered and evaporated to dryness to yield N,N-diallyl valine as a viscous yellow oil.

Yield : 1.7 g (86 %), **Rf** : 0.58 (BAW), 0.35 (EA).

IR : $\nu_{(\text{-OH})}$ = 3380 cm^{-1}, $\delta_{(\text{-OH})}$ = 1214, 946 cm^{-1},$\nu_{(\text{-C=O})}$ = 1736 cm^{-1}, $\nu_{(\text{-CH, CH3})}$ = 2974, 2886 cm^{-1}, $\nu_{(\text{-CH, CH2})}$ = 2920.1 and 2886 cm^{-1}, $\delta_{(\text{-CH, CH2})}$ = 1461 cm^{-1}, $\nu_{(\text{-C=C-})}$ = 1645 cm^{-1}, $\delta_{(\text{-CH, vinyl})}$ = 3080.0 cm^{-1}, $\delta_{(\text{-CH out of plane, vinyl})}$ = 999 cm^{-1}.

NMR ^{1}H (CDCl$_3$): δ (ppm) : 1.14, 1.23 (6H$_f$, d), 2.39 (H$_e$, m), 3.79 (H$_d$, m), 3.9 (4H$_c$, dq), 5.5 (4H$_a$, dd), 6.2 (2H$_b$, ddt).

NMR ^{13}C : δ (ppm) : 18.43 (C$_f$), 21.27, (C$_f$), 27.169 (C$_e$), 54.267 (2C$_c$), 69.612 (C$_d$), 125.406 (2C$_a$), 127.239 (2C$_b$), 168.586 (C$_g$).

2.1.3.2.10 Synthesis of N,N,N-triallyl valine betaine bromide

$C_{14}H_{24}NO_2Br$, M = 317.909 g.mol^{-1},

$(CH_2{=}CH{-}CH_2)_3\overset{+}{N}{-}CH(CH(CH_3)_2)CO_2H,\ Br^-$
a b c d e f g

The method used to synthesise N,N,N-triallyl valine betaine bromide was by quaternising the diallyl compound as described in section 2.1.3.2.4. N,N,N-triallyl valine betaine bromide was a brown oil.

Yield : 58 %, **Rf** : 0.35 (EA).

IR : $\nu_{(-OH)} = 3380$ cm^{-1}, $\delta_{(-OH)} = 1214, 920$ cm^{-1}, $\nu_{(-C=O)} = 1706$ cm^{-1}, $\nu_{(-CH,\ CH3)} = 2962.3$, 2885 cm^{-1}, $\nu_{(-CH,\ CH2)} = 2920.1$ and 2885 cm^{-1}, $\delta_{(-CH,\ CH2)} = 1468$ cm^{-1}, $\nu_{(-C=C-)} = 1642$ cm^{-1}, $\delta_{(-CH,\ vinyl)} = 3079.6$ cm^{-1}, $\delta_{(-CH\ out\ of\ plane,\ vinyl)} = 993$ cm^{-1}.

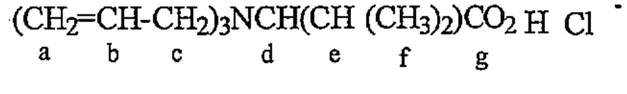
NMR ^{1}H (CDCl$_3$): δ (ppm) : 0.96, 1.03 (6H$_f$, d), 2.08 (H$_e$, m), 3.30 (6H$_c$, dq), 3.17 (H$_d$, m), 5.29 (6H$_a$, dd), 5.82 (3H$_b$, ddt).

NMR ^{13}C : δ (ppm) : 19.031 (C$_f$), 19.912 (C$_f$), 27.567 (C$_e$), 53.119 (3C$_c$), 69.974 (C$_d$), 119.963 (3C$_a$), 133.765 (3C$_b$), 174.75 (C$_g$).

Infra-red and NMR showed that the product contained about 10% free betaine. Because of this, it was decided to convert product and free betaine to the betaine chloride salt.

2.1.3.2.11 Synthesis of N,N,N- triallyl valine betaine chloride

$C_{14}H_{24}NO_2Cl$, MW = 273.5,



$$\underset{a}{(CH_2}=\underset{b}{CH}-\underset{c}{CH_2)_3}\overset{+}{N}\underset{d}{CH}(\underset{e}{CH}\ (\underset{f}{CH_3})_2)\underset{g}{CO_2}\,H\ Cl^{-}$$

The impure N,N,N-Triallyl valine betaine bromide (1.3 g, 4.089 mmol) was dissolved in chloroform (50 ml) and treated with gaseous hydrogen chloride (Vogel, 1980) generated by adding, drop-wise, sulphuric acid (40ml) to sodium chloride (100g) in concentrated hydrochloric acid (100ml). The chloroform solution was evaporated to dryness to yield N, N, N-triallyl valine betaine chloride as a viscous yellow oil.

Yield : 1.1 g (98.5 %), **Rf** : 0.35 (EA).

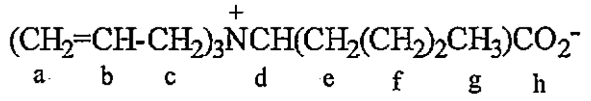
IR : $\nu_{(-OH)}$ = 3380 cm^{-1}, $\delta_{(-OH)}$ = 1201, 945.7 cm^{-1}, $\nu_{(-C=O)}$ = 1738.8 cm^{-1}, $\nu_{(-CH,\ CH3)}$ = 2972.8, 2880 cm^{-1}, $\nu_{(-CH,\ CH2)}$ = 2923.1 and 2880 cm^{-1}, $\delta_{(-CH,\ CH2)}$ = 1455 cm^{-1}, $\nu_{(-C=C-)}$ = 1645 cm^{-1}, $\delta_{(-CH,\ vinyl)}$ = 3086.6 cm^{-1}, $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 997.5 cm^{-1}.

NMR ^{1}H ($CDCl_3$): δ (ppm) : 1.17, 1.28 ($6H_f$, d), 2.48 (H_e , m), 3.95 ($6H_c$, m), 3.93 (H_d , dq), 5.51 ($6H_a$, dd), 6.26 ($3H_b$, ddt).

NMR ^{13}C : δ (ppm) : 19.031 (C_f), 19.912 (C_f), 27.567 (C_e), 53.119 ($3C_c$), 69.974 (C_d), 119.963 ($3C_a$), 133.765 ($3C_b$), 174.75 (C_g).

2.1.3.2.12 Synthesis of N,N,N-triallyl norleucine betaine

$C_{15}H_{25}O_2N$, MW = 254.19,



$$\underset{a}{(CH_2}\underset{b}{=CH}-\underset{c}{CH_2)_3}\overset{+}{N}\underset{d}{CH}(\underset{e}{CH_2}\underset{f}{(CH_2)_2}\underset{g}{CH_3})\underset{h}{CO_2^-}$$

This compound was synthesised using the method described in section 2.1.3.2.4 with slight modifications. The reaction was carried out in high dilution (20 mmol dm^{-3} norleucine in methanol with sodium carbonate as base. These modifications tripled the yield of the reaction. Pure N,N,N-triallyl norleucine betaine was a viscous yellow oil.

Yield : 31 %, **Rf** : 0.60 (BAW), 0 (EA).

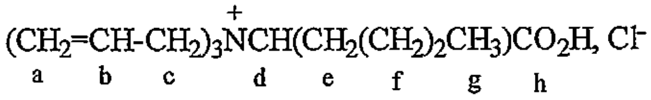
IR : $\nu_{(COO-)}$ = 1627.8, 1354.3 cm^{-1}, $\nu_{(-CH, CH3)}$ = 2959.4, 2854 cm^{-1}, $\nu_{(-CH, CH2)}$ = 2925.3 and 2871.0 cm^{-1} , $\delta_{(-CH, CH2)}$ = 1463.0cm^{-1}, $\delta_{(-CH, vinyl)}$ = 3085.4 cm^{-1} , $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 997.1 cm^{-1}.

NMR ^{1}H (CDCl$_3$): δ (ppm) : 0.78 (3H_g , t), 1.26 (H_f , broad s), 1.4 (H_f , m) 1.8 (2H_e , broad s),. 3.60 (1H_d , dd), 4.17 (6H_c , dq), 5.52 (6H_a , dd), 6.07 (3H_b , ddt).

NMR ^{13}C : δ (ppm) : 13.73 (C_g), 22.51, 27.4 (C_f), 28.55 (C_e), 61.60 (3C_c), 76.317 (C_d), 126.11 (3C_b), 127.69 (3C_a), 168.69 (C_h).

2.1.3.2.13 Synthesis of N,N,N-triallyl norleucine betaine chloride

$C_{15}H_{26}NO_2Cl$, MW = 290.69,



$$\underset{a}{(CH_2}=\underset{b}{CH}-\underset{c}{CH_2})_3\overset{+}{N}\underset{d}{CH}(\underset{e}{CH_2}(\underset{f}{CH_2})_2\underset{g}{CH_3})\underset{h}{CO_2H}, Cl^-$$

N, N, N-Triallyl norleucine betaine chloride was synthesised by the method described in section 2.1.3.2.2. Pure N,N,N-triallyl norleucine betaine chloride was a viscous yellow oil.

Yield : 90 %, **Rf** : 0.60 (BAW), 0 (EA).

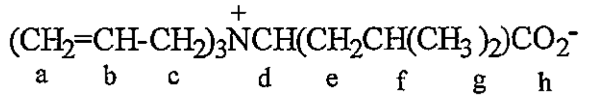
IR : $\nu_{(-OH)}$ = 3375 cm^{-1}, $\delta_{(-OH)}$ = 1195.4, 957.6. cm^{-1}, $\nu_{(-C=O)}$ = 1734.2 cm^{-1}, $\nu_{(-CH, CH3)}$ = 2962.9.6, 2872.7 cm^{-1}, $\nu_{(-CH, CH2)}$ = 2926.7 and 2872.7 cm^{-1}, $\delta_{(-CH, CH2)}$ = 1464.0 cm^{-1}, $\nu_{(-C=C-)}$ = 1641.6 cm^{-1}, $\delta_{(-CH, vinyl)}$ = 3085.2 cm^{-1}, $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 996.0 cm^{-1}.

NMR ^{1}H (D$_6$MSO): δ (ppm) : 0.85 (3H_g , t), 1.25 (4H_f , m), 2.0 (2H_e , m), 4.0 (1H_d, dd), 4.25 (6H_c , d), 5.65 (6H_a , dd), 6.15 (3H_b , ddt).

NMR ^{13}C : δ (ppm) : 13.71 (C_g), 21.72, 26.09 (C_f), 27.62 (C_e), 61.94 ($3C_c$), 70.91 (C_d), 126.34 ($3C_b$), 127.35 ($3C_a$), 168.71 (C_h).

2.1.3.2.14 Synthesis of N,N,N-triallyl leucine betaine

$C_{15}H_{25}O_2N$, MW = 254.19,



$$\underset{a}{(CH_2}{=}\underset{b}{CH}-\underset{c}{CH_2)_3}\overset{+}{N}\underset{d}{CH}(\underset{e}{CH_2}\underset{f}{CH}\underset{g}{(CH_3)_2})\underset{h}{CO_2^-}$$

N,N,N-Triallyl leucine betaine was synthesised according to the method described in section 2.1.3.2.12. Pure N,N,N-triallyl leucine betaine was a yellow viscous oil.

Yield : 24 %, **Rf** : 0.44 (BAW), 0 (EA).

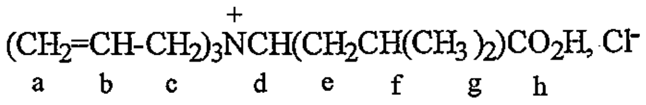
IR : $\nu_{(COO-)}$ = 1624.9, 1367.0 cm^{-1}, $\nu_{(-CH,\ CH3)}$ = 2959.8, 2872.3 cm^{-1}, $\nu_{(-CH,\ CH2)}$ = 2926.0 and 2872.4 cm^{-1}, $\delta_{(-CH,\ CH2)}$ = 1466.8cm^{-1}, $\delta_{(-CH,\ vinyl)}$ = 3085.1 cm^{-1}, $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 996.0 cm^{-1}.

NMR ^1H ($CDCl_3$) : δ (ppm) : 0.81 ($6H_g$, d), 1.4 (H_f, m), 1.7 (H_e, m) , 3.65 ($1H_d$, dd), 4.04 ($6H_c$, dq), 5.54 ($6H_a$, dd), 5.95 ($3H_b$, ddt).

NMR ^{13}C : δ (ppm) : 20.72, 23.69 (C_g), 25.79, (C_f), 35.62 (C_e), 62.12 ($3C_c$), 74.59 (C_d), 125.93 ($3C_b$), 128.14 ($3C_a$), 172.47 (C_h).

2.1.3.2.15 Synthesis of N,N,N-triallyl leucine betaine chloride

$C_{15}H_{26}NO_2Cl$, MW = 290.69,



$$\underset{a}{(CH_2}{=}\underset{b}{CH}-\underset{c}{CH_2)_3}\overset{+}{N}\underset{d}{CH}(\underset{e}{CH_2}\underset{f}{CH}\underset{g}{(CH_3)_2})\underset{h}{CO_2H}, Cl^-$$

N,N,N-Triallyl leucine betaine chloride was synthesised according to the method described in section 2.1.3.2.2. Pure N,N,N-Triallyl leucine betaine chloride was a viscous yellow oil.

Yield : 87 %, **Rf** : 0.46 (BAW), 0 (EA).

IR : $\nu_{(\text{-OH})} = 3367.6\ \text{cm}^{-1}$, $\delta_{(\text{-OH})} = 1202.4, 957.1.\ \text{cm}^{-1}$, $\nu_{(\text{-C=O})} = 1735.6\ \text{cm}^{-1}$, $\nu_{(\text{-CH, CH3})} = 2964.5, 2877.8\ \text{cm}^{-1}$, $\nu_{(\text{-CH, CH2})} = 2934.1$ and $2878\ \text{cm}^{-1}$, $\delta_{(\text{-CH, CH2})} = 1464\ \text{cm}^{-1}$, $\nu_{(\text{-C=C-})} = 1638.1\ \text{cm}^{-1}$, $\delta_{(\text{-CH, vinyl})} = 3087.0\ \text{cm}^{-1}$, $\delta_{(\text{-CH out of plane, vinyl})} = 994.5\ \text{cm}^{-1}$.

NMR ^{1}H (D_2O): δ (ppm) : 0.78 (6H_g , d), 1.39 (H_f, m), 1.89 (2H_e , m), 3.92 (1H_d, dd), 4.06 (6H_c , d), 5.49 (6H_a , dd), 5.91 (3H_b , ddt).

NMR ^{13}C : δ (ppm) : 20.71, 23.54 (C_g), 25.58, (C_f), 35.39 (C_e), 62.88 (3C_c), 71.06 (C_d), 125.41 (3C_b), 129.11 (3C_a), 170.69 (C_h).

2.1.3.2.16 Synthesis of α-bromoheptanoic acid

$C_7H_{13}O_2Br$, MW = 209.229,

$CH_3(CH_2)_3CH_2CH(Br)CO_2H$
a b c d e

n-Heptanoic acid (40 g, 0.31 mol) was melted into a round bottom flask. Bromine (25 ml, 0.46 mol), previously washed several times with sulphuric acid, was added to the acid. Phosphorus trichloride (0.6 ml) was then added drop-wise to the stirred reaction mixture. The reaction was refluxed at 90°C until no more hydrogen bromide was liberated by the reaction (about 3 hours). The reaction mixture was dissolved in diethyl ether and washed several times with water to remove excess bromine. The organic layer was dried with anhydrous sodium sulphate, filtrated and evaporate to yield α-bromoheptanoic acid as a yellow oil.

Yield : 62.5 g (97 %), **bp** : 90°C/ 2mm Hg (Lit. 147°C/12 mmHg), **Rf** : 0.75 (BAW).

IR : $\nu_{(\text{-OH})}$ = 2672.6 cm^{-1}, $\delta_{(\text{-OH})}$ = 923.4 cm^{-1}, $\nu_{(\text{-CH})}$ = 3097, 2958.6 cm^{-1}, $\nu_{(\text{-CH, CH2})}$ = 2930.3, 2854.5 cm^{-1}, $\delta_{(\text{-CH , CH2})}$ = 1459.8 cm^{-1}, $\nu_{(\text{-C=O})}$ = 1717.8 cm^{-1}, $\nu_{(\text{-C-0})}$, $\delta_{(\text{OH})}$ = 1423.3 cm^{-1}, $\nu_{(\text{-C-0})}$, $\delta_{(\text{OH})}$ = 1285.5 cm^{-1}.

NMR ^{1}H (CDCl$_3$): δ (ppm) : 0.88 (3H$_a$, t), 1.33 (6H$_b$, broad s), 2.03 (2H$_c$, dt), 4.22 (H$_d$, t), 11.93 (H$_e$, s).

NMR ^{13}C : δ (ppm) : 176.93 (C$_e$), 45.99 (C$_d$), 35.13 (C$_c$), 31.52, 27.43, 22..92 (C$_b$), 14.51 (C$_a$).

2.1.3.2.17 Synthesis of α-aminoheptanoic acid

$C_7H_{15}NO_2$, MW = 145.19,

$CH_3(CH_2)_3CH_2CH(NH_2)CO_2H$
a b c d e

α-Bromoheptanoic acid (30 g, 0.14 mol) was added to a solution of ammonia (35% solution, 168 ml). The reaction mixture was heated at 55°C for 24 hours, in a sealed round bottom flask. The reaction mixture was then washed successively with water to remove ammonium bromide formed by the reaction and diethyl ether to remove any remaining α-bromoheptanoic acid (these different washings formed an emulsion which was separated by centrifugation). Pure α-aminoheptanoic acid was obtained as white crystals.

Yield : 7.5 g (95 %), **mp** : 274°C (Lit. 275°C), **Rf** : 0.40 (BAW/ ninhydrin).

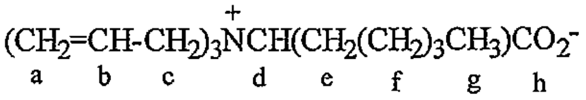
IR : $\nu_{(NH3+)}$ = 3000, 2101.8 cm^{-1} , $\delta_{(NH3+)}$ =1514.3 cm^{-1}, $\nu_{(COO-)}$ = 1416.5, 1345.4 cm^{-1} , $\nu_{(-CH, CH3)}$ = 2956.6, 2858.8 cm^{-1}, $\nu_{(-CH, CH2)}$ = 2931.0 and 2858.2 cm^{-1}, $\delta_{(-CH, CH2)}$ = 1459.0 cm^{-1}.

NMR ^{1}H (D_2O, 1eq KOH): δ (ppm) : 0.67 (3H_a , t), 1.12 (6H_b , broad s), 1.40 (2H_c , dt), 3.05 (H_d , t).

NMR ^{13}C : δ (ppm) : 184.51 (C_e), 56.55 (C_d), 35.15 (C_c), 31.56, 25.13, 22.38 (C_b), 13.86 (C_a).

2.1.3.2.18 Synthesis of N,N,N-triallyl α-aminoheptanoic acid betaine

$C_{16}H_{27}O_2N$, MW = 265.19,



$$(\underset{a}{CH_2}=\underset{b}{CH}-\underset{c}{CH_2})_3\overset{+}{N}\underset{d}{CH}(\underset{e}{CH_2}(\underset{f}{CH_2})_3\underset{g}{CH_3})\underset{h}{CO_2^-}$$

N,N,N-triallyl α-aminoheptanoic acid betaine was synthesised according to the method described in section 2.1.3.2.12. Pure N,N,N-triallyl α-aminoheptanoic acid betaine was a viscous yellow oil.

Yield : 18 %, **Rf** : 0.65 (BAW), 0 (EA).

IR : $\nu_{(COO-)}$ = 1627.6, 1361.2 cm^{-1}, $\nu_{(-CH, CH3)}$ = 2958.2, 2866.5 cm^{-1}, $\nu_{(-CH, CH2)}$ = 2930.6 and 2866.0 cm^{-1}, $\delta_{(-CH, CH2)}$ = 1460.7 cm^{-1}, $\delta_{(-CH, vinyl)}$ = 3086.7 cm^{-1}, $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 996.8 cm^{-1}.

NMR ^{1}H ($CDCl_3$): δ (ppm) : 0.69 (3H_g , t), 1.15 (6H_f, broad s), 1.74 (2H_e , m), 3.56 (1H_d , dd), 4.98 (6H_c , dq), 5.50 (6H_a , dd), 5.96 (3H_b , ddt).

NMR ^{13}C : δ (ppm) : 13.64 (C_g), 22.14, 25.70, 31.07 (C_f), 27.03 (C_e), 62.34 ($3C_c$), 75.62 (C_d), 126.08 ($3C_b$), 128.14 ($3C_a$), 172.8 (C_h).

2.1.3.2.19 Synthesis of N,N,N-triallyl α-aminoheptanoic acid betaine chloride

$C_{16}H_{28}O_2NCl$, MW = 301.59,

$$(\underset{a}{CH_2}=\underset{b}{CH}-\underset{c}{CH_2})_3\overset{+}{N}\underset{d}{CH}(\underset{e}{CH_2}\underset{f}{(CH_2)_3}\underset{g}{CH_3})\underset{h}{CO_2H},\ Cl^-$$

N,N,N-triallyl α-aminoheptanoic acid betaine (1.12 g, 4.22 mmol) was dissolved in chloroform (30 ml) and treated with gaseous hydrogen chloride. The HCl gas was generated using the method described in section 2.1.3.2.11. The chloroform solution was evaporated to dryness to yield N,N,N-triallyl α-aminoheptanoic acid betaine chloride as a viscous yellow oil.

Yield : 0.65 g (98 %), **Rf** : 0.65 (BAW), 0 (EA).

IR : $\nu_{(-OH)}$ = 3345 cm^{-1}, $\delta_{(-OH)}$ = 1211.5, 955.4 cm^{-1}, $\nu_{(-C=O)}$ = 1735.7 cm^{-1}, $\nu_{(-CH,\ CH3)}$ = 2960.5, 2878.5 cm^{-1}, $\nu_{(-CH,\ CH2)}$ = 2926.7 and 2878.3 cm^{-1}, $\delta_{(-CH,\ CH2)}$ = 1462.5 cm^{-1}, $\nu_{(-C=C-)}$ = 1650.0 cm^{-1}, $\delta_{(-CH,\ vinyl)}$ = 3080.0 cm^{-1} , $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 996.1 cm^{-1}.

NMR ^{1}H (D_2O): δ (ppm) : 0.78 ($3H_g$, t), 1.25 ($6H_f$, broad s), 2.00 ($2H_e$, broad s), 3.94 ($1H_d$, dd), 4.16 ($6H_c$, dq), 5.61 ($6H_a$, dd), 6.01 ($3H_b$, ddt).

NMR ^{13}C : δ (ppm) : 14.03 (C_g), 22.52, 25.74, 31.38 (C_f), 27.34 (C_e), 63.2 ($3C_c$), 72.89 (C_d), 125.96 ($3C_b$), 129.24 ($3C_a$), 171.49 (C_h).

2.1.3.2.20 Synthesis of N,N,N-triallyl α-aminocaprylic acid betaine

$C_{17}H_{29}NO_2$, MW = 279.42,

$$(CH_2{=}CH{-}CH_2)_3\overset{+}{N}CH(CH_2(CH_2)_4CH_3)CO_2^-$$

a b c d e f g h

N,N,N-Triallyl α-aminocaprylic acid was synthesised according to the method described in section 2.1.3.2.12. Pure N,N,N-triallyl α-aminocaprylic acid was a viscous yellow oil.

Yield : 35%, **Rf** : 0.62 (BAW), 0 (EA).

IR : $\nu_{(COO-)}$ = 1630, 1353 cm^{-1}, $\nu_{(-CH,\ vinyl)}$ = 3084 cm^{-1}$\nu_{(-CH,\ CH3)}$ = 2960, 2861 cm^{-1}, $\nu_{(-CH,\ CH2)}$ = 2927 and 2861 cm^{-1} , $\delta_{(-CH,\ CH2)}$ = 1460 cm^{-1}, $\delta_{(-CH,\ out\ of\ plane)}$ = 1001.8 cm^{-1}, $\nu_{(-C=C-)}$ = 1650 cm^{-1}, Skeletal vibr.$_{(-CH2-)4}$ = 729 cm^{-1}.

NMR ^{1}H (CDCl$_3$): δ (ppm) : 0.68 (3H$_g$, t), 1.11 (8H$_f$, broad s), 1.76 (2H$_e$, broad s), 3.54 (1H$_d$, dd), 3.97 (6H$_c$, dq), 5.48 (6H$_a$, d), 5.93 (3H$_b$, ddt).

NMR ^{13}C : δ (ppm) : 12.8 (C$_g$), 21.26, 24.96, 26.04, 27.52 (C$_f$), 30.16 (C$_e$), 61.32 (3C$_c$), 74.6 (C$_d$), 125.05 (3C$_b$), 127.14 (3C$_a$), 171.74 (C$_h$).

2.1.3.2.21 Synthesis of N,N,N-triallyl α-aminocaprylic acid betaine chloride

$C_{17}H_{30}NO_2$, MW = 315.88,

$$(CH_2{=}CH{-}CH_2)_3\overset{+}{N}CH(CH_2(CH_2)_4CH_3)CO_2H,\ Cl^-$$

a b c d e f g h

N,N,N-triallyl α-amino caprylic acid betaine chloride was synthesised using the method described in section 2.1.3.2.12. Pure N,N,N-triallyl α-aminocaprylic acid betaine chloride was a viscous yellow oil.

Yield : 83 %, **Rf** : 0.60 (BAW), 0 (EA).

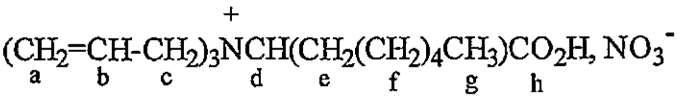
IR : $\nu_{(-OH)}$ = 3381 cm^{-1}, $\nu_{(-C=O)}$ = 1728.6 cm^{-1}, $\nu_{(-CH, CH3)}$ = 2960, 2862 cm^{-1}, $\nu_{(-CH, CH2)}$ = 2930 and 2862 cm^{-1} , $\delta_{(-CH, CH2)}$ = 1460 cm^{-1}, $\nu_{(-C=C-)}$ = 1650 cm^{-1}, Skeletal vibr.$_{(-CH2-)4}$ = 729.9 cm^{-1}, $\nu_{(-CH, vinyl)}$ = 3084 cm^{-1}, $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 1000.8 cm^{-1}.

NMR ^{1}H (D_2O): δ (ppm) : 0.64 ($3H_g$, t), 1.07 ($8H_f$, broad s), 1.82 ($2H_e$, broad s), 3.81 ($1H_d$, dd), 4.00 ($6H_c$, dq), 5.45 ($6H_a$, d), 5.90 ($3H_b$, ddt).

NMR ^{13}C : δ (ppm) : 14.70 (C_g), 23.19, 26.60, 27.89, 29.35 (C_f), 32.04 (C_e), 63.62 ($3C_c$), 74.02 (C_d), 126.56 ($3C_b$), 129.58 ($3C_a$), 172.38 (C_h).

2.1.3.2.22 Synthesis of N,N,N-triallyl α-aminocaprylic acid betaine nitrate

$C_{17}H_{30}N_2O_5$, MW = 342.0,



$$(\underset{a}{CH_2}=\underset{b}{CH}-\underset{c}{CH_2})_3\overset{+}{N}\underset{d}{CH}(\underset{e}{CH_2}(\underset{f}{CH_2})_4\underset{g}{CH_3})\underset{h}{CO_2H}, NO_3^-$$

N,N,N-triallyl α-aminocaprylic acid betaine nitrate was prepared according to the method described in section 2.1.3.2.3. Pure N,N,N-triallyl α-aminocaprylic acid betaine nitrate was a viscous yellow oil.

Yield : 72 %, **Rf** : 0.62 (BAW), 0 (EA).

IR : $\nu_{(\text{-OH})}$ = 3453 cm^{-1} ,$\nu_{(\text{-C=O})}$ = 1728 cm^{-1}, $\nu_{(\text{-CH, CH3})}$ = 2960, 2861 cm^{-1}, $\nu_{(\text{-CH, CH2})}$ = 2927 and 2861 cm^{-1} , $\delta_{(\text{-CH, CH2})}$ = 1460 cm^{-1} ,$\nu_{(\text{-CH, vinyl})}$ = 3089 cm^{-1}, $\delta_{(\text{-CH out of plane, vinyl})}$ = 998.6 cm^{-1}, $\nu_{(\text{-C=C-})}$ = 1650 cm^{-1}, $\nu_{(\text{NO3-})}$ = 1357 cm^{-1}, Skeletal vibr.$_{(\text{-CH2-})4}$ = 729 cm^{-1}.

NMR ^{1}H (D_2O): δ (ppm) : 0.68 ($3H_g$, t), 1.11 ($8H_f$, broad s), 1.76 ($2H_e$, broad s), 3.54 ($1H_d$, dd), 3.97 ($6H_c$, dq), 5.48 ($6H_a$, dd), 5.93 ($3H_b$, ddt).

NMR ^{13}C : δ (ppm) : 12.8 (C_g), 21.26, 24.96, 26.04, 27.52 (C_f), 30.16 (C_e), 61.32 ($3C_c$), 74.6 (C_d), 125.05 ($3C_b$), 127.14 ($3C_a$), 171.74 (C_h).

2.1.3.2.23 Synthesis of α-bromolauric acid

$C_{12}H_{25}O_2Br$, MW = 279.229,

$$\underset{a}{CH_3}\underset{b}{(CH_2)_8}\underset{c}{CH_2}\underset{d}{CHBr}\underset{e}{CO_2H}$$

α-Bromo lauric acid was prepared according to the method described in section 2.1.3.2.16. α-Bromolauric acid was an off-white solid at room temperature.

Yield : 55 %, **bp** : 158°C/ 0.2mm Hg (Lit. 161-164°C/0.4 mmHg), **mp** : 30°C (Lit. 32°C), **Rf** : 0.75 (BAW).

IR : $\nu_{(\text{-OH})}$ = 2674.1 cm^{-1}, $\delta_{(\text{-OH})}$ = 927.1 cm^{-1}, $\nu_{(\text{-CH})}$ = 3093, 3007 cm^{-1}, $\nu_{(\text{-CH, CH2})}$ = 2925.3, 2854.5 cm^{-1}, $\delta_{(\text{-CH , CH2})}$ = 1475, 1457 cm^{-1}, $\nu_{(\text{-C=O})}$ = 1717.3 cm^{-1},

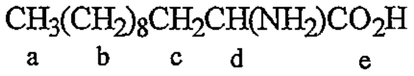
$\nu_{(\text{-C-0})}$, $\delta_{(\text{OH})}$ = 1422.7 cm^{-1}, $\nu_{(\text{-C-0})}$, $\delta_{(\text{OH})}$ = 1286.3 cm^{-1}.

NMR ^{1}H ($CDCl_3$): δ (ppm) : 0.87 ($3H_a$, t), 1.25 ($16H_b$, broad s), 2.01 ($2H_c$, dt), 4.2 (H_d, t), 10.84 (H_e, s).

NMR ^{13}C : δ (ppm) : 176.13 (C_e), 45.41 (C_d), 34.61 (C_c), 31.86, 29.50, 29.43, 29.25, 29.00, 28.77, 27.15, 22.64 (C_b), 14.07 (C_a).

2.1.3.2.24 Synthesis of α-aminolauric acid

$C_{12}H_{27}NO_2$, MW = 215.229,



$\underset{a}{CH_3}(\underset{b}{CH_2})_8\underset{c}{CH_2}\underset{d}{CH}(NH_2)\underset{e}{CO_2H}$

α-Aminolauric acid was prepared according to the method described in section 2.1.3.2.17. Pure α-aminolauric acid was a white solid.

yield : 95 %, **mp** : 258-259°C (Lit. 264°C), **Rf** : 0.80 (BAW/ninhydrin).

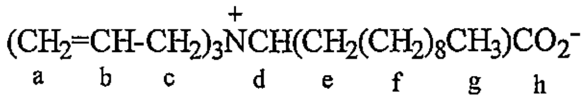
IR : $\nu_{(NH3+)}$ = 3000, 2103.4 cm^{-1}, $\delta_{(NH3+)}$ =1511.5 cm^{-1}, $\nu_{(COO-)}$ = 1414, 1343.5 cm^{-1}, $\nu_{(-CH, CH3)}$ = 2955.6, 2849.7 cm^{-1}, $\nu_{(-CH, CH2)}$ = 2918.0 and 2849.7 cm^{-1}, $\delta_{(-CH, CH2)}$ = 1465.8 cm^{-1}.

NMR ^{1}H (D_2O / 1eq. KOH): δ (ppm) : 0.68 (3H_a, t), 1.10 (16H_b, broad s), 1.40 (2H_c, dt), 3.0 (H_d, t).

NMR ^{13}C : δ (ppm) : 182.77 (C_e), 56.41 (C_d), 35.98 (C_c), 31.97, 29.92, 29.80, 29.51, 26.04, 22.63 (C_b), 13.81 (C_a).

2.1.3.2.25 Synthesis of N,N,N-triallyl α-amino lauric acid betaine

$C_{21}H_{37}O_2N$, MW = 335.229,



$(\underset{a}{CH_2}=\underset{b}{CH}-\underset{c}{CH_2})_3\overset{+}{N}\underset{d}{CH}(\underset{e}{CH_2}(\underset{f}{CH_2})_8\underset{g}{CH_3})\underset{h}{CO_2^-}$

N,N,N-Triallyl α-aminolauric acid betaine was synthesised according to the method described in section 2.1.3.2.12. Pure N,N,N-Triallyl α-aminolauric acid betaine was a vicous yellow oil.

Yield : 21 %, **Rf** : 0.71 (BAW), 0 (EA).

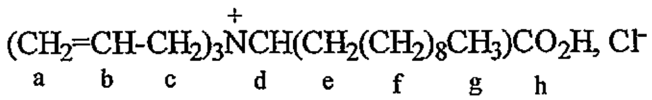
IR : $\nu_{(COO-)}$ = 1628.3, 1356.3 cm^{-1}, $\nu_{(-CH, CH3)}$ = 2955.6, 2854 cm^{-1}, $\nu_{(-CH, CH2)}$ = 2925.3 and 2854.4 cm^{-1}, $\delta_{(-CH, CH2)}$ = 1464.8 cm^{-1},$\nu_{(-C=C-)}$ = 1650 cm^{-1}, $\delta_{(-CH, vinyl)}$ = 3085.3 cm^{-1}, $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 994.5 cm^{-1}.

NMR ^{1}H (CDCl$_3$): δ (ppm) : 0.84 (3H$_g$, t), 1.19 (16H$_f$, broad s), 1.61 (2H$_e$, broad s), 3.66 (1H$_d$, dd), 4.12 (6H$_c$, dq), 5.59 (6H$_a$, dd), 6.03 (3H$_b$, ddt).

NMR ^{13}C : δ (ppm) : 14.01 (C$_g$), 22.57, 26.6, 27.92, 29.18, 29.29, 29.45, 29.66 (C$_f$), 31.77 (C$_e$), 61.66 (3C$_c$), 76.99 (C$_d$), 126.10 (3C$_b$), 127.93 (3C$_a$), 168.14 (C$_h$).

2.1.3.2.26 Synthesis of N,N,N-triallyl α-aminolauric acid betaine chloride

$C_{21}H_{38}NO_2Cl$, MW = 371.69,



$$(\underset{a}{CH_2}=\underset{b}{CH}-\underset{c}{CH_2})_3\overset{+}{N}\underset{d}{CH}(\underset{e}{CH_2}(\underset{f}{CH_2})_8\underset{g}{CH_3})\underset{h}{CO_2H},\ Cl^-$$

N,N,N-Triallyl α-aminolauric acid betaine chloride was prepared according to the method described in section 2.1.3.2.19. Pure N,N,N-Triallyl α-aminolauric acid betaine chloride was a viscous oil.

Yield : 84 %, **Rf** : 0.71 (BAW), 0 (EA).

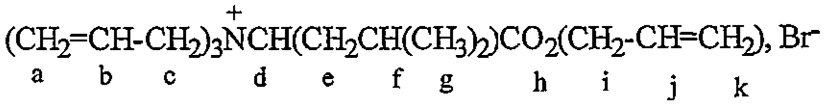
IR : $\nu_{(-OH)} = 3396\ cm^{-1}$, $\delta_{(-OH)} = 1205,\ 950.4\ cm^{-1}$, $\nu_{(-C=O)} = 1725.6\ cm^{-1}$, $\nu_{(-CH,\ CH3)} = 2955.6,\ 2855.3\ cm^{-1}$, $\nu_{(-CH,\ CH2)} = 2926.7$ and $2855.3\ cm^{-1}$, $\delta_{(-CH,\ CH2)} = 1465.4\ cm^{-1}$, $\nu_{(-C=C-)} = 1641.1\ cm^{-1}$, $\delta_{(-CH,\ vinyl)} = 3087.2\ cm^{-1}$, $\delta_{(-CH\ out\ of\ plane,\ vinyl)} = 994.6\ cm^{-1}$.

NMR ^{1}H (D_2O): δ (ppm) : 0.71 ($3H_g$, t), 1.11 ($16H_f$, broad s), 1.83 ($2H_e$, broad s), 3.70 ($1H_d$, dd), 4.05 ($6H_c$, dq), 5.53 ($6H_a$, dd), 5.98 ($3H_b$, ddt).

NMR ^{13}C : δ (ppm) : 14.42 (C_g), 23.13, 26.58, 29.82, 30.2 (C_f), 32.44 (C_e), 62.84 ($3C_c$), 78.64 (C_d), 125.91 ($3C_b$), 128.88 ($3C_a$), 170.64 (C_h).

2.1.3.2.27 Synthesis of N,N,N-triallyl leucine betaine allyl ester bromide

$C_{18}H_{30}O_2NBr$, MW = 371.9,


$(CH_2{=}CH{-}CH_2)_3\overset{+}{N}CH(CH_2CH(CH_3)_2)CO_2(CH_2{-}CH{=}CH_2),\ Br^-$

Leucine (10.0 g, 76 mmol) was added to methanol (400 ml) followed by sodium carbonate (80.6 g, 0.76 mol) and allyl bromide (87 ml , 1.06 mol) successively to the stirred solution.

After 1 day reflux, the mixture was evaporated to dryness. The residue was extracted with chloroform (350 ml). The chloroform extract was dried with anhydrous sodium sulphate, filtered and evaporated to dryness. The residue was dissolved in ethyl acetate and extracted with water. The water extract was filtered and evaporated down to dryness to yield N, N, N-triallyl leucine betaine allyl ester bromide salt as a brown oil.

Yield : 8.0 g (29 %), **Rf** : 0.58 (BAW).

IR : $\nu_{(COOR)}$ = 1743 cm^{-1}, $\nu_{(-CH, CH3)}$ = 2959.8, 2872.3 cm^{-1}, $\nu_{(-CH, CH2)}$ = 2926.0 and 2872.4 cm^{-1} , $\delta_{(-CH, CH2)}$ = 1466.8cm^{-1}, $\delta_{(-CH, vinyl)}$ = 3085.1 cm^{-1}, $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 996.0 cm^{-1}.

NMR ^{1}H (D_2O): δ (ppm) : 0.81 (6H_g, d), 1.4 (H_f, m), 1.96 (2H_e, m) , 4.0 (1H_d, dd), 4.1 (6H_c, dq), 4.62 (2H_i, dq), 5.30 (2H_k, dd), 5.60 (6H_a, dd), 5.84 (H_j, ddt), 5.99 (3H_b, ddt).

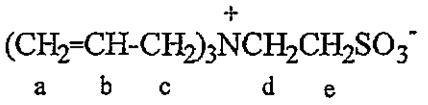
NMR ^{13}C : δ (ppm) : 20.65, 23.62 (C_g), 25.65, (C_f), 35.49 (C_e), 63.009 (3C_c), 68.346 (C_i), 70.30 (C_d), 121.265 (C_k), 125.20 (3C_b), 129.33 (3C_a), 130.879(C_j), 172.47 (C_h).

CNHBr : Found : %C : 56.97; %H : 8.24, %N : 3.76 and %Br : 19.62.

Calculated : %C : 58.08, % H : 8.09, %N : 4.49 and %Br : 21.4. Product contained 10% of starting material (Leucine).

2.1.3.2.28 Synthesis of N,N,N-triallyl taurine betaine

$C_{11}H_{19}NSO_3$, MW = 245.34,



$$\underset{a}{(\mathrm{CH_2}}{=}\underset{b}{\mathrm{CH}}{-}\underset{c}{\mathrm{CH_2})_3}\overset{+}{\mathrm{N}}\underset{d}{\mathrm{CH_2}}\underset{e}{\mathrm{CH_2}}\mathrm{SO_3^-}$$

N,N,N-Triallyl taurine betaine was synthesised according to the method described in section 2.1.3.2.1. Pure compound was a white granular solid.

Yield : 32%, **mp** : 171-172°C, **Rf** : 0.3(BAW), 0 (EA).

IR : $\nu_{(-O-S-O-O-)}$ = 1197, 1038, 698 cm^{-1}, $\nu_{(-CH, vinyl)}$ = 3091 cm^{-1}$\nu_{(-CH, CH2)}$ = 2927 and 2861 cm^{-1}, $\delta_{(-CH, CH2)}$ = 1460 cm^{-1}, $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 1000.8 cm^{-1}, $\nu_{(-C=C-)}$ = 1650 cm^{-1}.

NMR ^{1}H (D_2O): δ (ppm) : 3.19, 3.35 (2H_d, 2H_e , t), 3.70 (6H_c , d), 5.51 (6H_a , dd), 5.82 (3H_b , ddt).

NMR ^{13}C : δ (ppm) : 44.46 (C_d), 54.02 (C_e), 61.87 (3C_c), 124.50 (3C_b), 130.21 (3C_a).

2.1.3.2.29 Synthesis of N,N,N-triallyl taurine betaine chloride

$C_{11}H_{20}ClNSO_3$, MW = 281.8,

$(\underset{a}{CH_2}=\underset{b}{CH}-\underset{c}{CH_2})_3\overset{+}{N}\underset{d}{CH_2}\underset{e}{CH_2}SO_3H, Cl^-$

N,N,N-Triallyl taurine betaine chloride was synthesised according to the method described in section 2.1.3.2.2. Pure N,N,N-triallyl taurine betaine chloride was a white granular solid.

Yield : 75%, **mp** : 205°C, **Rf**: 0.38 (BAW), 0 (EA).

IR : $\nu_{(-OH)} = 3434\ cm^{-1}\nu_{(-CH)} = 3096, 2994\ cm^{-1}$, $\nu_{(-CH,\ CH2)} = 2932, 2850$, $\delta_{(-CH,\ CH2)} = 1475\ cm^{-1}$, $\nu_{(-CH,\ CH2\ in\ plane)} = 1425\ cm^{-1}$, $\nu_{(-C=C-)} = 1639\ cm^{-1}$, $\nu_{(-CH,\ vinyl)} = 3092\ cm^{-1}$, $\delta_{(-CH\ out\ of\ plane,\ vinyl)} = 1000.8\ cm^{-1}$,$\nu_{(-C=C-)} = 1650\ cm^{-1}$, $\nu_{(-O-S-O-O-)} = 1196, 1040, 695\ cm^{-1}$.

NMR ^{1}H (D_2O): δ (ppm) : 3.30, 3.47 ($2H_d$, $2H_e$, t), 3.81 ($6H_c$, d), 5.59 ($6H_a$, dd), 5.892 ($3H_b$, ddt).

NMR ^{13}C : δ (ppm) : 44.46 (C_d), 54.02 (C_e), 61.87 ($3C_c$), 124.50 ($3C_b$), 130.21 ($3C_a$).

2.2 Membrane fabrication and method of evaluation

2.2.1 Membrane composition

2.2.1.1 Krynac 50.75 membranes

The preparation of membranes fabricated with Krynac 50.75 follows that used in previous work (Frampton, 1992 and Braven *et al.*, 1996).

Purified Krynac (0.53 g, 46 % m/m) was dissolved in THF (6 ml). Free radical initiator (0.0864 g, 7.5 % m/m) and solvent mediator, (2-NPOE unless stated) (0.478 g, 41.5 %) were added, followed by sensor molecule (0.0576 g, 5 % m/m). The mixture was shaken until homogeneous. The solvent was then removed by drying to constant weight in a vacuum oven at room temperature (5 days) over phosphorus pentoxide to give an uncross-linked paste.

2.2.1.2 SBS membranes

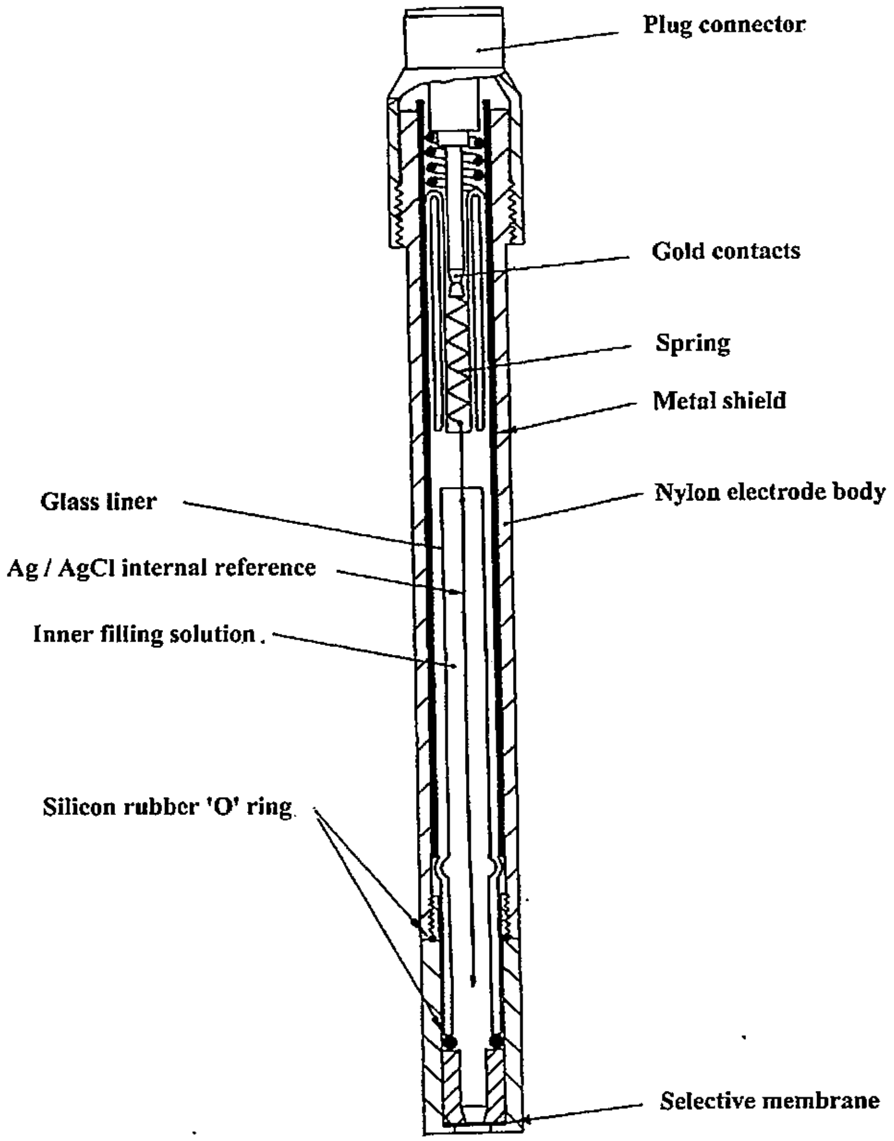
The composition of SBS membranes was similar to that of previous studies (Ellis, 1980, King, 1985 and Frampton, 1992).

SBS (0.565 g, 43.5 % m/m), free radical initiator, DCP (0.13 g, 10 % m/m), solvent mediator, 2-NPOE (0.52 g, 40 %) and sensor molecule (0.085 g, 6.5 % m/m).

2.2.2 Membrane fabrication by hot-pressing

Hot-pressed membranes were prepared according to previous studies (Frampton, 1992), except that a BYTEC industrial heated press (BYTEC Ltd., London, UK) was used instead of a purpose built hydraulic press (E1180, George Moore Ltd, Birmingham, UK). After drying the uncross-linked paste was placed between two sheets of Melanex film (ICI films division, Dumfries, UK) and pressed in a custom built steel die (Schiemann Tools Ltd, Bodmin, UK) at a temperature of 150 ± 3 °C for 7 minutes and at a pressure of 220 KN. The pressure was increased slowly for the first minute to expel any air pockets. The temperature was monitored using a thermocouple (Conway microprocessor thermometer, Conway Ltd., UK). Hot pressed membranes were 3mm in thickness. It is important to notice that longer curing times resulted in membrane charring.

Figure 2.2 Sensing electrode arrangement


Plug connector
Gold contacts
Spring
Metal shield
Nylon electrode body
Glass liner
Ag / AgCl internal reference
Inner filling solution
Silicon rubber 'O' ring
Selective membrane

2.2.3 Technique for membranes evaluation

2.2.3.1 Equipment

2.2.3.1.1 Nitrate-selective electrode construction

Discs of 7 mm diameter were punched from hot pressed master membranes and conditioned in 0.1 mol dm^{-3} potassium nitrate (AnalaR grade, BDH chemicals, Poole, Dorset, UK) solution. SBS membranes fabricated using N,N,N-triallyl α-amino-acid betaine chlorides required approximately 48 hours conditioning to replace the chloride by nitrate, but it was found that Krynac membranes required 7 days. The conditioned membrane was assembled into the tip of a commercially available electrode body (IS560, Philips Analytical, Cambridge, UK) as shown in figure 2.2. The inner filling solution was composed of 10^{-2} mol dm^{-3} potassium nitrate and potassium chloride (AnalaR grade, BDH chemicals, Poole, Dorset, UK) solutions (1+1).

A commercial nitrate-selective electrode (ELITE, Merck Ltd, Lutterworth, Leicestershire, UK) was used for performance comparison purposes. The electrode membrane was made of PVC and the sensor molecule was believed to be a quaternary ammonium salt.

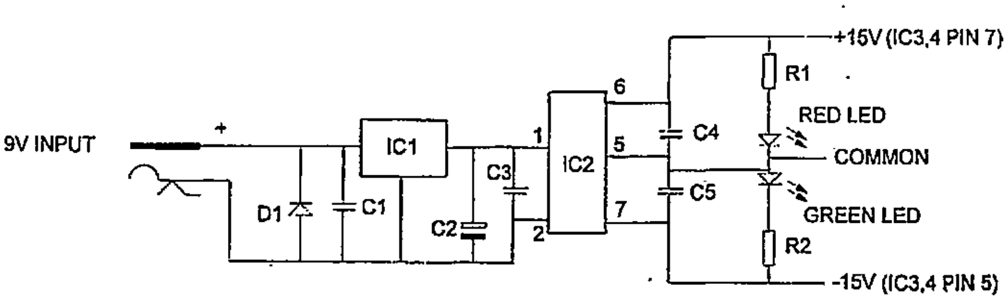
2.2.3.1.2 The reference electrode

The reference electrode selected for this work was a double junction reference electrode (model 90-02, Orion research, Cambridge, MA, USA). The electrical contact was made with an outer filling solution of 4×10^{-2} mol dm^{-3} ammonium sulphate (AnalaR grade, BDH chemicals, Poole, Dorset, UK). The reference electrode was carefully cleaned with milli-Q water before each set of measurements.

2.2.3.1.3 Meters and electronics

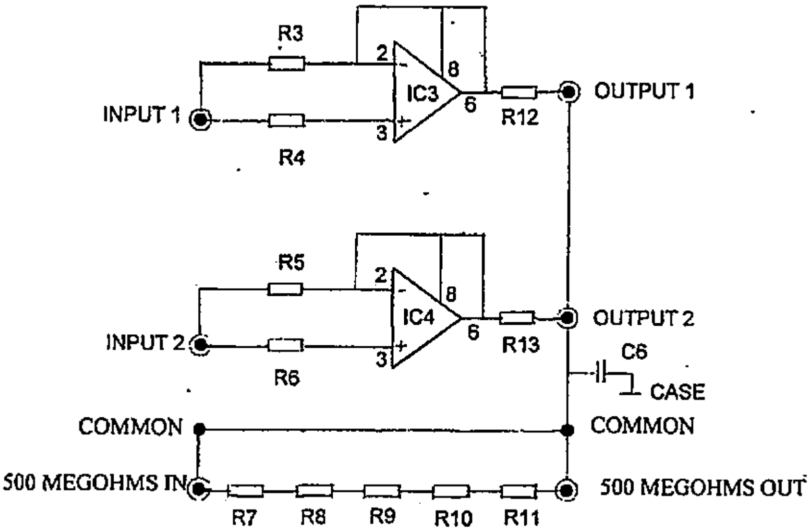
The measurements of electromotive force (EMF) were made using a high impedance voltmeter (model 931402, Hanna instruments, Bedfordshire, UK) connected to a custom built pre-amplifier (Wood, Research Instrument Design, Penmoth, Ruthern Bridge, Bodmin, UK) as shown in figure 2.3.

Figure 2.3 **Schematic of the pre-amplifier unit**



+15V (IC3,4 PIN 7)
R1
6
RED LED
9V INPUT
+
IC1
1
IC2
5
C4
COMMON
C3
D1
C1
C2
2
7
C5
GREEN LED
R2
-15V (IC3,4 PIN 5)

ISOLATED POWER SUPPLY



R3
2
8
IC3
6
OUTPUT 1
INPUT 1
3
R12
R4
R5
2
8
IC4
OUTPUT 2
INPUT 2
6
R13
3
C6
R6
CASE
COMMON
COMMON
500 MEGOHMS IN
500 MEGOHMS OUT
R7
R8
R9
R10
R11

This custom built pre-amplifier is composed of two outputs for two ISEs and a common for the reference electrode and was used to improved the stability of the cell potential reading. This was achieved by the two operational amplifiers (IC3 and IC4 shown in figure 2.3) which enable to decrease the current in the circuitry reducing electrostatic noise. The meter was used to a precision of ± 0.1 mV.

2.2.3.1.4 Ancillary equipment

The EMF measurements were carried out on solutions maintained at 25 ± 0.5 °C and constantly stirred with a PTFE coated stirrer bar. Measurements were made with 100 cm^3 test solution in 250 cm^3 pyrex beaker. All glassware was decontaminated (Decon 90, Decon Laboratories Ltd, Sussex, UK), washed several times with milli-Q water and dried in an oven at 150°C overnight prior to use.

2.2.3.2 Experimental procedures

2.2.3.2.1 Measurement of cell potential

The following electrode arrangement was used

$$\text{Ag}\,\Big|\,\overbrace{\text{AgCl}\,\Big|\,\text{Satd. KCl}\,\Big\|\,\text{Outer filling solution}}^{E_{Ref1}}\,\Big\|\,\text{Sample}\,\Big|\,\underbrace{\text{Nitrate ion-selective membrane}}_{E_m}\,\Big|\,\overbrace{\text{Internal filling solution}\,\Big|\,\text{AgCl}\,\Big|\,\text{Ag}}^{E_{Ref2}}$$

Outer filling solution: 4×10^{-2} mol dm^{-3} ammonium sulphate

Internal filling solution: 1.0×10^{-2} mol dm^{-3} KCl + KNO_3 (1+1)

The overall observed potential is given by the following equation 2.1:

$$E_{Cell} = E_{Ref1} + E_j + E_m - E_{Ref2} \qquad (2.1)$$

Where :

E_{Ref1} = External reference electrode potential (double junction electrode)

E_j = Liquid junction potentials

E_m = Membrane potential

E_{Ref2} = Internal reference electrode potential

As mentioned in the introduction it can be assumed that E_{Ref1}, E_{Ref2} and liquid junction potentials remain constant at constant temperature. Therefore E_{cell} will be a function of E_m. We also described that the membrane potential could be related to the activity of the analyte (a) using the Nernst equation. At a constant temperature of 298K and for a monovalent anion the Nernst equation is reduced to:

$E_{cell} = E° - 59.12 \log a$

and if a graph of E against log a is plotted, a slope of -59.12 mV per activity decade should be obtained. However a slope between -55 and -60 mV dec^{-1} is considered as Nernstian.

2.2.3.2.2 Membrane Conditioning

The conditioning of the membranes was made in 1×10^{-1} mol dm^{-3} nitrate solution in order to exchange the chloride to the nitrate ion. This time varied according to the

polymeric material used. Typically Krynac membranes required a conditioning of 1 week prior to use and SBS membranes only two days.

2.2.3.2.3 Standard solutions

The standard solutions were prepared daily using analytical reagents of the highest purity commercially available. Standard solutions were prepared with potassium nitrate (AnalaR grade, BDH chemicals, Poole, Dorset, UK) dissolved in Milli-Q water (Milli-Q, Millipore (UK) Ltd., Watford, Hertfordshire, UK). A range of standards 10^{-1}-10^{-8} mol dm^{-3} were prepared by serial dilution of 10^{-1} mol dm^{-3} stock solution. Potassium dihydrogen orthophosphate (AnalaR grade, BDH chemicals, Poole, Dorset, UK) was used as ISAB. The commercial nitrate electrode and equipment were checked using the standard solutions.

2.2.3.2.4 Linear Nernstian range and limit of detection

The range of linear response was measured over the region of the calibration curve exhibiting a Nernstian slope (Amman, 1986). The limit of detection was measured from the experimental data as the point of the intersection between a linear extrapolation of the Nernstian slope (AB), and the horizontal part of the upper curve where the EMF is a constant value (DE) (Amman, 1986), as shown graphically in figure 1.7.

2.2.3.2.5 Interference study

The determination of selectivity coefficient ($k^{pot}_{NO3-,\,J}$) were made accordingly to IUPAC recommandations using the fixed interferent method (FIM) (Guilbaut, 1976). A constant level of interferent, 0.01 mol dm^{-3}, was used as an interferent unless stated otherwise. Selectivity coefficients were calculated using the intersect method as illustrated in figure 1.7. The 18 mV method (Bailey, 1980) was also used for comparison purposes.

2.2.3.2.6 pH studies

pH Dependency experiments were carried out on blank cross-linked membranes fabricated with 50 % m/m polymeric material, 43 % m/m 2-NPOE and 7 % DCP and on membranes containing the sensor molecules. The pH was adjusted by using 50 mmol dm^{-3} citric acid / trisodium citrate / sodium hydroxide buffer solutions (AnalaR grade, BDH chemicals, Poole, Dorset, UK) containing 1 mmol dm^{-3} potassium nitrate. The pH was monitored with a pH electrode (Gelplas, General Purpose Combination, BDH, Lutterworth) and a high impedance pH meter (model 290, PYE UNICAM, Cambridge, UK) and the cell potential recorded as described in section 2.2.3.1.3. Three polymeric materials were examined, SBSa containing a phenolic based anti-oxidant, anti-oxidant free SBS and poly(acrylonitrile-butadiene)copolymer (Krynac 50.75).

2.2.3.2.7 Stability of response

Stability of the response was determined using an autologger (CR10X, Campbell Scientific, Shepshed, Leicestershire, UK). Potentials were recorded automatically every

30 minutes, in 1 x 10^{-3} mol dm^{-3} potassium nitrate solution for at least 3 days. For this experiment the outer filling solution of the double junction reference electrode was prepared in agar gel to overcome interference by electrode bleed.

2.2.3.2.8 Response times

Speed of response was determined by measuring the time required for the electrode to equilibrate in a solution of 10^{-5} mol dm^{-3} nitrate solution having previously been immersed into a 10^{-6} mol dm^{-3} nitrate solution. From this, as IUPAC recommends, it is possible to determine t_{90} response time value i.e the time required by the electrode to reach 90 % of the equilibrium potential value.

2.2.3.2.9 Lifetimes of electrode membranes

The assessment of the limetime of an ion-selective electrodes is difficult. Ideally they should be tested in flow systems under the same conditions. Unfortunately such a device was not available and would be unpractical to use with a great number of membranes to be tested.

The lifetime of the membranes were determined either by leaving them in a laboratory solution containing 10^{-1} mol dm^{-3} potassium nitrate or under environmental conditions by leaving them in agricultural run-off waters or rivers.

CHAPTER 3

NITRATE-SELECTIVE ELECTRODES: LABORATORY RESULTS AND DISCUSSION

3.1 Studies using Krynac 50.75 membranes

3.1.1 Blank membranes

Previous work (Ebdon *et al.*, 1990, 1991; Braven *et al.*, 1996) on covalently bound sensors had used a block copolymer of butadiene and acrylonitrile, Krynac 50.75. The study of blank membranes was necessary to ensure that the response obtained from the nitrate-selective membranes was attributed to the sensor molecule and not the polymeric material.

3.1.1.1 Blank membrane composition and response

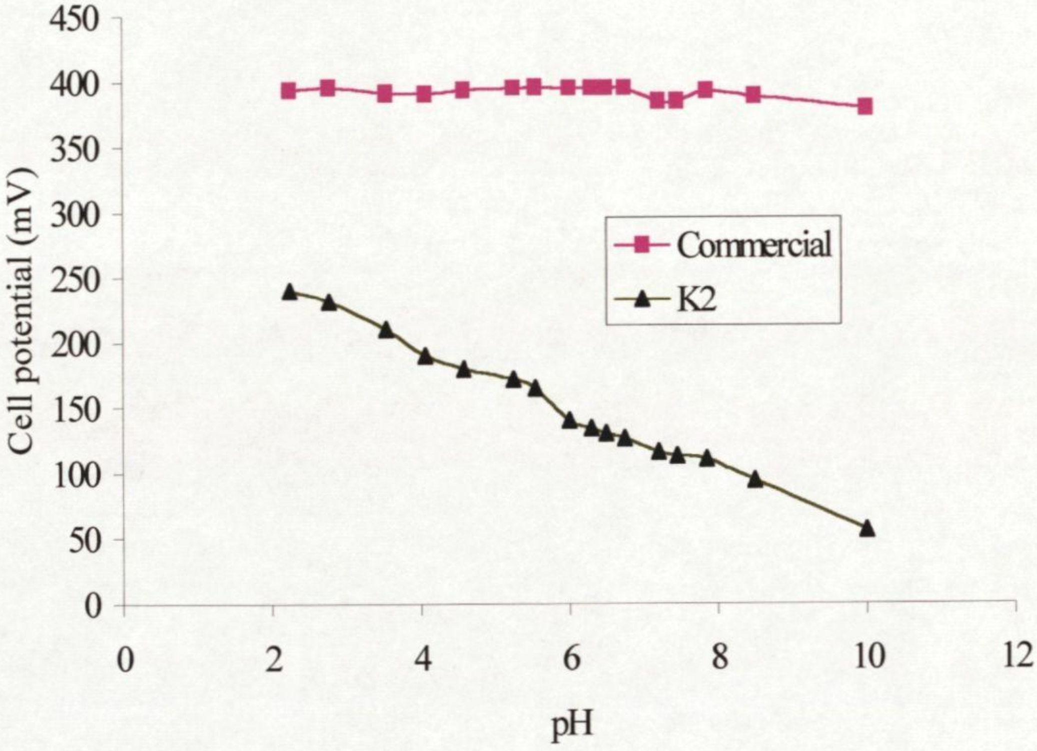
Table 3.1 Composition and response of blank Krynac 50.75 membranes

Membrane number	Free radical initiator (% m/m)	Solvent mediator (% m/m)	Krynac 50.75 (% m/m)	Slope (mV dec^{-1})	Limit of detection (μmol dm^{-3})
K1	DCP (8)	0	92	-58.3	34.0
K2	DCP (8)	2-NPOE (39)	53	-58.3	11.0
K3	DCP (8)	0	92 (impure)	Sub-Nernstian -34.3	N.D
K4	DCP (8)	2-NPOE (39)	53 (impure)	Sub-Nernstian -34.3	N.D

Table 3.1 shows that the pure polymer exhibits a response to nitrate greater than that of the impure material. The reason for this is not known. Repeat purification caused loss of polymer additives yielding a brittle material unsuitable for membrane use. However in all cases this nitrate response from blank membranes was only observed for a short time and the Nernstian response disappeared after 2 weeks. Krynac blank membranes with or without solvent mediator gave similar Nernstian slopes clearly showing that the solvent mediator can not be responsible for the nitrate response.

3.1.1.2 pH Dependency of blank membranes

Figure 3.1 pH Dependence of membrane K2 and a commercial nitrate-selective electrode in a 1 mmol dm^{-3} nitrate solution



The blank membranes fabricated with Krynac 50.75 seem to be pH dependent whereas commercial nitrate-selective electrode membrane (made of PVC) was perfectly stable over a wide pH range (2-10), as shown in figure 3.1.

3.1.2 Krynac membranes containing an immobilised N.N.N-triallyl α-amino-acid betaine salt

3.1.2.1 Membrane composition and response

Table 3.2 Membrane composition for sensors containing N,N,N-triallyl glycine betaine chloride (TAGBCl), N, N, N-triallyl 2-aminocaprylic acid betaine chloride (TA2CABCl) and N, N, N-triallyl taurine betaine chloride (TATBCl)

Membrane number	TAGBCl (%m/m)	TA2CABCl (%m/m)	TATABCl (%m/m)	Krynac (%m/m)	DCP (%m/m)	2-NPOE (%m/m)
K5	5.0	0	0	46.0	7.5	41.5
K6	0	5.0	0	46.0	7.5	41.5
K7	0	3.0	0	47.0	7.5	42.5
K8	0	6.5	0	45.6	7.2	40.7
K9	0	9.0	0	44.6	7.0	39.4
K10	0	0	5.0	46.0	7.5	41.5

Table 3.2 shows the sensors and the percentage composition of the first series of membranes studied. Three different N,N,N-triallyl betaine chloride salts were successfully bonded to Krynac 50.75. The amount of sensor molecule was optimised for the best performing sensor available at the time i.e. N,N,N-triallyl 2-aminocaprylic acid betaine chloride (TA2ACABCl). The ratio Krynac 50.75 / 2-NPOE was kept constant. The results obtained for the sensors are shown in table 3.3.

Table 3.3 Nitrate electrode response of Krynac membranes containing immobilised betaine salts compared with a commercial electrode

Membrane number	Slope (mV dec^{-1})	Limit of detection (μmol dm^{-3})	$k^{pot}_{NO3-, Cl-}$ (10^{-2} mol dm^{-3})	Linear Nernstian range (mol dm^{-3})
Commercial electrode	-59.0	7.0	5.5×10^{-3}	$0.1 – 1.0 \times 10^{-5}$
K5	-55.0	34.0	1.05×10^{-2}	$0.1 - 1.0 \times 10^{-4}$
K6	-56.0	5.0	4.0×10^{-3}	$0.1 - 5.0 \times 10^{-5}$
K7	-55.0	7.5	5.0×10^{-3}	$0.1 – 1.0 \times 10^{-4}$
K8	-56.5	5.5	4.5×10^{-3}	$0.1 – 5.0 \times 10^{-5}$
K9	-53.5	15.0	1.0×10^{-2}	$0.1 – 1.0 \times 10^{-4}$
K10	-56.0	50.0	1.0×10^{-2}	$0.1 – 5.0 \times 10^{-4}$

Table 3.3 shows the electrochemical data obtained from membranes K5-K10. The membranes were evaluated after full conditioning (7 days). At this stage, only three N,N,N-triallyl α-amino-acid betaine salts were fully examined using Krynac 50.75. All the compounds gave a Nernstian response. The limit of detection has been improved from 34.0 μmol dm^{-3} nitrate for N,N,N-triallyl glycine betaine chloride (membrane K5) to 5.0 μmol dm^{-3} nitrate for triallyl α-amino caprylic acid betaine chloride (membrane K6). The selectivity for nitrate against chloride has been also improved from 1.05×10^{-2} for membrane K5 to 4.0×10^{-3} for membrane K6. These improvements for membranes K6-K9 compared with K5 clearly show the importance of incorporating an alkyl side chain in the betaine.

Membranes K6 and K8 fabricated with 5 and 6.5% m/m sensor molecule gave very similar electrochemical performances. It can be seen that membranes K9 fabricated with 9% m/m betaine gave inferior results to membrane K6, probably due to a greasy layer present on the surface. Membrane K7 fabricated with 3.0 % m/m sensor molecule had a very similar selectivity coefficient for nitrate over chloride as membranes K6 and K8 but a weaker Nernstian slope and higher limit of detection. N,N,N-triallyl taurine betaine (membrane K10) was insoluble in THF and a homogeneous mixture of this sensor with the other components of the membrane could not be obtained. This resulted in a poor quality of the hot-pressed membranes which is probably the reason for the poor results obtained for membrane K10.

The electrochemical performances of the nitrate-selective electrode fabricated using membrane K6 were comparable or better than the commercial electrode used throughout this study as shown in figures 3.2 and 3.3.

Figure 3.2 **Nitrate response for a commercial nitrate electrode and membranes K6 & K5**

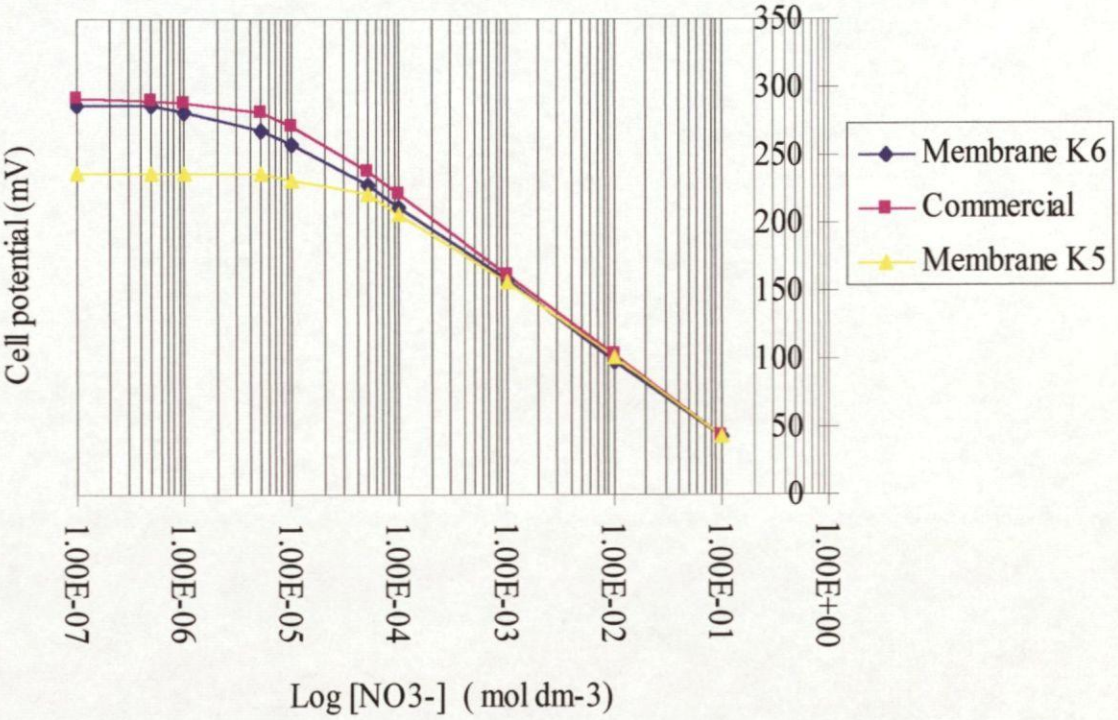
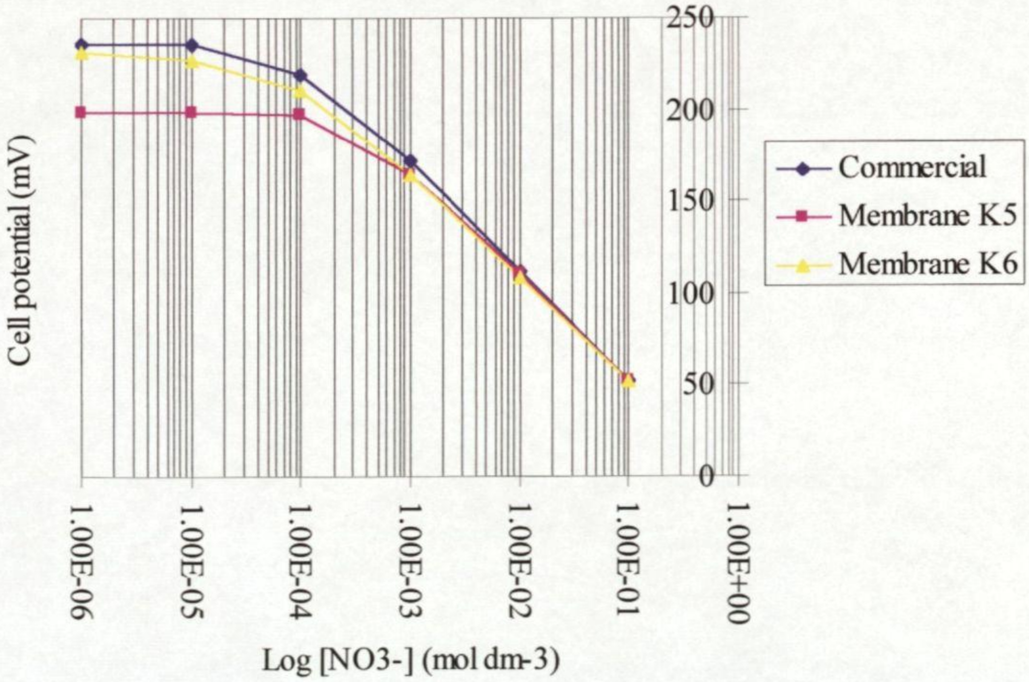


Figure 3.3 **Selectivity data obtained for a commercial nitrate electrode and membranes K6 & K5 (for 1 x 10^{-2} mol dm^{-3} chloride as interferent)**



3.1.2.2 Lifetime studies

Table 3.4 Lifetime studies of Krynac membranes containing immobilised betaine chloride salts

Number of days after appropriate conditioning	Electrochemical data	K5	K6	K10
Original response	Nernstian slope (mV dec^{-1})	-55.0	-56.0	-56.0
	Limit of detection (μmol dm^{-3})	34.0	5.0	50.0
Day 70	Nernstian slope (mV dec^{-1})	-55.3	-57.0	*
	Limit of detection (μmol dm^{-3})	33.0	6.5	*
Day 200	Nernstian slope (mV dec^{-1})	-55.2	-56.7	*
	Limit of detection (μmol dm^{-3})	32.0	6.0	*

* Unresponsive

The membranes were evaluated after full conditioning (7 days). Previous workers (Frampton, 1992 and Braven *et al.*, 1996) showed that membranes containing trapped betaine-based sensors quickly lost activity due to leaching of the sensor from the membrane. The covalent binding of the betaine to the polymer via the three allyl groups increased the lifetime of the membranes from a few days to more than two hundred days as shown in table 3.4. After more than six months, membranes K5 and K6 still exhibited very similar electrochemical properties to those originally observed, proving the value of covalent sensor attachment in improving membrane lifetime.

3.1.2.3 Membrane conditioning

Figure 3.4 Nitrate response for membrane K6 after 2, 5 and 7 days of conditioning

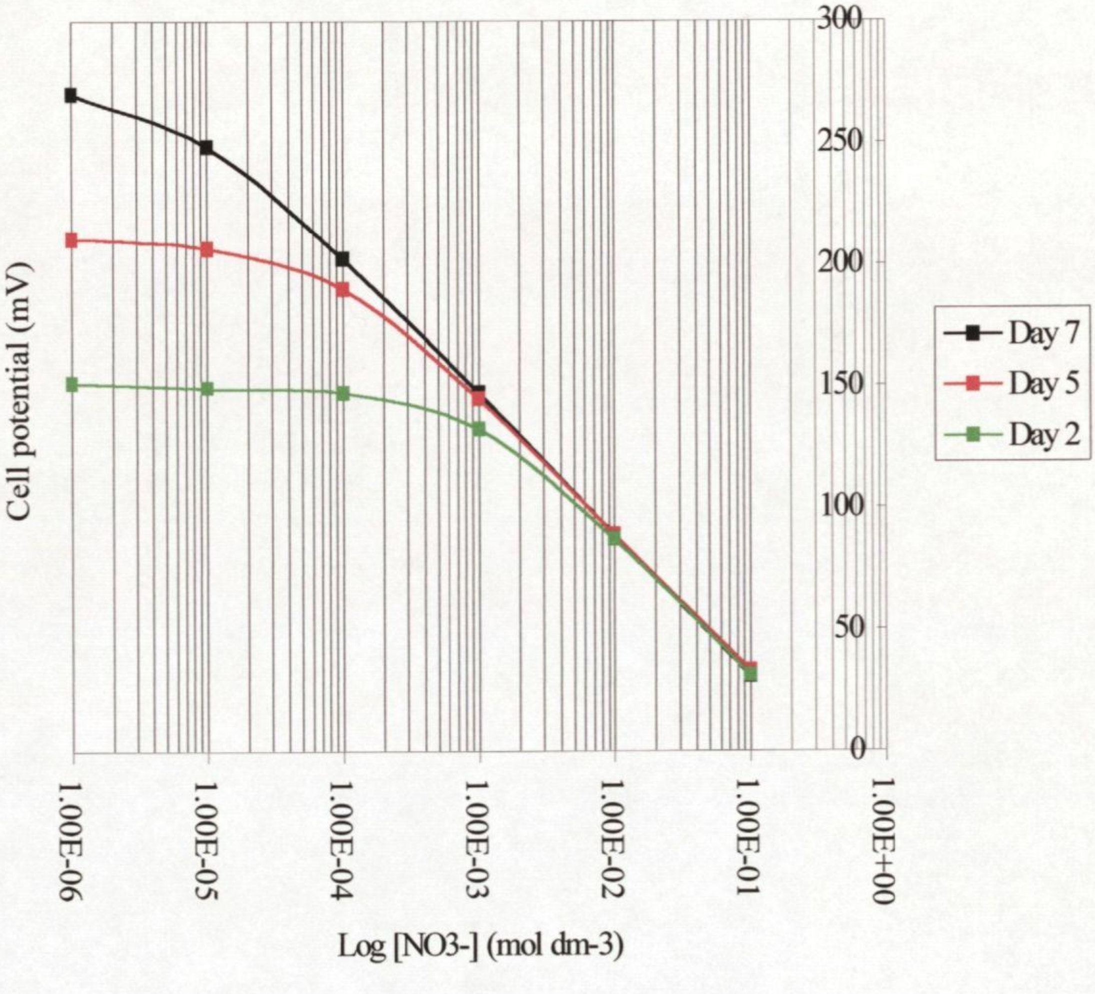


Figure 3.4 shows that the membranes had to be conditioned in 1 x 10^{-1} mol dm^{-3} nitrate solution for a week to optimise the exchange of chloride to nitrate. Previous workers (Sutton *et al.*, 1996) had shown that it was possible to reduce that time of conditioning by directly incorporating the nitrate salt of the betaine into the membrane.

3.1.2.4 Use of N,N,N-triallyl α-amino-acid betaine nitrate

3.1.2.4.1 Membrane composition and response

Table 3.5 Membrane composition of sensors containing N,N,N-triallyl α-amino-acid betaine nitrates.

Membrane number	TAGBN (%m/m)	TA2CABN (%m/m)	TATABN (%m/m)	Krynac (%m/m)	DCP (%m/m)	2-NPOE (%m/m)
K11	5.0	0	0	46.0	7.5	41.5
K12	0	5.0	0	46.0	7.5	41.5
K13	0	0	5.0	46.0	7.5	41.5

Table 3.5 shows the membrane composition for betaine nitrate salts which is identical to the optimum membrane composition used for betaine chloride salts. All the betaine nitrate salts were insoluble in THF resulting in poor quality heterogeneously dispersed membranes. It was also observed that during the hot-pressing of the membranes, a decomposition of the sensor molecule occurred. Lower temperatures of hot-pressing were tried but without any improvements in the quality of the cross-linked membranes. The betaine nitrate salts were only soluble in water and alcohol and some attempts were made to mix solvents in the production of uncross-linked membranes. N,N,N-triallyl α-amino-acid salt (100 mg) was dissolved in the minimum amount of solvent (water, 0.5 ml or methanol, 1 ml) and added to a solution of THF containing free radical initiator, Krynac 50.75 and solvent mediator. Unfortunately during the drying process the sensor molecule

re-precipitated and the same problems as described above were encountered. The electrochemical data obtained for these membranes are shown in table 3.6.

Table 3.6 **Nitrate electrode response of Krynac membranes containing immobilised betaine nitrate salts**

Membrane number	Slope (mV dec^{-1})	Limit of detection (μmol dm^{-3})	Linear Nernstian range (mol dm^{-3})
K11	Sub-Nernstian -46.0	110.0	$0.1 - 1.0 \times 10^{-3}$
K12	Sub-Nernstian -45.0	100.0	$0.1 - 1.0 \times 10^{-3}$
K13	Sub-Nernstian -47.0	110.0	$0.1 - 1.0 \times 10^{-3}$

Table 3.6 shows that all N,N,N-triallyl α-amino-acid betaine nitrate salts gave sub-Nernstian response to nitrate, whereas conditioned betaine chloride salts gave Nernstian response. This undoubtedly arose from the poor quality of the membranes. They contained a lot of air bubbles resulting in a lack of robustness, whereas hot-pressing of membranes containing betaine chloride salts, in the same conditions, did not exhibit this problem. For this reason chloride salts followed by nitrate exchange were used for this work.

3.1.2.4.2 Membrane conditioning and lifetime studies

The membranes containing betaine nitrate salts required only 3 hours of conditioning but, as mentioned previously, they exhibited a sub-Nernstian response to nitrate. Table 3.7

shows that these membranes were unresponsive after only 70 days more like blank membranes suggesting a decomposition of the sensor molecule during the hot-pressing process. Therefore the use of nitrate salts for the membrane fabrication was stopped.

Table 3.7 Lifetime studies of Krynac membranes containing immobilised betaine nitrate salts

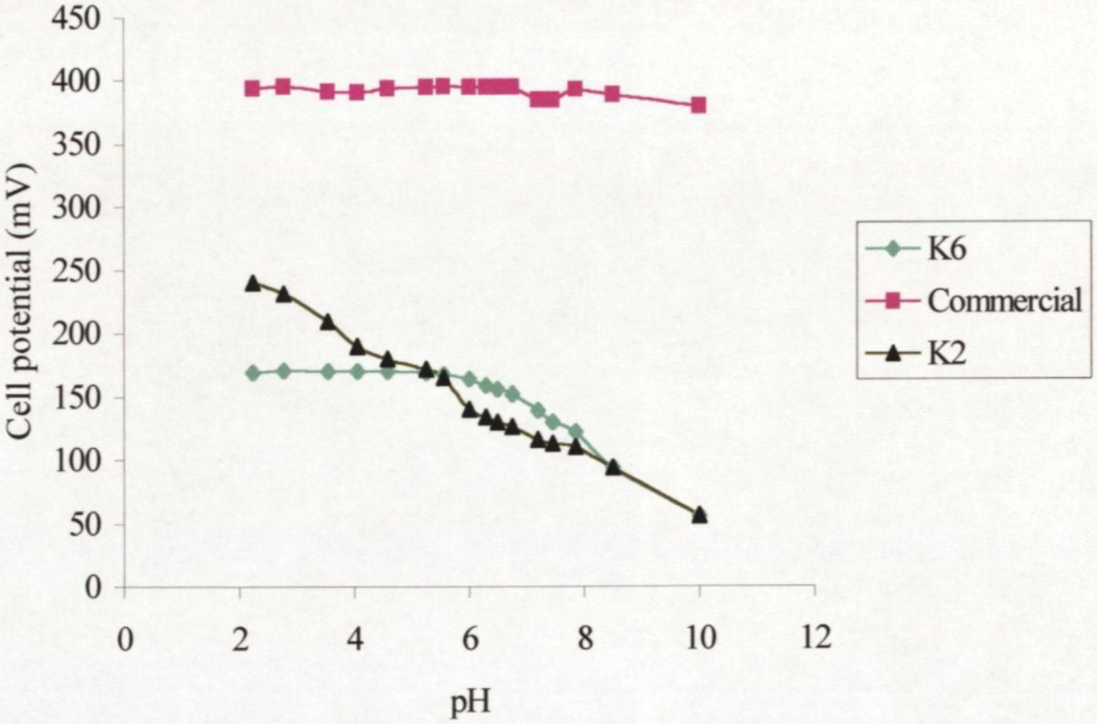
Number of days after conditioning	Electrochemical data	K11	K12	K13
Original response	Slope ($mV\ dec^{-1}$)	-46.0	-45.0	-47.0
	Limit of detection ($\mu mol\ dm^{-3}$)	110.0	100.0	110.0
Day 21	Slope ($mV\ dec^{-1}$)	-28.5	-30.0	-29.0
	Limit of detection ($\mu mol\ dm^{-3}$)	N.D	N.D	N.D
Day 70	Slope ($mV\ dec^{-1}$)	*	*	*
	Limit of detection ($\mu mol\ dm^{-3}$)	*	*	*

* Unresponsive

3.1.2.5 pH Dependency

It has been shown in section 3.1.1.2 and figure 3.1 that blank Krynac membranes were pH dependent from pH 2 upwards. A pH evaluation was carried out on Krynac membranes containing nitrate exchanged immobilised N,N,N-triallyl 2-aminocaprylic acid betaine chloride (K6). These were shown to be pH dependent only above pH 6 as shown in figure 3.5. However, a pH independent polymer was required to extend the electrode performance over a greater pH range; pH 6 being too low for the majority of uses especially those of an environmental nature.

Figure 3.5 pH Dependence of a commercial nitrate electrode and membranes K6 & K2



3.2 Studies using SBS membranes

Krynac-based membranes showed an excellent stability over a long period of time but their pH dependency limited their use in environmental waters. Therefore, efforts were concentrated on the search for a pH independent polymeric material. For this reason SBS was re-investigated as a possible candidate for the production of a pH independent

nitrate-selective electrode. SBS had previously proved to be a very successful membrane matrix when used for both calcium and nitrate-selective electrodes (Ebdon *et al.*, 1979, 1982, 1985; Ellis *et al.*, 1980). However, a relatively poor selectivity for nitrate in the presence of interferents was a recognised limitation (King, 1985; Frampton, 1992) due to an incompatibility between SBS and 2-NPOE the solvent mediator.

3.2.1 Blank membranes

Two slightly different polystyrene-*block*-polybutadiene-*block*-polystyrene polymers were investigated. The first one contained 30 % styrene (SBSa) plus anti-oxidant. In the second one the level of phenolic based anti-oxidant was reduced, but it contained the same polystyrene content (SBS).

3.2.1.1 Blank membrane composition and response

Incompatibility between 2-NPOE and SBS was re-investigated and also 2-F-2'-NDPE was investigated for this study. 2-NPOE used with Krynac gave robust membranes with good sensitivity and selectivity. 2-F-2'-NDPE had never been investigated with SBS but proved to be a good solvent mediator when used with Krynac (Sutton, 1996).

Table 3.8 Composition of SBS blank membranes

Membrane number	Polymeric material (%m/m)	Solvent mediator (%m/m)	DCP (%m/m)
S1	SBSa (50)	2-NPOE (42.0)	8.0
S2	SBS (50)	2-NPOE (42.0)	8.0
S3	SBSa (50)	2-F-2'-NDPE (42.0)	8.0
S4	SBS (50)	2-F-2'-NDPE (42.0)	8.0
S5	SBS (92)	nil	8.0

Preliminary studies showed that 2-NPOE did not appear to be incompatible with SBS as previously indicated so the use of this polymer was re-investigated and a series of membranes with compositions shown in table 3.8 were fabricated.

Membranes S3 and S4 were not sufficiently robust to allow subsequent evaluation. This was caused by a poor compatibility between 2-F-2'-NDPE and the polymer. Membrane S5 without the solvent mediator was unresponsive. Membranes S1 and S2 composed of SBS, SBSa and 2-NPOE were robust after hot-pressing.

Table 3.9 **Lifetime studies of blank membranes**

Time of conditioning	Electrochemical parameters	S1	S2
Day 1	Slope (mV/dec)	-41.7	-30.0
	Linear range (mol dm^{-3})	0.1 – 10^{-3}	0.1 – 10^{-3}
Day 20	Slope (mV/dec)	-30.2	-22.0
	Linear range (mol dm^{-3})	0.1 – 10^{-3}	0.1 – 10^{-3}
Day 30	Slope (mV/dec)	-12.0	-10.0
	Linear range (mol dm^{-3})	0.1 – 10^{-3}	0.1 – 10^{-2}

Table 3.9 shows that membranes S1 and S2 exhibit sub-Nernstian response to nitrate lasting for about two weeks. The ideal matrix should be unresponsive to the analyte. Both SBS-types were good candidates, from this standpoint, because of their poor response to nitrate.

3.2.1.2 pH Dependency

Membrane S1 and S2 were investigated using the method described in section 2.2.3.2.6. A nitrate solution of 1 mmol dm^{-3} was used and the results are shown in figure 3.6.

Figure 3.6 pH Dependence of blank membranes S1 and S2

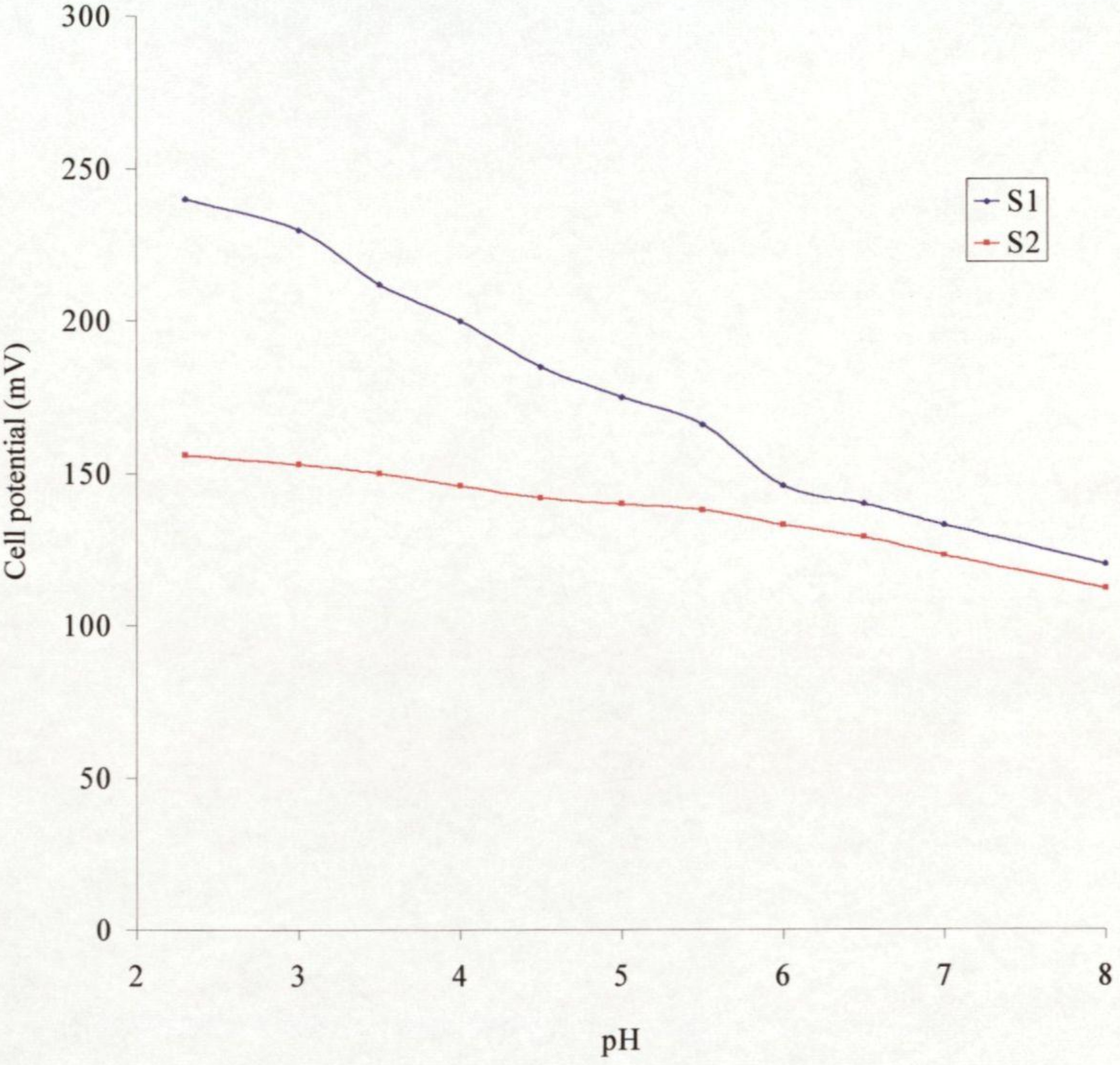


Figure 3.6 shows that the polymeric material with a low phenolic based anti-oxidant content (SBS, membrane S2) only had a small pH dependency over a pH range from 2 to 8 whereas the one containing more of the phenolic based anti-oxidant (SBSa, membrane S1) was very pH dependent. This suggests that the anti-oxidant was responsible for the pH dependency of the SBSa and Krynac-based blank membranes because Krynac also contained a phenolic-based anti-oxidant.

In conclusion, SBS blank membranes were robust, exhibited a poor response to nitrate, and were compatible with 2-NPOE offering the prospect of a good selectivity for nitrate

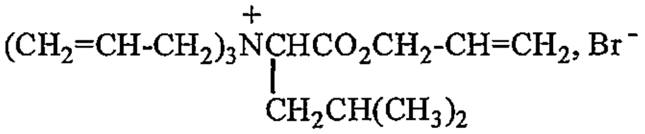
over interferents and pH independent over a wide pH range. For all these reasons SBS was chosen for the rest of the study.

3.3 SBS membranes containing an immobilised N,N,N-triallyl α-amino-acid betaine salt

Because of the improvement in electrode performance observed by the introduction of a hexyl side-chain into the glycine betaine sensor molecule, (table 3.3: K5 had a limit of detection of 32.0 μmol dm^{-3} nitrate and a $k^{pot}_{NO3-, Cl-}$ of 1.05×10^{-2} and K6 has a limit of detection of 5.0 μmol dm^{-3} nitrate and a $k^{pot}_{NO3-, Cl-}$ of 4.0×10^{-3}), it was decided to investigate a series of betaines $((CH_2{=}CH\text{-}CH_2)_3\overset{+}{N}CHRCO_2H, Cl^-, (V))$. N,N,N-triallyl α-amino-acid betaine chlorides with the following side-chains (R) which were investigated using SBS with a low content of phenolic based anti-oxidant (SBS).

R = H, CH_3CH_2, $CH_3(CH_2)_2$, $(CH_3)_2CH$, $CH_3(CH_2)_3$, $(CH_3)_2CHCH_2$, $CH_3(CH_2)_4$, $CH_3(CH_2)_5$, $CH_3(CH_2)_9$.

Additionally the ester (VI), with the structure shown below, was examined:



$$(CH_2{=}CH\text{-}CH_2)_3\overset{+}{N}\underset{CH_2CH(CH_3)_2}{\underset{|}{C}}HCO_2CH_2\text{-}CH{=}CH_2,\ Br^-$$

All the membranes investigated were composed of 43.5 % m/m purified SBS, 10 % m/m free radical initiator (DCP), 40.0 % m/m solvent mediator (2-NPOE) and 6.5 % m/m sensor molecule.

3.3.1 Electrode evaluation

After appropriate conditioning, the membranes were evaluated as described in section 2.2.3.2. The results of the electrochemical evaluation of each sensor are shown in table 3.10. All the membranes gave Nernstian responses and the best electrochemical performances were obtained from N,N,N-triallyl leucine betaine chloride.

Table 3.10 Performances of nitrate selective electrodes

Membrane number	Electrode Sensor (R)	Linear Nernstian range (mol dm^{-3} NO_3^-)	Nernstian slope (mV/dec)	Limit of detection (µmol dm^{-3} NO_3^-)	$k^{pot}_{NO_3^-, Cl^-}$ (10^{-2} mol dm^{-3} Cl^-)
	Commercial	1×10^{-1} to 1×10^{-5}	-59.0	7.0	5.50×10^{-3}
S6	H	1×10^{-1} to 1×10^{-4}	-55.3 ± 0.4	30.0 ± 2.9	12.0×10^{-3}
S7	CH_3CH_2	1×10^{-1} to 5×10^{-4}	-50.5 ± 0.5	25.0 ± 7.0	20.0×10^{-3}
S8	$CH_3(CH_2)_2$	1×10^{-1} to 5×10^{-5}	-53.4 ± 0.5	6.6 ± 0.7	6.0×10^{-3}
S9	$(CH_3)_2CH$	1×10^{-1} to 1×10^{-5}	-58.1 ± 0.2	1.5 ± 0.2	6.0×10^{-3}
S10	$CH_3(CH_2)_3$	1×10^{-1} to 5×10^{-5}	-57.6 ± 0.6	3.4 ± 0.5	4.0×10^{-3}
S11	$(CH_3)_2CHCH_2$	1×10^{-1} to 5×10^{-6}	-59.1 ± 0.2	0.34 ± 0.05	3.4×10^{-3}
S12	$CH_3(CH_2)_4$	1×10^{-1} to 5×10^{-5}	-55.0 ± 0.5	8.0 ± 1.3	6.0×10^{-3}
S13	$CH_3(CH_2)_5$	1×10^{-1} to 5×10^{-5}	-57.0 ± 0.5	7.4 ± 0.6	6.0×10^{-3}
S14	$CH_3(CH_2)_9$	1×10^{-1} to 5×10^{-5}	-56.6 ± 0.6	11.2 ± 0.8	8.0×10^{-3} (5×10^{-3})*
S15	(VI)	1×10^{-1} to 5×10^{-5}	-57.5 ± 0.1	2.9 ± 0.6	4.5×10^{-3}

* : -18 mV method

Table 3.10 shows that all the betaines studied are nitrate responsive with Nernstian slopes and also shows the improvement obtained for membranes containing N,N,N-triallyl leucine betaine chloride as sensor compared with the commercial nitrate-selective electrode used for this work. The linear Nernstian range, 0.1 - 5.0 x 10^{-6} mol dm^{-3} nitrate, is wider than that obtained for the commercial nitrate-selective electrode (0.1 - 10^{-5} mol dm^{-3} nitrate). The limit of detection of this sensor was 3.4 x 10^{-7} mol dm^{-3} nitrate which is better than the commercial nitrate-selective electrode (7.0 x 10^{-6} mol dm^{-3} nitrate) representing an improvement in the sensitivity of more than one order of magnitude. The selectivity for nitrate against chloride of the best betaine sensor (3.4 x 10^{-3}) is better than that obtained with the commercial sensor (5.5 x 10^{-3}). The slope over the linear range is -59.1 mV dec^{-1} that is similar to the theoretical Nernstian slope calculated for a singly charged anion at 25°C (-59.12 mV dec^{-1}). Structure-activity relationship will be discussed in details in section 3.4.

Table 3.11 Reproducibility of membrane performance for five nitrate-selective membranes containing N,N,N-triallyl leucine betaine sensor

[NO_3^-]	Cell potential (mV)					Average	Standard	RSD
(mol dm^{-3})	(1)	(2)	(3)	(4)	(5)	Potential (mV)	deviation	%
1.00E-01	57.0	57.0	57.0	57.0	57.0	57.0	0.00	0.00
1.00E-02	117.0	117.0	117.0	117.0	117.0	117.0	0.00	0.00
1.00E-03	177.9	177.9	176.3	177.8	177.6	177.5	0.68	0.38
1.00E-04	237.5	237.4	235.3	236.8	236.0	236.6	0.94	0.40
5.00E-05	255.3	255.3	252.7	255.2	254.5	254.6	1.11	0.44
1.00E-05	295.6	294.8	292.6	294.6	293.9	294.3	1.13	0.38
5.00E-06	312.9	312.1	309.6	312.1	310.7	311.5	1.32	0.42
1.00E-06	346.8	343.8	341.7	345.5	342.4	344.0	2.12	0.62
5.00E-07	361.5	356.8	352.6	356.5	352.3	355.9	3.75	1.05
1.00E-07	378.1	370.8	367.7	375.0	367.2	371.8	4.71	1.27
4.00E-08	388.0	379.6	376.5	382.2	374.9	380.2	5.17	1.36
1.00E-08	388.0	379.6	376.5	382.2	375.0	380.3	5.15	1.35

Figure 3.7 shows the excellent sensitivity for nitrate obtained for 5 membranes S11 compared with a commercial electrode. Table 3.11 shows the good reproducibility of the membrane's performance. The relative standard deviation of the cell potential does not exceed 0.5% over the linear Nernstian range (0.1 - 5 x 10^{-6} mol dm^{-3} nitrate). This shows the good homogeneity of the membranes.

The selectivity coefficients were also determined for a variety of anions usually given in the Hofmeister series and other anions of environmental importance.

Table 3.12 Selectivity data for a variety of anions using membrane S11

[J] in mol dm^{-3}	$k^{pot}_{NO3-, J}$	
	S11	Commercial
F^- (10^{-2})	3.0×10^{-4}	(10^{-4})
Cl^- (10^{-2})	3.4×10^{-3}	5.5×10^{-3} (5.6×10^{-3})
Br^- (10^{-2})	7.3×10^{-2}	*
I^- (10^{-4})	14	*
SCN- (10-5)	37	*
ClO_4^- (10^{-5})	400	*
HCO_3^- (10^{-2})	3.0×10^{-2}	(5×10^{-3})
NO_2^- (10^{-2})	3.9×10^{-2}	*
SO_4^{2-} (10^{-2})	4.6×10^{-5}	1.6×10^{-4} (10^{-4})
Phthalate (10^{-5})	15	*

(): Manufacturer selectivity coefficient; *: no data

Table 3.12 shows that the interferences from the anions increase in the following order:

$SO_4^{2-} < F^- < Cl^- < HCO_3^- < NO_2^- < Br^- < I^- <$ phthalate $< SCN^- < ClO_4^-$

This selectivity pattern is consistent with the empirical series suggested by Hofmeister (Hofmeister, 1888) shown below:

$SO_4^{2-} < F^- < Cl^- < Br^- < I^- < SCN^- < ClO_4^-$

3.3.2.3 pH Dependence of the electrode

pH Dependency studies were performed using the method described in section 2.2.3.2.6. Table 3.13. gives the membrane composition of N,N,N-triallyl norleucine betaine in three different polymeric materials SBS, SBSa and Krynac 50.75.

Table 3.13 Composition of membranes containing N,N,N-triallyl norleucine betaine

Membrane number	Sensor (% m/m)	Solvent mediator (% m/m)	Free radical initiator (% m/m)	Polymer (% m/m)
S16	6.5	2-NPOE (40.0)	DCP (10.0)	SBSa (43.5)
S10	6.5	2-NPOE (40.0)	DCP (10.0)	SBS (43.5)
K14	6.5	2-NPOE (41.5)	DCP (7.5)	Krynac 50.75 (46.0)

Figure [illegible]

[illegible]

Figure 3.10 pH Dependency of immobilised N,N,N-triallyl leucine betaine (S11) in a 1 mmol dm^{-3} nitrate solution

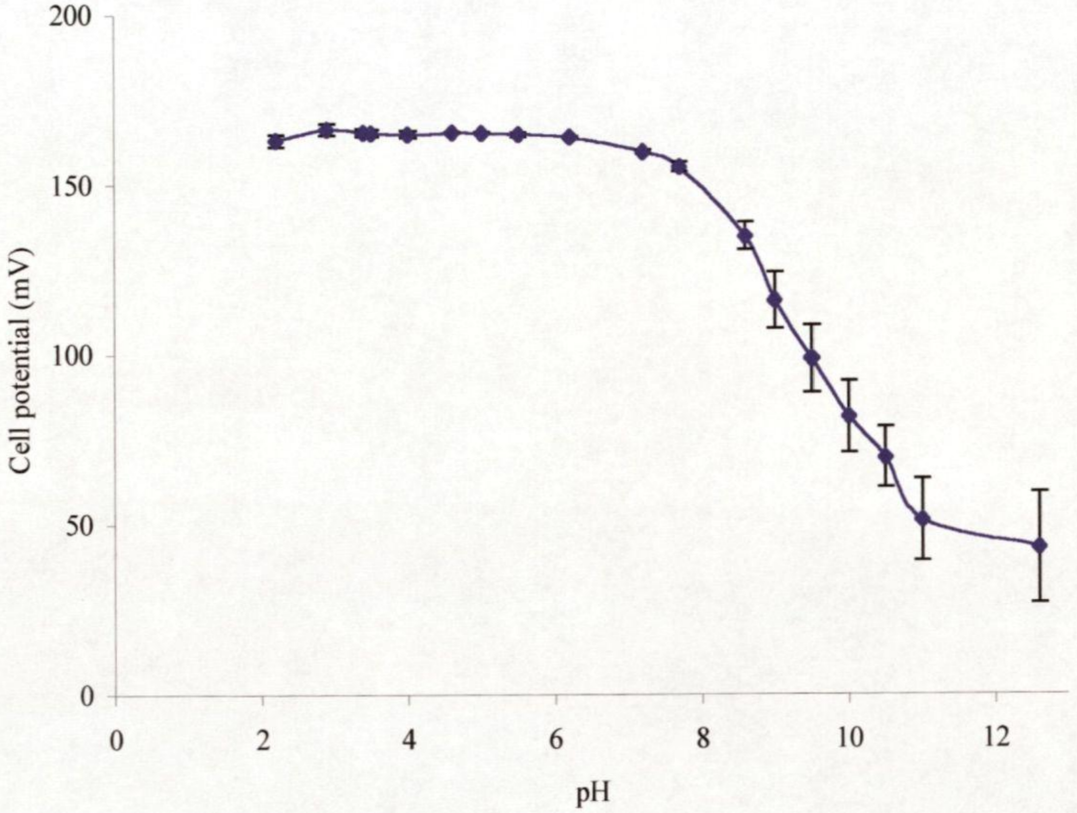


Figure 3.10 shows that the most sensitive betaine electrode obtained to date, *viz* that of leucine, has a range from pH 2-8 over which performance is independent of pH change. As observed previously, both Krynac and SBSa with the anti-oxidant gave a less satisfactory pH range. A working pH range of 2 to 8 would indicate that the electrode should be suitable for most non-saline environmental waters.

3.3.2.4 Lifetime of the electrodes

The electrochemical parameters for N,N,N-triallyl norleucine betaine in three different polymers are shown in table 3.14. These are compared with the best betaine sensor, N,N,N-triallyl leucine betaine.

Table 3.14 Lifetime studies for N,N,N-triallyl norleucine betaine in Krynac 50.75 (K14), SBSa (S16) and SBS (S10) compared with N,N,N-triallyl leucine betaine in SBS (S11)

Lifetime	studies	K14	S10	S16	S11
Day 7	Slope (mV dec^{-1})	-57.0	-57.6	-57.0	-59.1
	Limit of detection (x 10^{-6} mol dm^{-3} NO_3^-)	3.0	3.4	3.0	0.34
	$k^{pot}_{NO3-, Cl-}$	3.0×10^{-3}	4.0×10^{-3}	3.0×10^{-3}	3.4×10^{-3}
Day 48	Slope (mV dec^{-1})	-57.0	-57.0	-56.5	-56.9
	Limit of detection (x 10^{-6} mol dm^{-3} NO_3^-)	3.0	3.3	3.2	0.7
	$k^{pot}_{NO3-, Cl-}$	3.0×10^{-3}	3.0×10^{-3}	3.0×10^{-3}	3.0×10^{-3}
Day 100	Slope (mV dec^{-1})	-57.0	-56.0	-55.0	56.7
	Limit of detection (x 10^{-6} mol dm^{-3} NO_3^-)	4.0	5.0	3.5	1.0
	$k^{pot}_{NO3-, Cl-}$	4.0×10^{-3}	4.0×10^{-3}	3.3×10^{-3}	3.5×10^{-3}
Day 147	Slope (mV dec^{-1})	N.D	N.D	-45.0	-56.9
	Limit of detection (x 10^{-6} mol dm^{-3} NO_3^-)	N.D	N.D	3.5	0.9
	$k^{pot}_{NO3-, Cl-}$	N.D	N.D	*	4.0×10^{-3}
Day 240	Slope (mV dec^{-1})	-57.0	-53.0	*	-55.0
	Limit of detection (x 10^{-6} mol dm^{-3} NO_3^-)	5.5	13.0	*	1.2
	$k^{pot}_{NO3-, Cl-}$	5.0×10^{-3}	N.D	*	6.0×10^{-3}

*Unresponsive

The stability of the cell potential was measured using the method described in section 2.2.3.2.7. Figure 3.11 shows the variation of a given potential reading over 3 days. This potential was 160.0 mV and varies only by ± 1 % over this period of time. But the important conclusion of this experiment was the absence of drift.

3.3.2.6 Speed of response

Speed of response was determined using the method described in section 2.2.3.2.8. This was determined by measuring the time required for an electrode to equilibrate in a solution of 1 x 10^{-5} mol dm^{-3} nitrate with a cell potential of 294 mV having previously been immersed in a 5 x 10^{-6} mol dm^{-3} nitrate solution having a cell potential of 311 mV. This procedure will mimic the electrode performance under the condition of a sudden pollution incident in an environmental water such as a river.

Figure 3.12 Speed of response of triallyl Leucine betaine based electrode

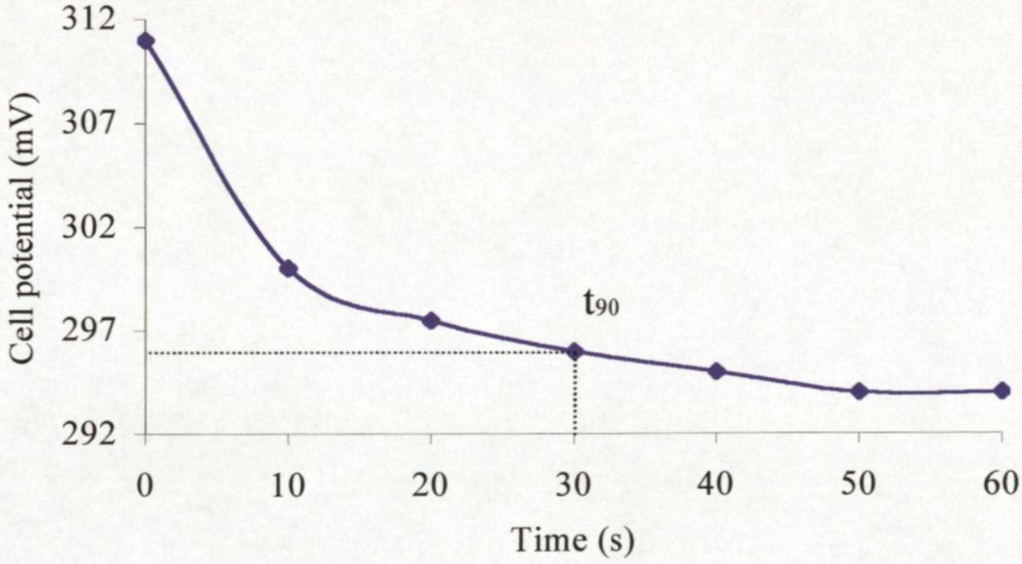


Figure 3.13 shows the good reproducibility of the membrane fabrication. The RSD of the cell potential at 1.0 μmol dm^{-3} nitrate is only 2 %.

3.3.2.8 Influence of the solvent mediator on the electrochemical properties of the electrode

3.3.2.8.1 Membrane composition

For this experiment, the best performing sensor (N,N,N-triallyl leucine betaine chloride) was used. The purpose of this study was to examine the relationship between the lipophilicity (log P_{TLC}) (Dinten *et al.*, 1991; Carey and Lewis, 1996) of the solvent mediator used in the membrane and the selectivity of the nitrate-selective electrode. It is also known that the change of solvent mediator in PVC-based membranes can improve the selectivity for nitrate against interfering anions but cannot modify the selectivity sequence (empirical Hofmeister series) (Wegmann *et al.*, 1984). Five different solvent mediators with a range of calculated logP_{TLC} from 2.9 to 8.3 P_{TLC} (Fluka, 1996) were evaluated for selectivity ($k^{pot}_{NO3-, Cl-}$). Table 3.15 shows the membrane composition and the different log P_{TLC} for the solvent mediators used.

Table 3.15 Membrane composition

Membrane Number	Sensor molecule (% m/m)	Solvent mediator (% m/m)	Log P_{TLC}	SBS (% m/m)	DCP (% m/m)
S11	6.5	2-NPOE (40.0)	5.9	43.5	10.0
S17	8.6	none	-	58.0	13.5
S18	6.5	2-F-2'-NDPE (40.0)	2.9	43.5	10.0
S19	6.5	2-NPDE (40.0)	8.3	43.5	10.0
S20	6.5	DBP (40.0)	4.7	43.5	10.0
S21	6.5	DBS (40.0)		43.5	10.0

3.3.2.8.2 Nitrate response and selectivity

Membrane S18 was not sufficiently robust to allow subsequent evaluation. This was caused by a poor compatibility between 2-F-2'-NDPE and the polymer. Membrane S17 gave poor nitrate response due to the absence of solvent mediator. DBS and DBP appeared to inhibit the nitrate response because membranes S20 and S21 were unresponsive.

Table 3.16 Performance of nitrate electrodes

Membrane Number	Linear Nernstian range (mol dm^{-3})	Nernstian slope (mV dec^{-1})	LOD (μmol dm^{-3})	$k^{pot}_{NO3-, Cl-}$	t_{90} (s)
S11	$1 \times 10^{-1} - 5.0 \times 10^{-6}$	-59.1	0.4	3.4×10^{-3}	30
S19	$1 \times 10^{-1} - 5.0 \times 10^{-6}$	-57.5	0.7	4.0×10^{-3}	20

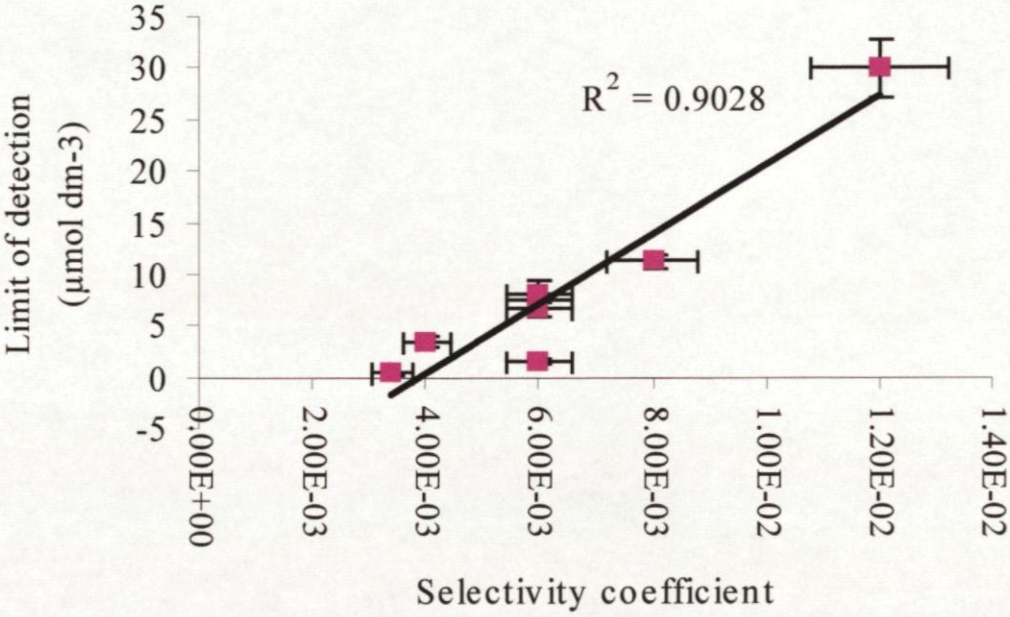
Table 3.16 shows that membranes S11 and S19 were the only ones to be evaluated due to the problem encountered with the other solvent mediators. Membranes S11 and S19 have very similar electrochemical parameters apart from the slope which was slightly less for membrane S19 (-57.5 mV dec^{-1}) than membrane S11 (- 59.1 mV dec^{-1}). From this limited study, the lipophilicity of the solvent mediator does not appear to affect the electrochemical parameters. Traditionally, the dielectric constant of plasticisers in PVC-based membranes is known to influence the selectivity (Hulanicki, 1978). The two solvent mediators used in membranes S11 and S19 had different dielectric constants 23.5 for 2-NPOE and 25 for 2-NPDE. However, the difference observed for $k^{pot}_{NO3-, Cl-}$ was not significant to conclude anything.

Table 3.16 also shows that the speed of response of membrane S19 was very slightly faster than membranes S11 (t_{90} determined from 5.0×10^{-6} to 1.0×10^{-5} mol dm^{-3} NO_3^-)

3.4 Structure-activity relationships

The purpose of this section is to understand how the structure of N,N,N-triallyl α-amino-acid betaine chlorides influence the electrochemical parameters of the nitrate-selective membrane. Table 3.10 can be visualised graphically by plotting Nernstian slope, limit of

Figure 3.17 **Limit of detection against selectivity coefficient for nitrate against chloride for membranes S6-S14 (except S7)**



All these graphs 3.14-3.16 are very similar showing an optimum performance with the C_4-branched side chain of leucine. Graph 3.17 shows the close relationship between selectivity and sensitivity. Membrane S7 has not been considered because of its sub-Nernstian response (Slope -50.5 mV dec^{-1}). Generally, for all the membranes studied, an improved selectivity results in an improved sensitivity.

Commercial sensors are hydrophobic N-alkyl quaternary ammonium salts e.g. tridodecylmethylammonium nitrate trapped in PVC (Fluka, 1996) or quaternary ammonium salts such as triallyl decyl ammonium nitrate covalently bound to polymers (Sutton *et al*., 1999). In the case of the betaines the performance slowly declined with increasing chain length after C_4. It has generally been assumed that the main factor of influence has been the hydrophobicity of the sensor molecules as covered in section 1.5.2. This does not seem to be the case with the betaine molecule. There were two isomers with

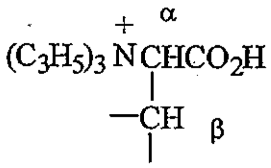
a C_4 side chain examined. Membrane S10 contained the C_4 straigth chain and had a LOD of 3.4 x 10^{-6} mol dm^{-3} nitrate, the C_4 branched chain of S11 had a LOD of 3.4 x 10^{-7} mol dm^{-3} nitrate an improvement of 10-fold. A similar increase in improvement with branching was observed between the two C_3 side chain isomers S8 and S9.

It has recently been shown (Braven *et al.*, 1999) using an immobilised quaternary ammonium salt that the mechanism of nitrate response is due to ion-exchange process with no diffusion involved. The identification of the structural features present in the betaine molecule influencing nitrate ion capture by the positively charged nitrogen atom will therefore be the key to the study. A comparison between S11 and S15 shows that converting the free carboxylic acid group to a carboxylate ester resulted in a decrease in limit of detection from 0.34 to 2.9 x 10^{-6} mol dm^{-3} nitrate. The difference in inductive effect between a free carboxyl group and an ester β to a reaction site will be rather small and unlikely to account for the change in response. The ester showed a somewhat narrower pH working range (2-7), this is well above the pK_a for CO_2H groups, so carboxyl ionization seems unlikely to be involved. There remains the possibility of a spatial effect, however since covalent bonding of the ester to the polymer may have taken place through the *O*-allyl groups. The free CO_2H group present in betaines could possibly improve the hydration or hydrogen bonding capacity near to the positively charged nitrogen atom.

As the side-chains (R) are all alkyl groups the only electronic effects will be a slight positive inductive effect (a field effect can be discounted in this case since the side chains have no charge (Ingold, 1969). The strongest inductive effect will be that from the side-chain of S9 (iso-propyl) not that of the isobutyl of S11 which gives the best membrane. An examination of table 3.10 does not suggest that the inductive effect of the side chain is a primary contribution to the performance.

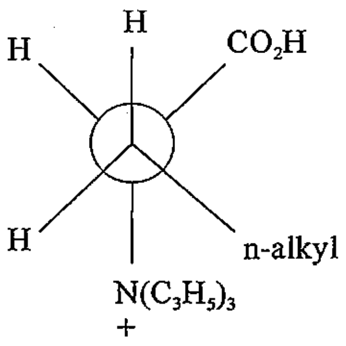
When the steric effects of R are considered there does not seem to be a direct relationship between the bulk of R and the performance.

The various betaines with the exception of glycine (S6) have the following common features:

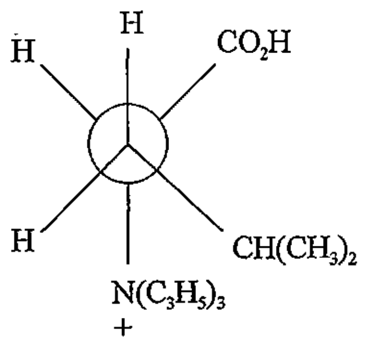


$$(C_3H_5)_3\overset{+}{N}\underset{-\underset{|}{CH}\ \beta}{\overset{\alpha}{C}}HCO_2H$$

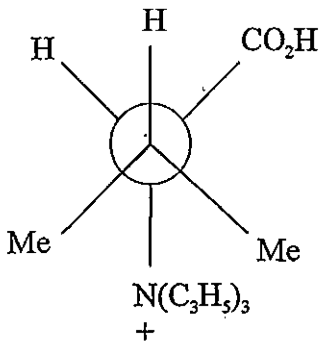
There does however seem to have a steric influence from the group occupying the β position. The following three projection structures of the various betaines will serve to illustrate the point:



(a)



(b)



(c)

(a) shows a projection diagram of betaines S7 to S14 with the exception of S9, (b) shows the projection diagram for S8, and (c) shows the projection diagram for S9, arising from the β carbon atom. From a β position the two structures (b) S11 and (c) S9 have a possibly greater steric effect on the vicinity of the cationic nitrogen atom than structure of the type (a) which have a primary alkyl group. It is not easy to predict which of structures (b) and (c) will have the greatest influence. S9 and S11 are the best performing membranes however.

A separate study (Chilcott *et al.*, 1999) investigated the influence of modification of the allyl group on membrane performance as shown in table 3.17.

Table 3.17 Performances of N,N,N-trialkenyl α-amino-acid betaine

Alkenyl group	LOD (μmol dm^{-3})	$k^{pot}_{NO3-, Cl-}$
CH_2=CH-CH_2-CH_2-	3.1	4 x 10^{-3}
CH_2=CH-CH(CH_3)-	7.0	6 x 10^{-3}
CH_2=C(CH_3)-CH_2-	4.0	5 x 10^{-3}

All of these molecules gave similar performance electrodes. It seems likely that the changes involved were too close to the point of attachment of the polymer to have a major effect.

In conclusion there seems to be no single factor which dominates the relationship between structure and activity.

CHAPTER 4

FIELD TRIALS

4.1 Background

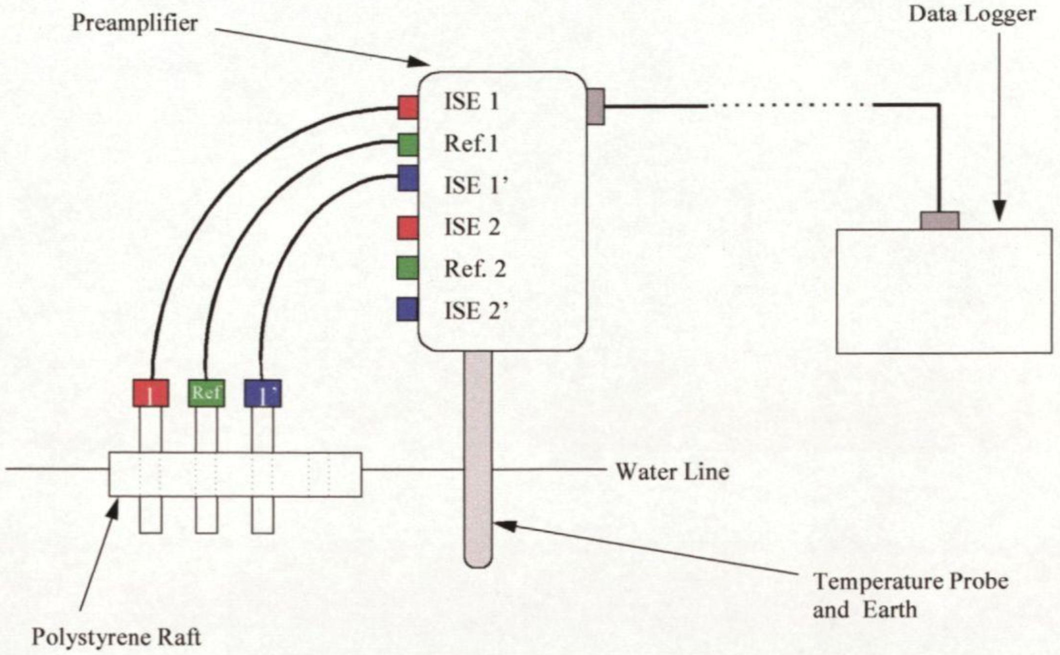
An evaluation of the performance of two betaine sensors, for the measurement of nitrate in environmental waters, was carried out during winters 1998 and 1999. N,N,N-triallyl norleucine betaine and N,N,N-triallyl leucine betaine were selected for this evaluation. They were both covalently attached to SBS. The membrane composition for both sensors were 43.5% m/m SBS, 10% m/m DCP, 40% m/m 2-NPOE and 6.5% m/m sensor.

4.2 Field instrument

Some laboratory voltmeters such as those for measuring pH are not suitable for field use because the reference electrode may be connected directly to earth. As effective insulation of field solutions from earth cannot be guaranteed a system was required to accommodate this. A field pre-amplifier system designed by John Wood (Research Instrument Design, Bodmin, UK) was used. The measurement unit comprised two sets of two nitrate ISEs, each with its own reference electrode, connected through a pre-amplifier to a datalogger and battery supply as shown in figure 4.1. This set-up allowed the two types of electrode to be compared under identical conditions in duplicate.

In the pre-amplifier, electrode potential is measured differentially with respect to the reference electrode and amplified by a factor of 10 to provide a good scaling match to the digital display. All electrodes are buffered by voltage followers (OPA 129, Burr-Brown International, Watford, UK), which have a very low input bias current and very high input resistance to minimise fluctuations. A thermal sensor is also connected to the pre-amplifier circuit, in order that temperature corrections can be applied to the mV response and also to provide an earth return. This sensor consists of a thermoconductor (AD590, Analog Devices Ltd., Walton on Thames, UK) inserted into a stainless steel thermal pocket (TP-250-X, Labfacility Ltd., Sheffield, UK) for environmental protection. The thermal sensor must be in contact with the solution being measured to maintain the earth contact. The pre-amplifier requires split (+15V and –15V) supply rails, which are derived from a low power voltage converter (DCPO105115DP, Burr-Brown International). The converter itself is supplied with a stable 5V from a low dropout regulator (LX8940CP, Linfinity Microelectronics Ltd., Surrey, UK) which allows a single 9V or 12V battery to power the complete system. The supply current is about 120mA.

Figure 4.1 **Diagram of the field instrument**



Preamplifier
Data Logger
ISE 1
Ref.1
ISE 1'
ISE 2
Ref. 2
ISE 2'
I
Ref
I'
Water Line
Temperature Probe and Earth
Polystyrene Raft

The pre-amplifier circuitry is enclosed in a sealed watertight box containing silica desiccant. The connectors between the box and the electrodes are co-axial and waterproof (Series 101, W. W. Fischer Ltd., Hampshire, UK), but the co-axial connectors to the electrodes needed to be sealed using heat shrinkable waterproof sleeving.

The field measurement system was linked to a modified data logger (CR10X1, Campbell Scientific, UK) that was enclosed in a sealed watertight box containing silica desiccant. The logger has a programmable switched 12V control allowing the powering up of the electrodes before a reading is recorded. Stored data can be retrieved using a cable connected to the logger by a waterproof 8-way connector (Mini-Buccaneer, Bulgin Component Ltd., Barking, UK) that attaches to the serial port of lap-top computer with the appropriate data communication software. This connection also allows real time monitoring and the uploading of the logger program. The logger was powered by a 12V,

24 Ah rechargeable lead-acid battery (NP24-12B, YUASA Corporation, Taiwan) via a power cable with a waterproof 3-way connector (Mini-Buccaneer, Bulgin Component Ltd., Barking, UK).

Such a system was used with the preamplifier fixed in a field run-off weir at the Institute of Grassland and Environmental Research (IGER), North Wyke with the temperature probe submerged in the water. Nitrate ISEs and their corresponding reference electrodes were supported by a floating polystyrene raft with approximately 2 cm of the electrodes immersed in the water. Preliminary work showed that potentiometric oscillations were obtained when an aqueous solution was used as outer filling solution in the double junction reference electrode. This problem was due to electrode bleeding (the reference was refilled regularly (every 3 days)). The use of a gel overcame the problem of oscillations and the maintenance of the reference electrode. The stability of the electrode response is illustrated in section 3.3.2.5.

4.2.1 Calibration of the field instrument

Prior to the field evaluation, each nitrate ISE was calibrated using the field instrument and the method described in section 2.2.3. The potential measurement of each standard solution was carried out in triplicate and the standard deviation for each potential reading was less than 0.5 mV in the Nernstian linear range. This represents a relative standard deviation of less than 2% for the nitrate concentration. A typical calibration graph for N,N,N-triallyl leucine betaine is shown in figure 4.2. In this chapter the nitrate concentrations are given preferentially in mg nitrate-N per litre (ppm nitrate-N) instead of

directly depend on these two variables and the cell potential measured in the field, according to equation 4.1 derived from the Nernst equation:

$$\left[NO_3^-\right]_{Molar} = 10^{((-1)-(\frac{A}{B}))} \qquad (4.1)$$

where,

A = Potential observed (mV) - potential reading for a 0.1 mol dm^{-3} NO_3^- (mV)

B = Slope (mV dec^{-1})

Prior to the field trials each nitrate ISEs were calibrated at 25°C. However, the temperature in the field is variable and was in a range from 2°C to about 20°C. For this reason, calibration graphs were obtained in a concentration range between 10^{-3} and 10^{-5} mol dm^{-3} NO_3^- (measuring range in the field) and a temperature range from 0 to 25°C. The slopes were plotted as functions of the temperature as shown in figure 4.3. The variation in cell potential as a function of the temperature for a 1.0 x 10^{-1} mol dm^{-3} nitrate solution was also studied and the results are presented in figure 4.4.

Figure 4.3 **Temperature dependence of the slope for N,N,N-triallyl leucine betaine as sensor over its linear Nernstian range**

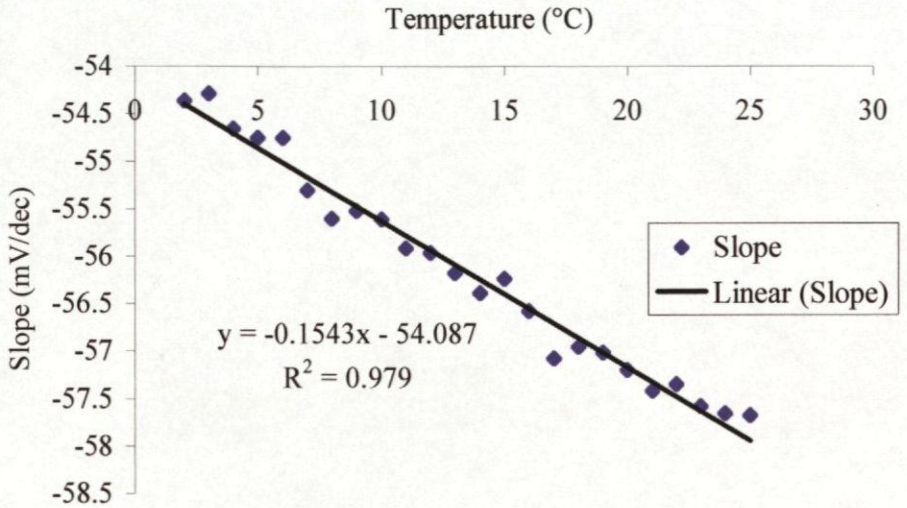
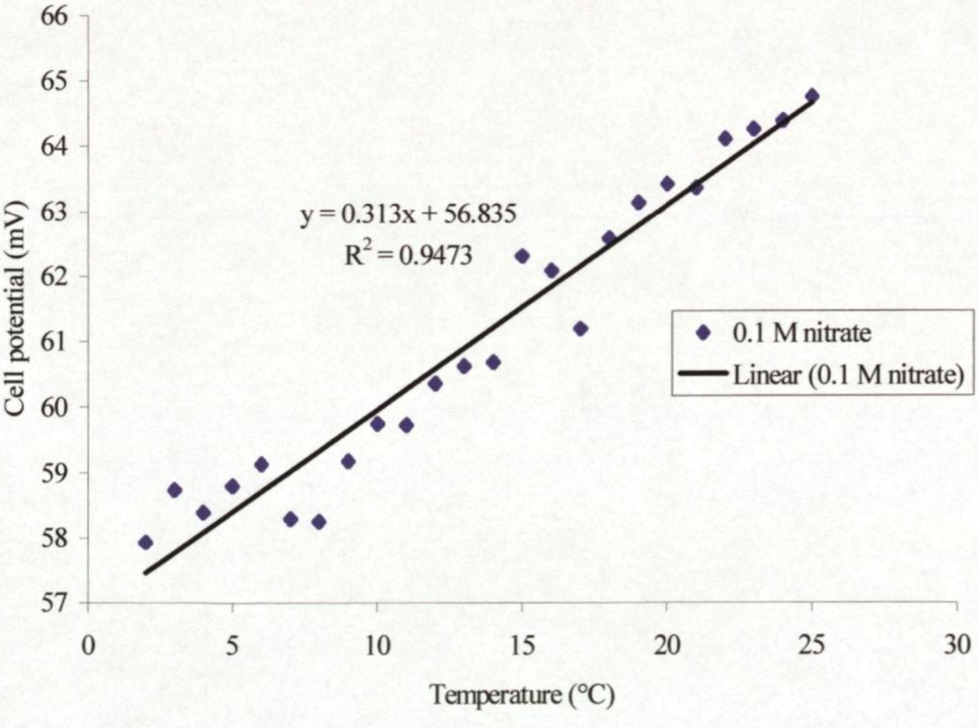


Figure 4.4 **Temperature dependence of the cell potential for a 1.0 x 10^{-1} mol dm^{-3} nitrate standard (N,N,N-triallyl leucine betaine as sensor)**



In a range from 0 to 25°C, the slope varies by -0.1543 mV dec^{-1} $°\text{C}^{-1}$ and the potential of the 0.1 mol dm^{-3} nitrate standard by 0.313 mV $°\text{C}^{-1}$. Therefore equation 4.1 can be modified to equation 4.2 to integrate the temperature correction.

$$\left[NO_3^-\right]_{Molar} = 10^{((-1)-(\frac{A-(0.313*(25-T°C))}{B-(0.1543*(25-T°C))}))} \tag{4.2}$$

And the conversion from nitrate concentration in mol dm^{-3} to ppm nitrate-N is done using equation 4.3.

$$\left[NO_3^-\right]_{ppm.nitrate-N} = \frac{\left[NO_3^-\right]_{Molar.nitrate}}{0.001} * 14 \tag{4.3}$$

4.3 Field performance evaluation

Prior to setting up, on site, the electrode response (mV per decade) and response time (30-40 seconds) were determined in the laboratory for each electrode.

The data logger took recorded measurements at hourly intervals. The logger would power up the pre-amplifier after which readings of the date, time, electrode potentials, temperature and battery power were recorded.

Readings were recorded from November 1998 to April 1999. A water sample was collected every few days at a specific time for the measurement the nitrate level using the segmented-flow instrument (SAN Plus Segmented Flow Analyser containing a SA4000/SA20000 Chemistry Unit; Skalar UK Ltd., York, UK) at IGER. The principle of this method is explained in section 1.2.1. The externally scrutinised quality control scheme under which the segmented-flow instrument is operated guarantees that 95% of readings above 0.1 ppm nitrate-N will be within 5% of the true value. Calibration checks of the nitrate ISEs were carried out every 2 weeks.

4.3.1 Field trials in V-notch weirs at IGER

4.3.1.1 Choice of the site and aim of the experiment

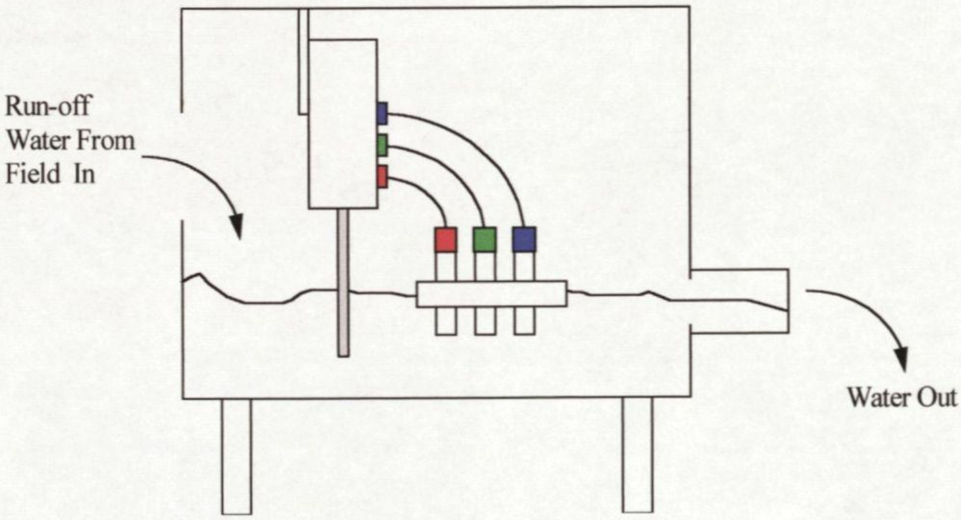
The V-notch weir was positioned at the drainage outflow of 1 ha grazed grassland lysimeter plot (Scholefield *et al.*, 1999). The plot was part of a long term field experiment set up at the Rowden Moor site of the IGER Station in order to develop understanding of the flows and losses of nitrogen within and from pasture systems. The plot was constantly monitored for parameters including nitrate levels. Previous papers give additional information of the drainage hydrology (Armstrong *et al.*, 1991) and of the effects of the treatments on pasture production (Tyson *et al.*, 1992) and nitrate leaching at the site (Scholefield *et al.*, 1993; Tyson *et al.*, 1997). From these previous studies, it was known that the nitrate concentration was in a range from 1 to 20 ppm nitrate-N which was ideal for an initial study.

This experiment had multiple aims. The first one was to test the electrodes under very demanding conditions e.g. membrane resistance to chemicals and biological degradation. The second one, and probably the most important, was the correlation between the nitrate ISEs and the segmented-flow instrument available at IGER. EC Nitrate Directive (CEC, 1991) requires better use of N in agriculture and also the application of a comprehensive nitrate monitoring scheme. And therefore, the overall goal of this work was to investigate if a nitrate ISE could be used as an alternative to costly segmented-flow instruments commonly used in the water industry. Such automated devices are expensive in labour and equipment but also generate some toxic waste.

4.3.1.2 Description of the setting-up

One measurement system, with two nitrate ISEs and one reference electrode, was deployed, in a V-notched weir, as shown in figure 4.5. The pre-amplifier was attached to the side of the weir and the electrodes were floating on a polystyrene raft. The weir was covered and protected from the rain.

Figure 4.5 Description of the set-up in V-notched weir at IGER

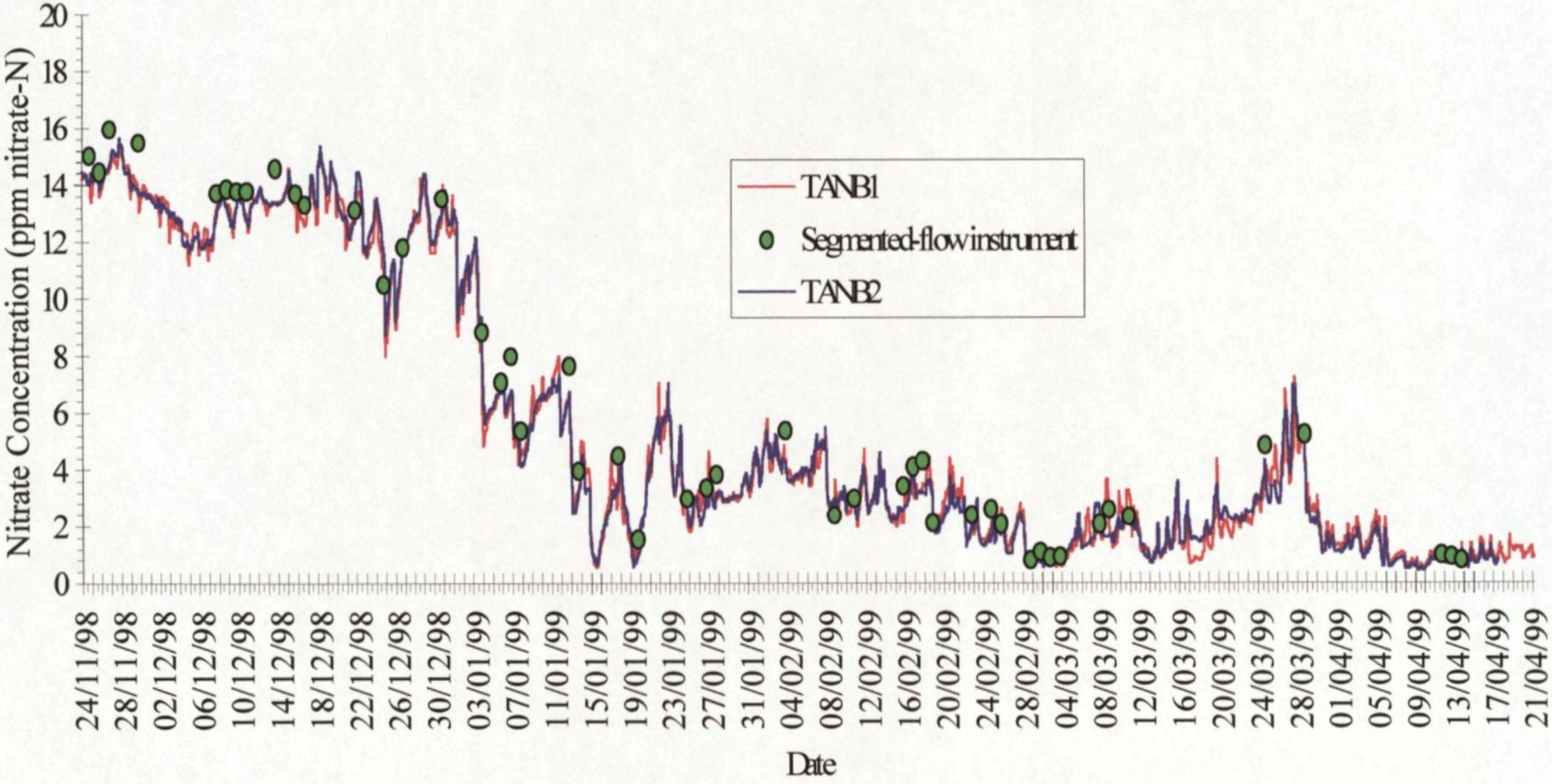


In blue: nitrate ISE 1, in red: nitrate ISE 2 and in green: double junction reference electrode

4.3.1.3 Results for N,N,N-triallyl norleucine betaine

Figure 4.6 shows the performance of two nitrate ISEs using N,N,N-triallyl norleucine betaine (TANB) as sensor compared with the segmented-flow instrument data over a period of 5 months. The range in concentration of nitrate-N in the weir was between 0.8 - 16 ppm, as measured by the laboratory instrument.

Figure 4.6 Field Trial Data for N,N,N-triallyl norleucine betaine (TANB) as sensor (in duplicate)

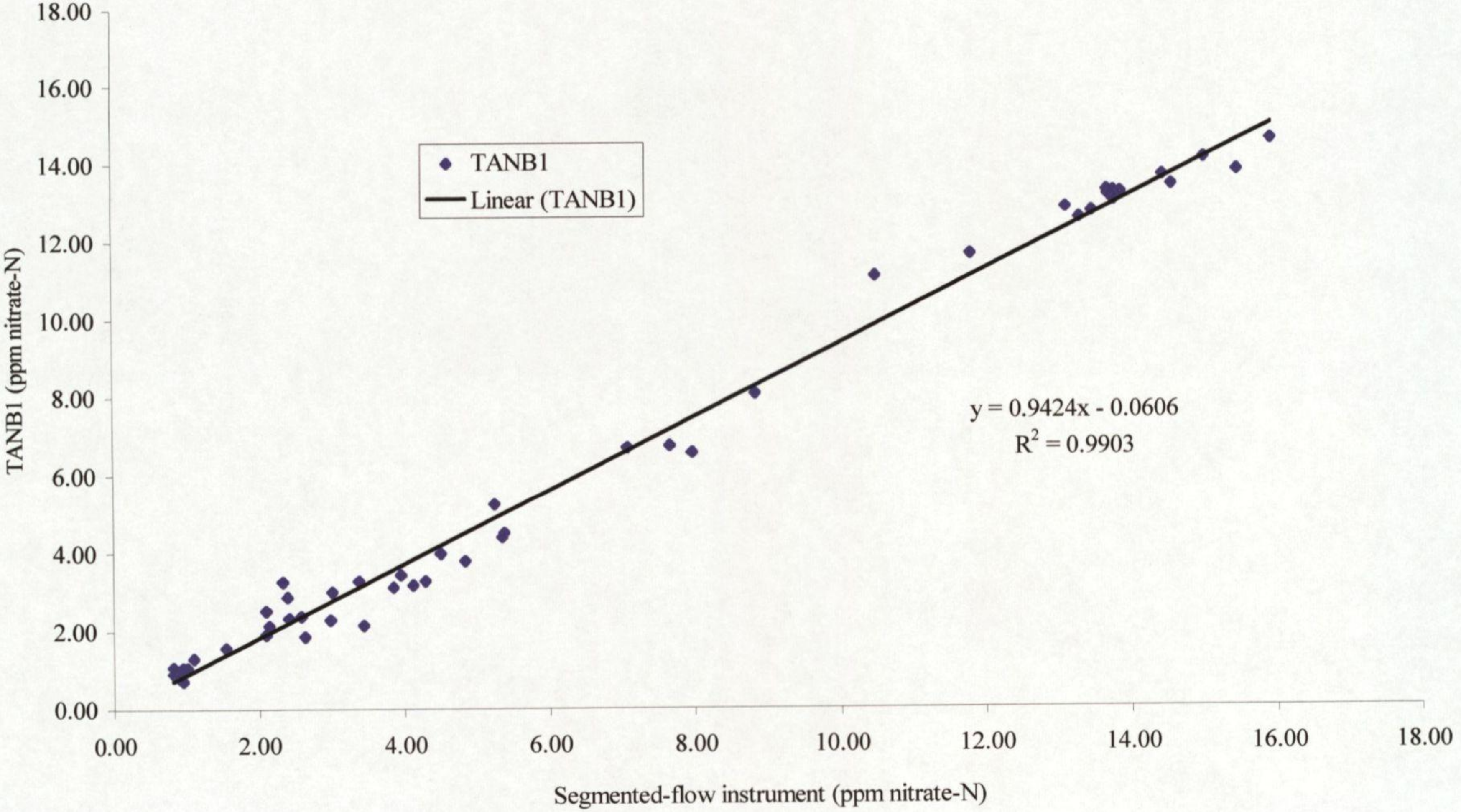


4.3.1.4 Discussion

Figure 4.6 shows that the outputs from the two nitrate ISEs have continued to follow accurately the nitrate levels given by the segmented-flow instrument during the whole period of the field trial. The nitrate concentrations were calculated using an algorithm that also corrected the results for temperature fluctuations as explained in section 4.2.2. The SBS used for this experiment is pH independent and therefore it was not necessary to correct for any pH changes. Figures 4.7 and 4.8 show graphical correlation between the nitrate levels as determined by the nitrate ISEs and the levels determined by the segmented-flow instrument. The graphs show that there is excellent correlation between the two techniques. The regression lines have gradients that do not differ significantly from 1 at the 95% confidence level indicating that there are no systematic errors.

The results of the fortnightly calibration checks were very satisfactory. Figures 4.9 and 4.10 show the initial calibration curves for each electrode and the results of subsequent calibration checks. The response times of the nitrate ISEs were less than 40 seconds in each case. The results of the calibration checks show negligible drift for both the electrodes for more than 4 months.

Figure 4.7 Correlation between segmented-flow instrument and TANB1



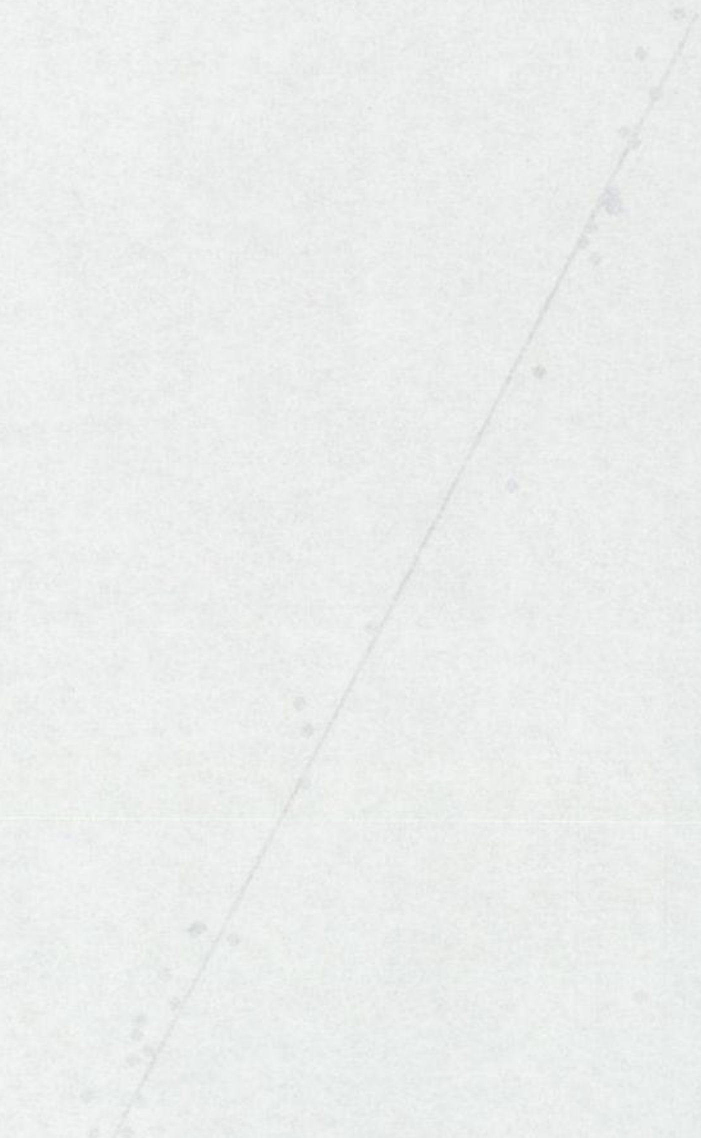


Figure 4.8 Correlation between segmented-flow instrument and TANB2

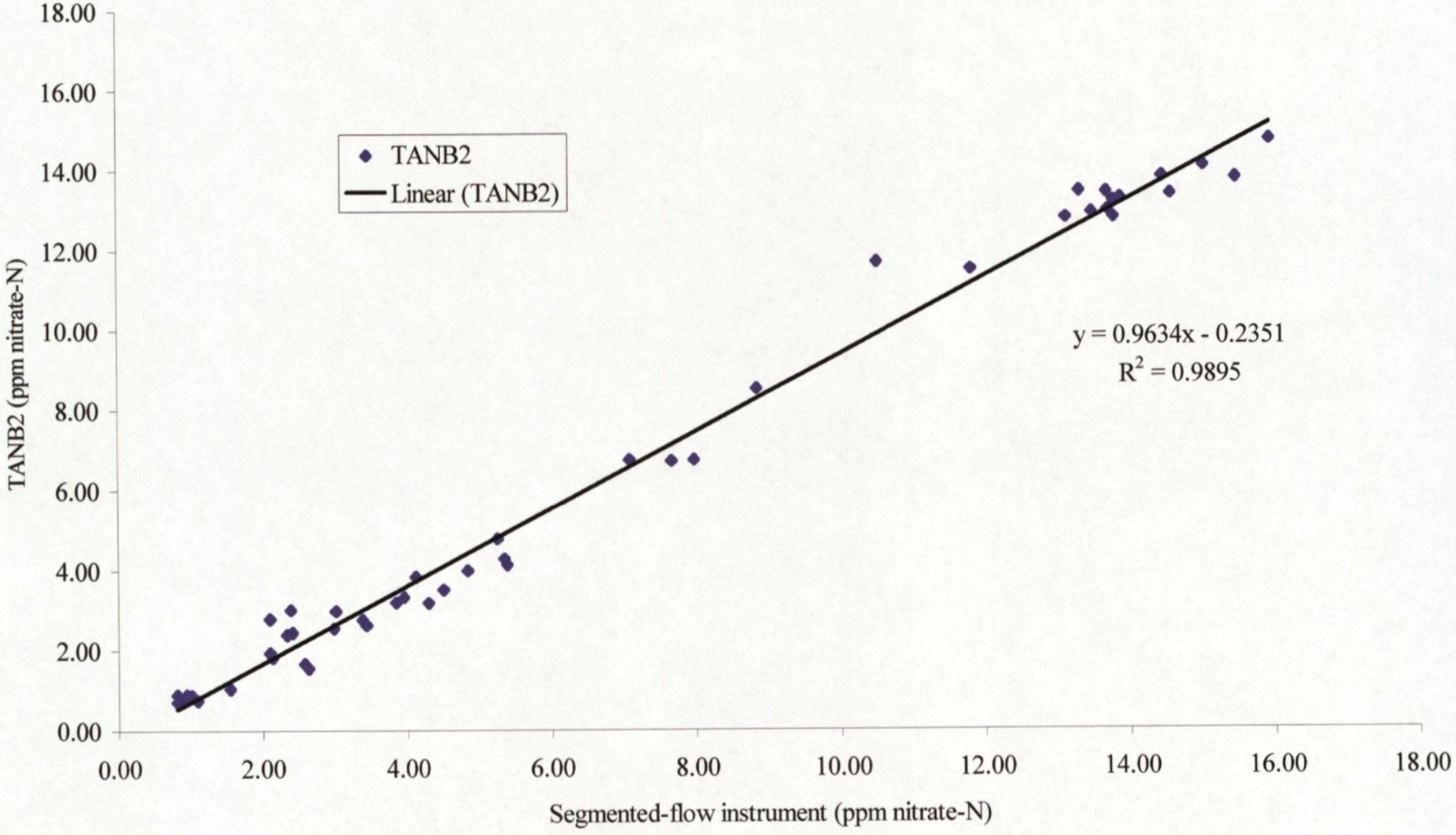


Figure 4.9 Initial calibration and subsequent checks for TANB1

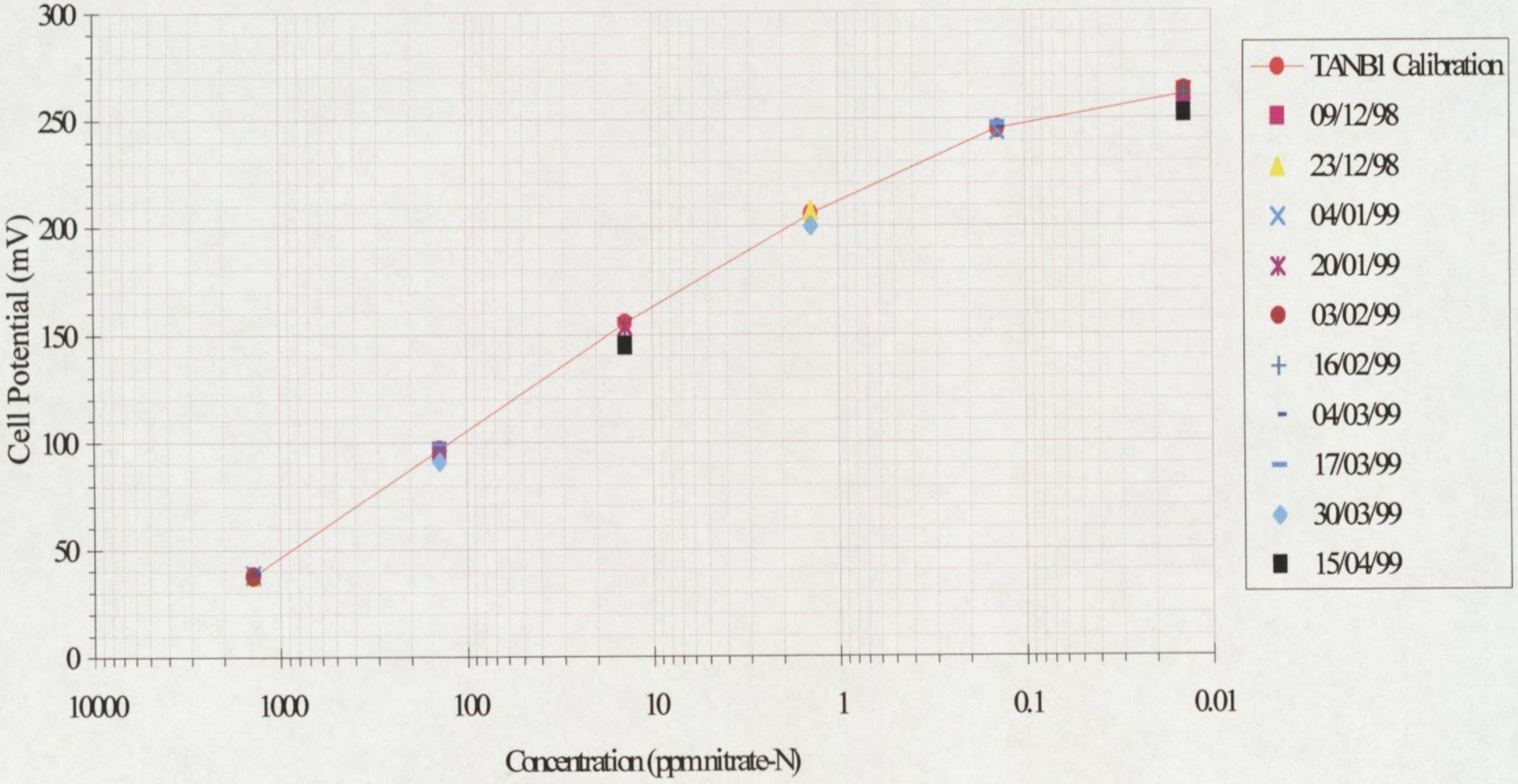
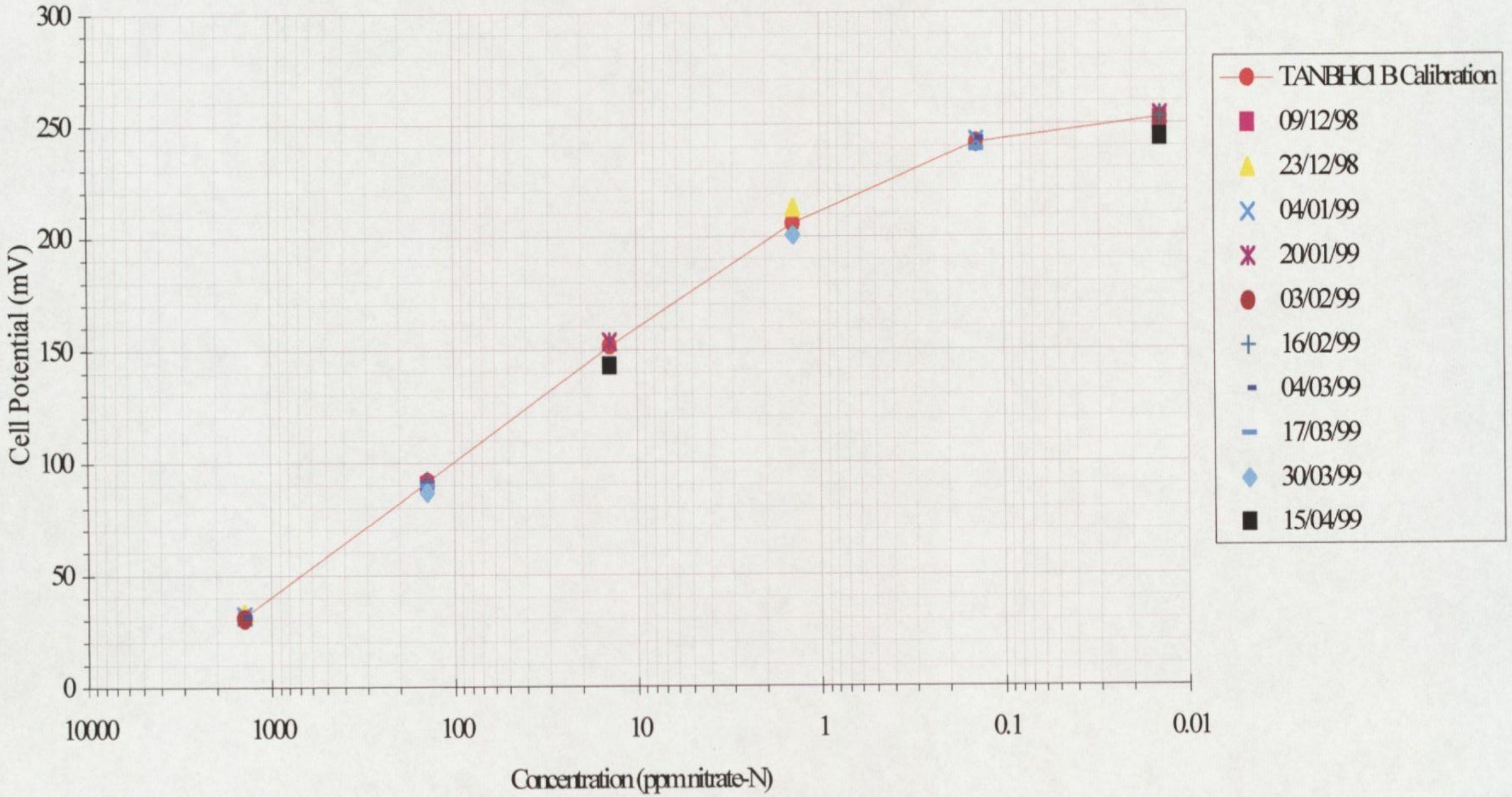


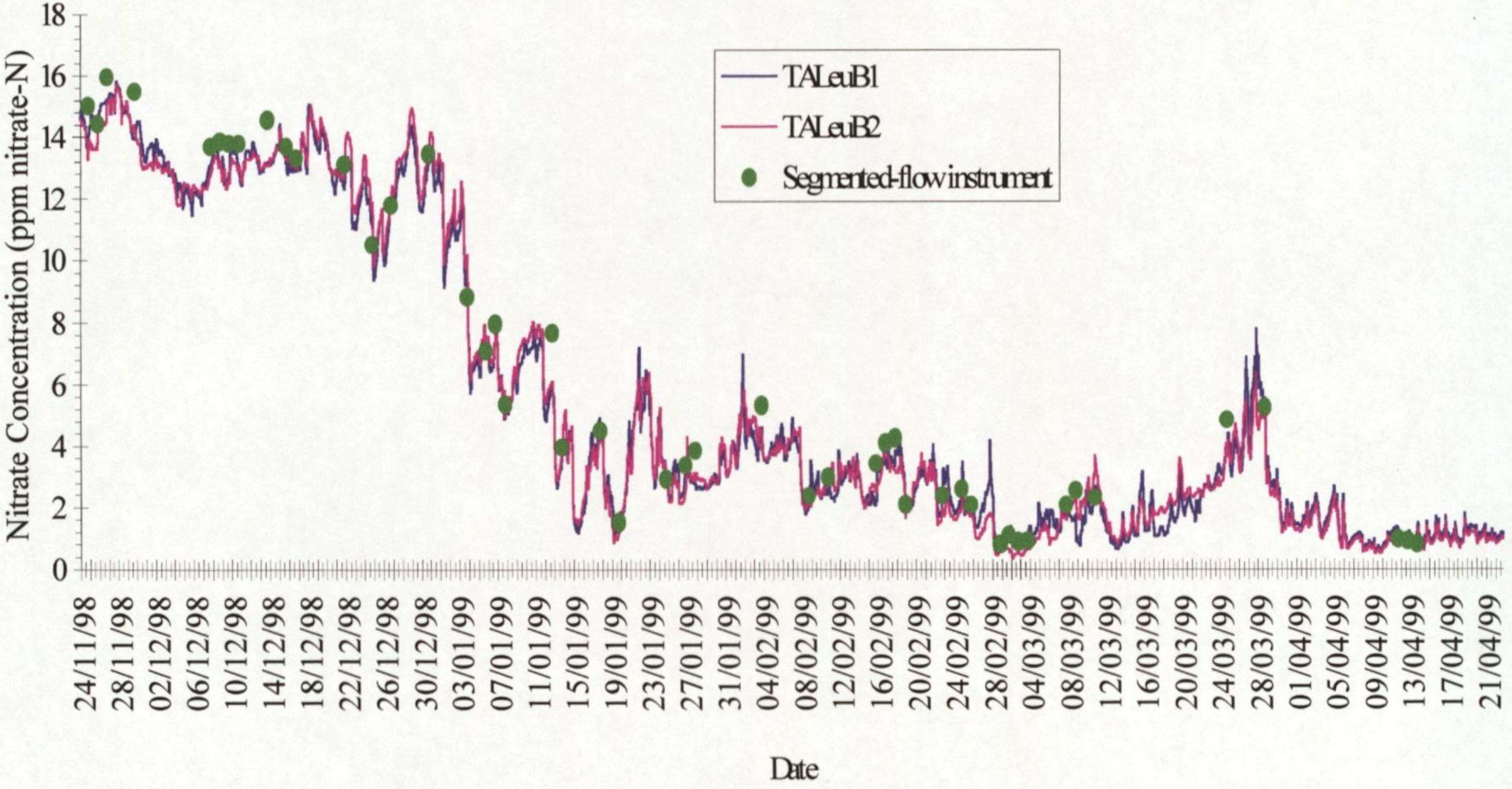
Figure 4.10 Initial calibration and subsequent checks for TANB2



4.3.1.5 Results for N.N.N-triallyl leucine betaine

Figure 4.11 shows the performance of two nitrate ISEs using N,N,N-triallyl leucine betaine as sensor (TALeuB) compared with the segmented-flow instrument data over a period of 5 months. This experiment was carried out in the same conditions as for the previous sensor. The range in concentration of nitrate-N in the weir was between 0.8 - 16 ppm, as measured by the laboratory instrument.

Figure 4.11 Field Trial Data for N,N,N-triallyl leucine betaine (TALeuB) as sensor (in duplicate)



4.3.1.6 Discussion

Figure 4.11 shows the results obtained using N,N,N-triallyl leucine betaine (TALeuB) were as good as those obtained for the previous sensor.

The outputs from the two nitrate ISEs have continued to track nitrate levels given by the segmented-flow instrument during the whole field trial. Figures 4.12 and 4.13 show the comparisons of the nitrate levels as determined by the nitrate ISEs and the levels determined by the segmented-flow instrument. The graphs show that there is excellent correlation between the two techniques. The regression lines have gradients that do not differ significantly from 1 at the 95% confidence level indicating that there are no systematic errors confirming the findings of the TANB.sensor in section 4.3.1.3.

The results of the fortnightly calibration checks were also very satisfactory. Figures 4.14 and 4.15 show the initial calibration curves for each electrode and the results of subsequent calibration checks. The response times of the nitrate ISEs were less than 40 seconds in each case. The results of the calibration check show negligible drift for more than 4 months.

Figure 4.12 Correlation between the segmented-flow instrument and TALeuB1

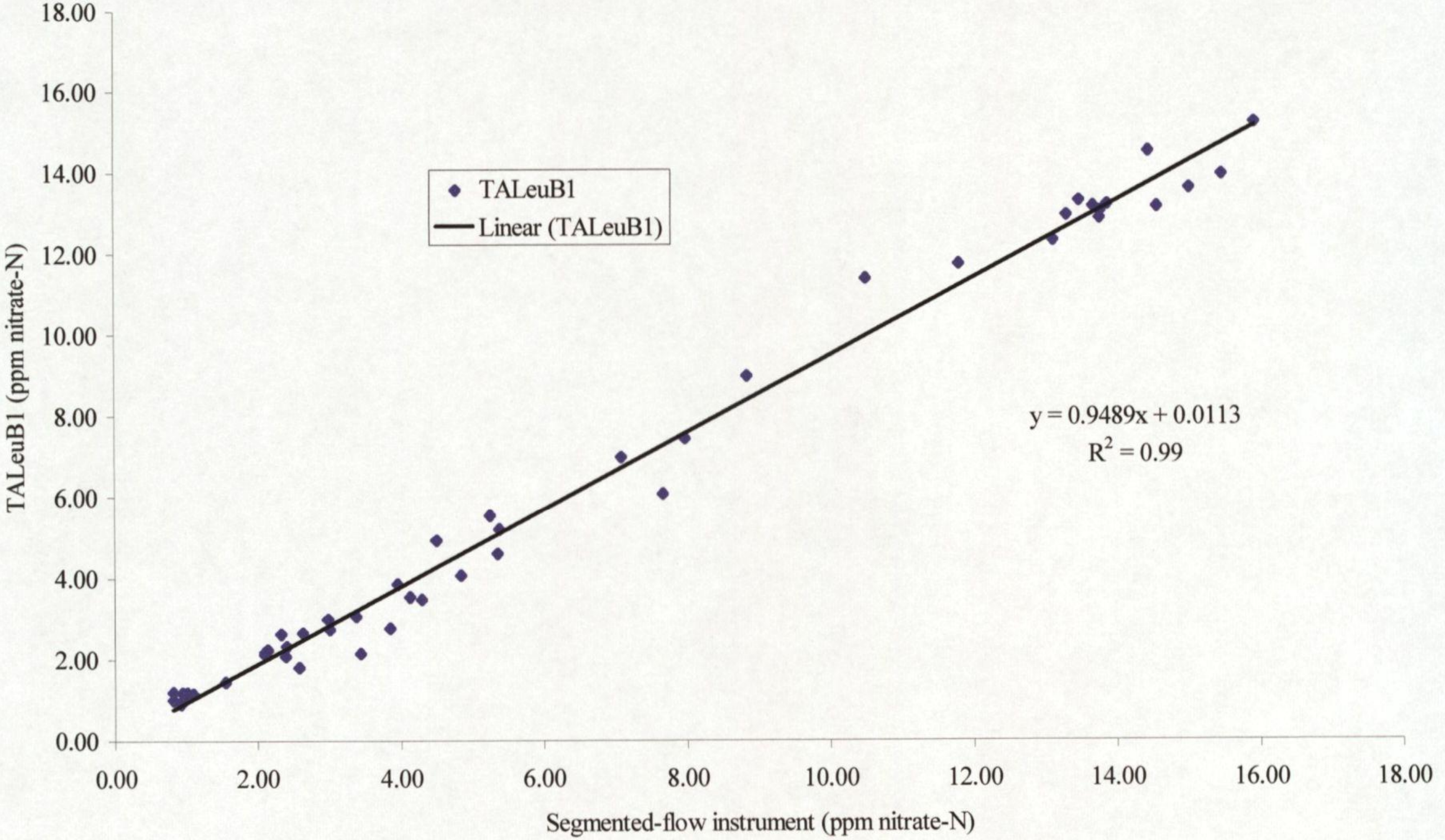


Figure 4.13 Correlation between the segmented-flow instrument and TALeuB2

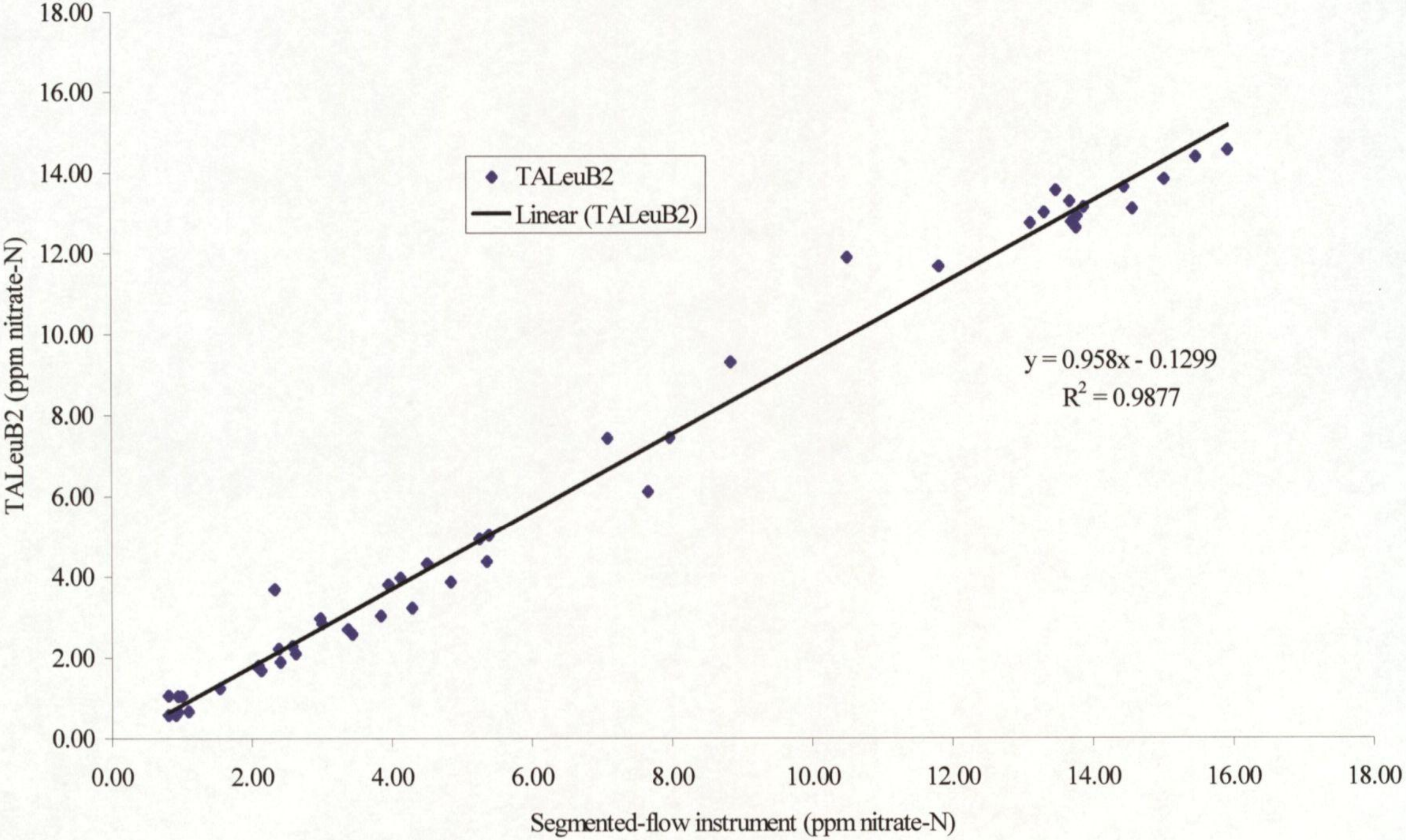


Figure 4.14 Initial calibration and subsequent checks for TALeuB1

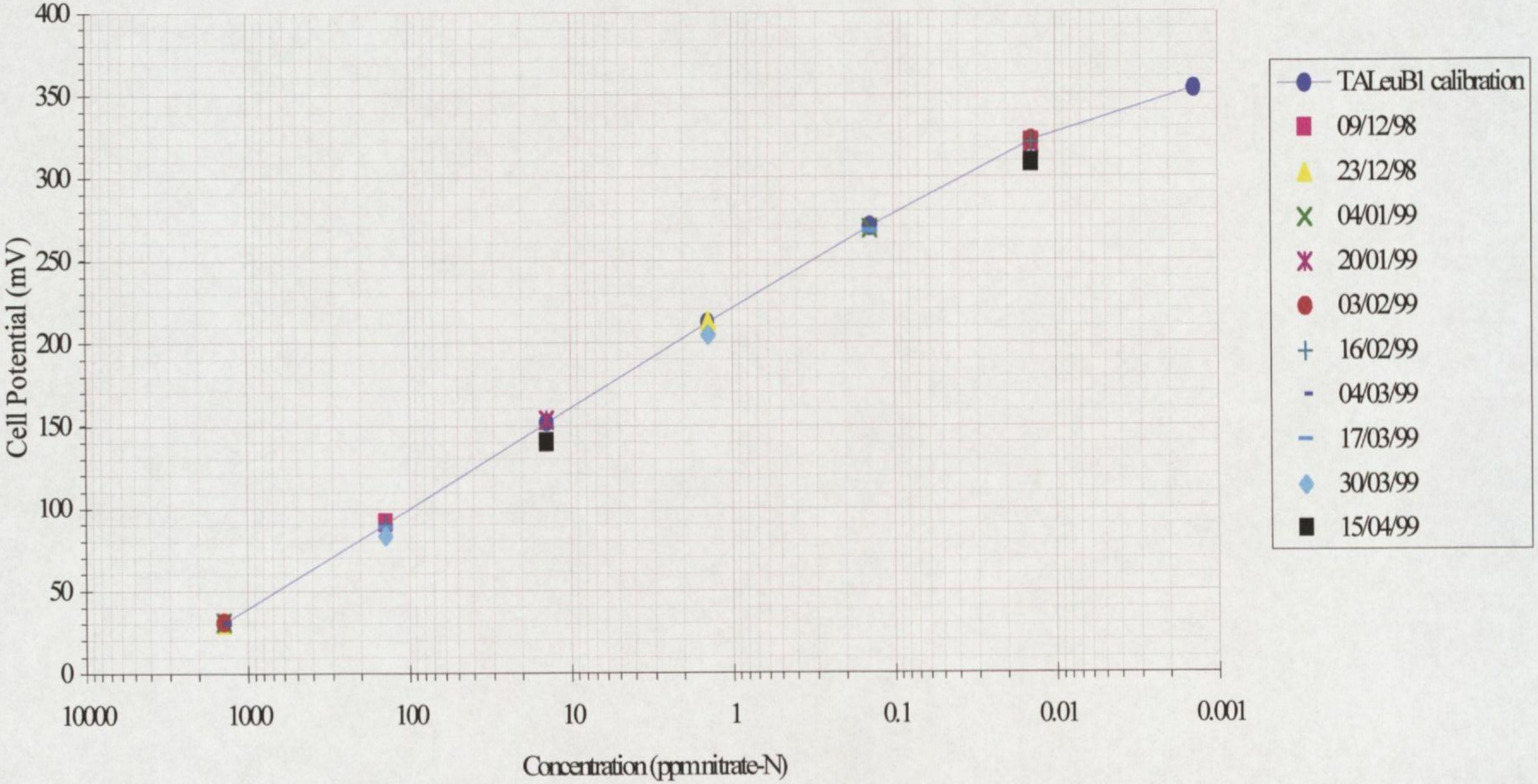
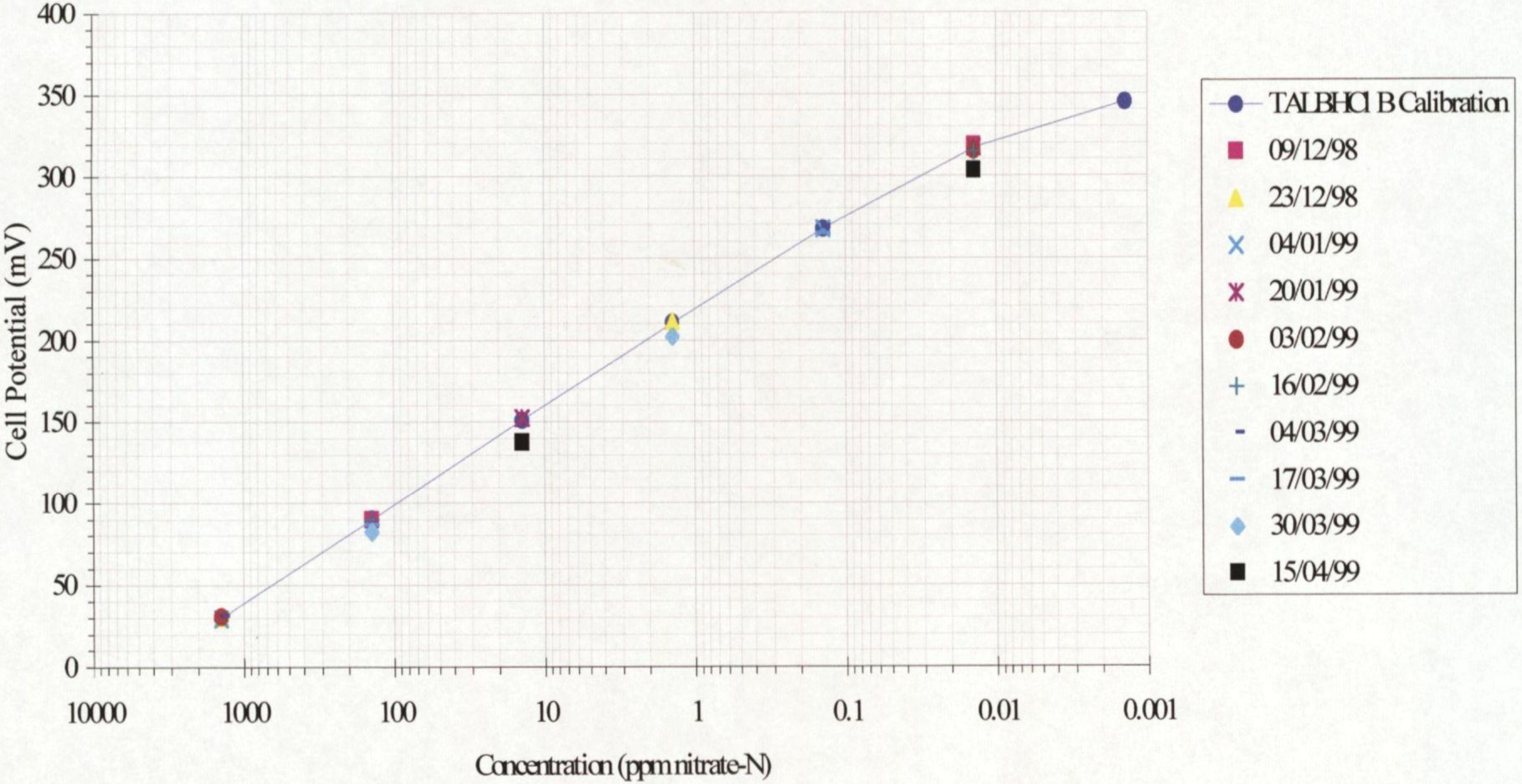


Figure 4.15 Initial calibration and checks for TALeuB2



4.3.2 River water monitoring

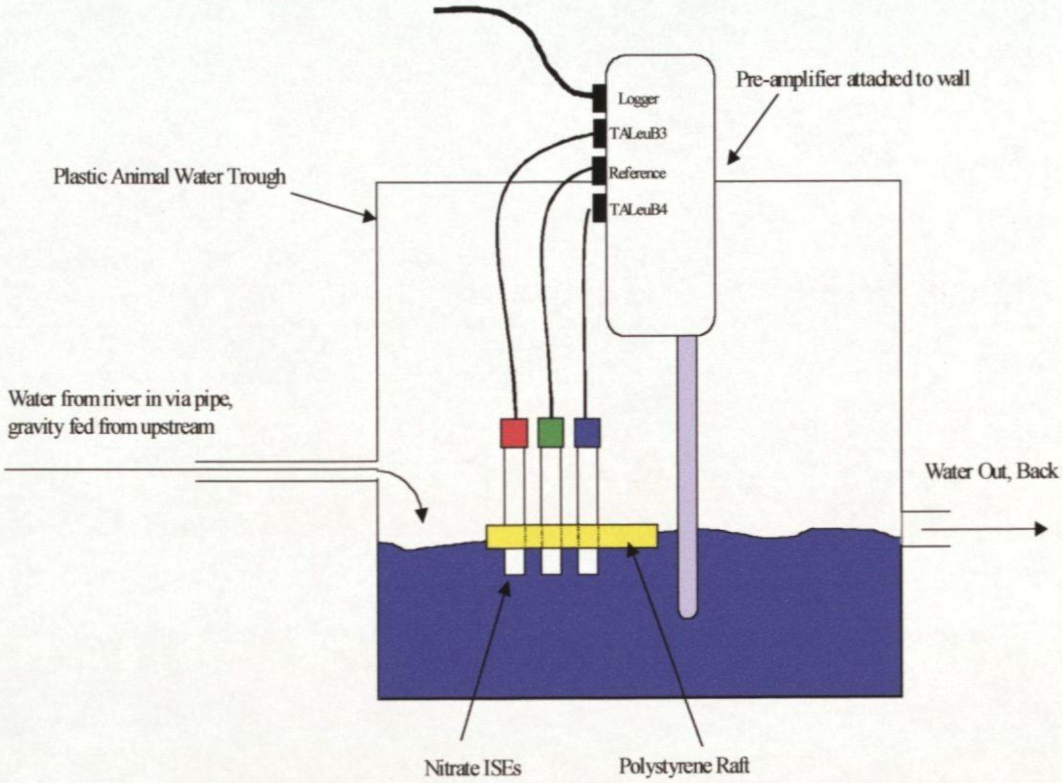
4.3.2.1 Description of the equipment

The equipment used for this field trial was the same as used for the V-notch weir experiment. Two nitrate ISEs were constructed with membranes containing N,N,N-triallyl leucine betaine. The cell was assembled with a double junction reference electrode filled with an agar gel.

4.3.2.2 Description of the site

The equipment was situated on a private stretch of the River Taw at IGER, North Wyke. The nitrate level in the river was expected to be between 0.1 and 2 ppm nitrate-N, much lower than that in the run-off weirs and closer to the electrode's limit of detection (7 ppb nitrate-N) but still in the linear Nernstian range (1400-0.07 ppm nitrate-N). Figure 4.16 shows the set-up used in this river trial. The water was gravity fed from further upstream by a siphon into a tank on the river bank containing the electrodes. As with the previous field trial the preamplifier was fixed with the temperature probe submerged in the water.

Figure 4.16 Diagram of the Equipment Used For the River Trial


Pre-amplifier attached to wall
Logger
TALeuB3
Reference
TALeuB4
Plastic Animal Water Trough
Water from river in via pipe, gravity fed from upstream
Water Out, Back into River
Nitrate ISEs
Polystyrene Raft

The electrodes were supported by a floating polystyrene raft with approximately 2 cm of the electrodes immersed in the water.

The field measurement system was linked a data logger similar to the one used in the previous field trial.

4.3.2.3 Results

Figure 4.17 shows the performance of the two nitrate ISEs compared with the segmented-flow instrument data over a period of two months. The range in concentration of nitrate-N in the river was between 0.4–1.9 ppm, as measured by the segmented-flow instrument. The outputs from the 2 electrodes have continued to track accurately that reference method within this range.

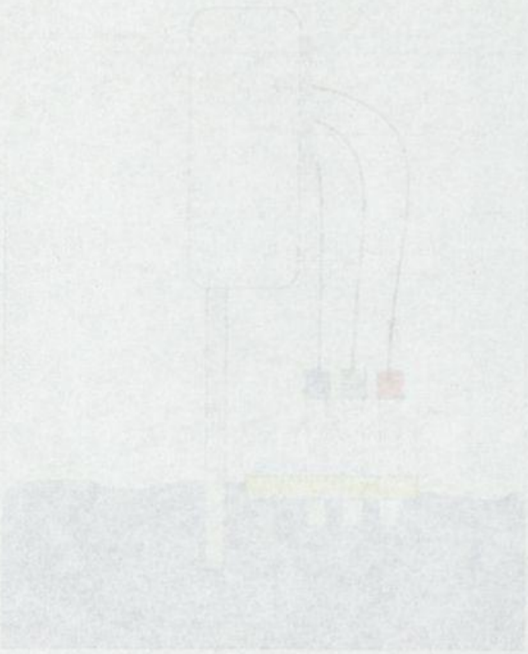
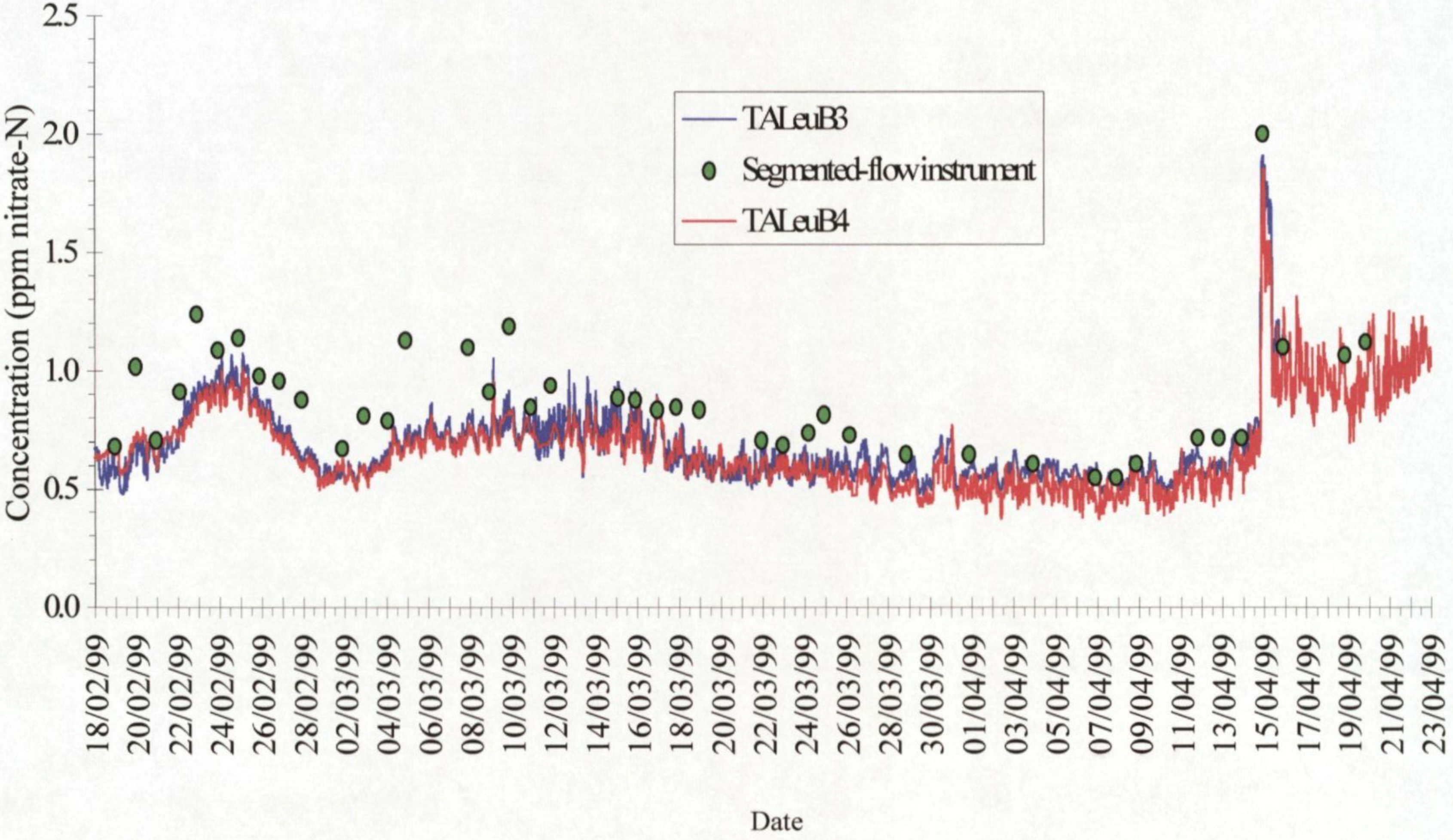
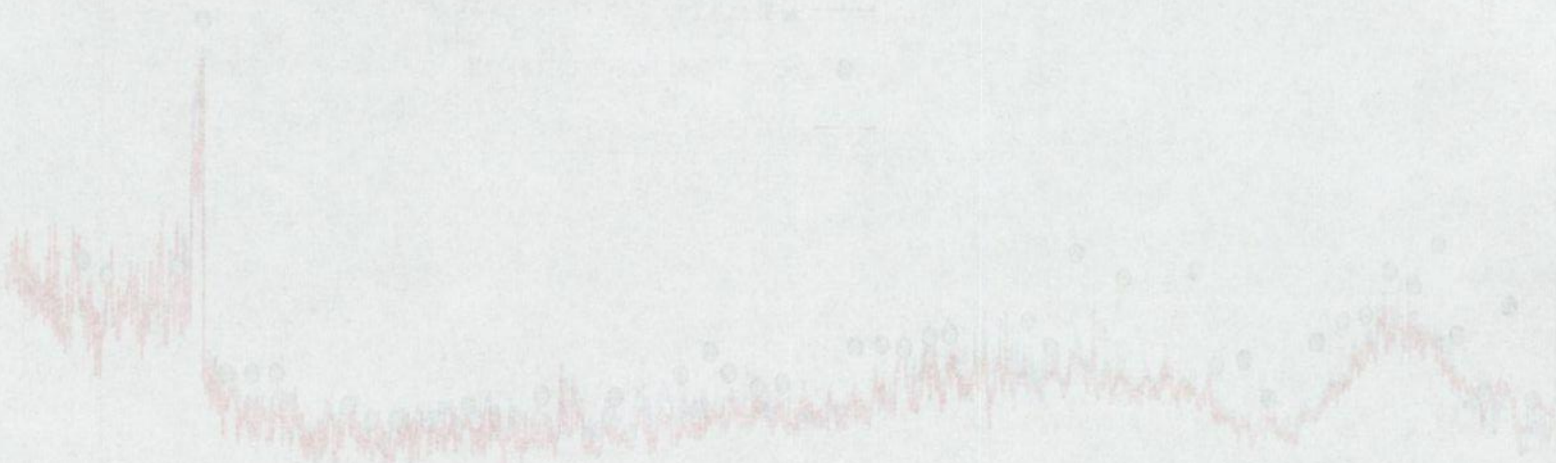


Figure 4.17 Field Trial Data in river Taw from two ISEs using TALeuB as sensor





4.3.2.4 Discussion

Figures 4.18 and 4.19 show the comparisons of the nitrate levels as determined by the nitrate ISEs and the levels determined by the segmented-flow instrument. The graphs show that there is good correlation between the two techniques. However, the regression lines have gradients that differ significantly from 1 at the 95% confidence level indicating the possibility of a systematic errors. However, the calibration check data as shown in figures 4.20 and 4.21 indicate a negligible drift in electrode performance during the course of the experiment.

Figure 4.18 Correlation between the segmented-flow instrument and TALeuB3

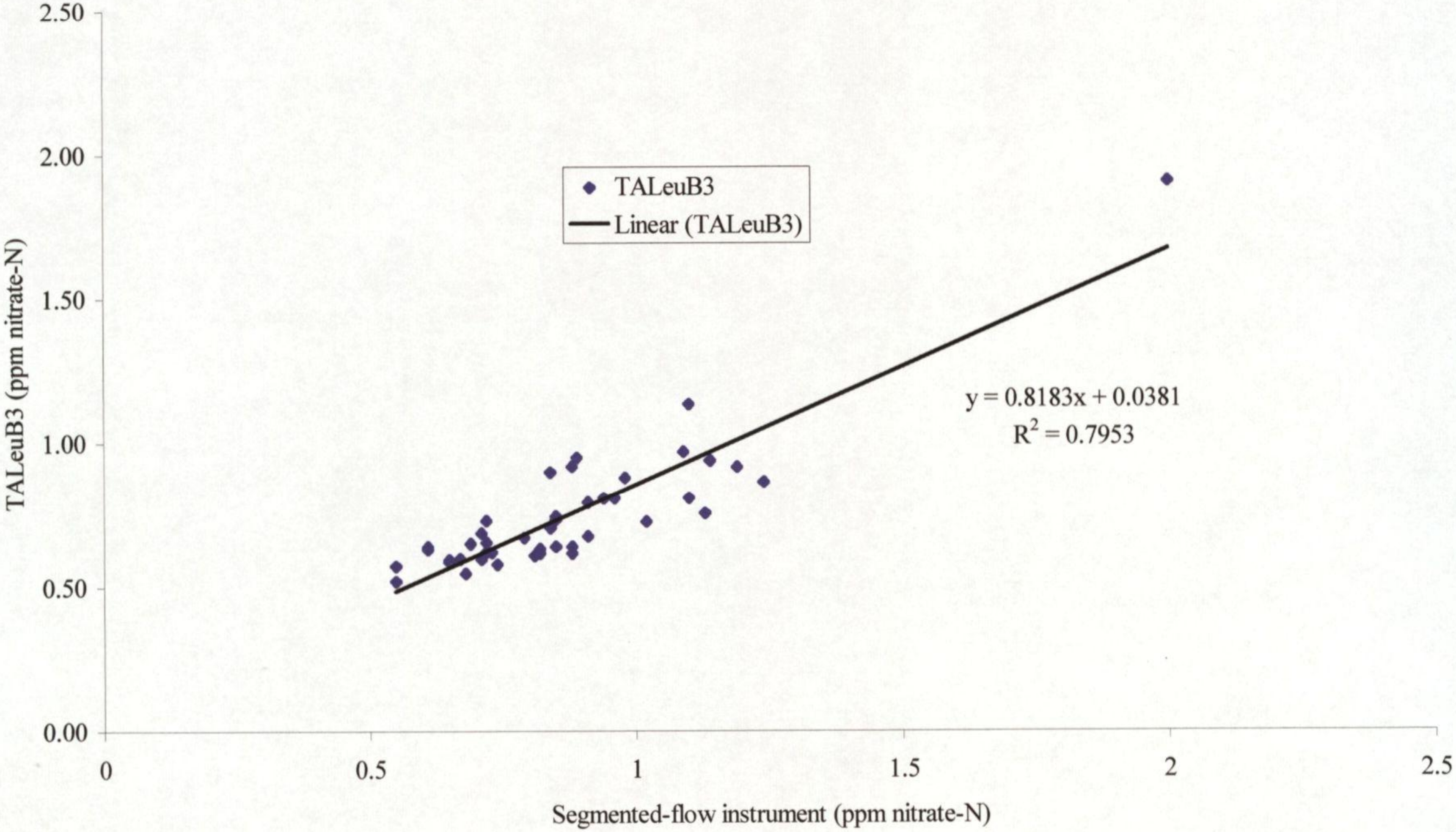


Figure 4.19 Correlation between the segmented-flow instrument and TALeuB4

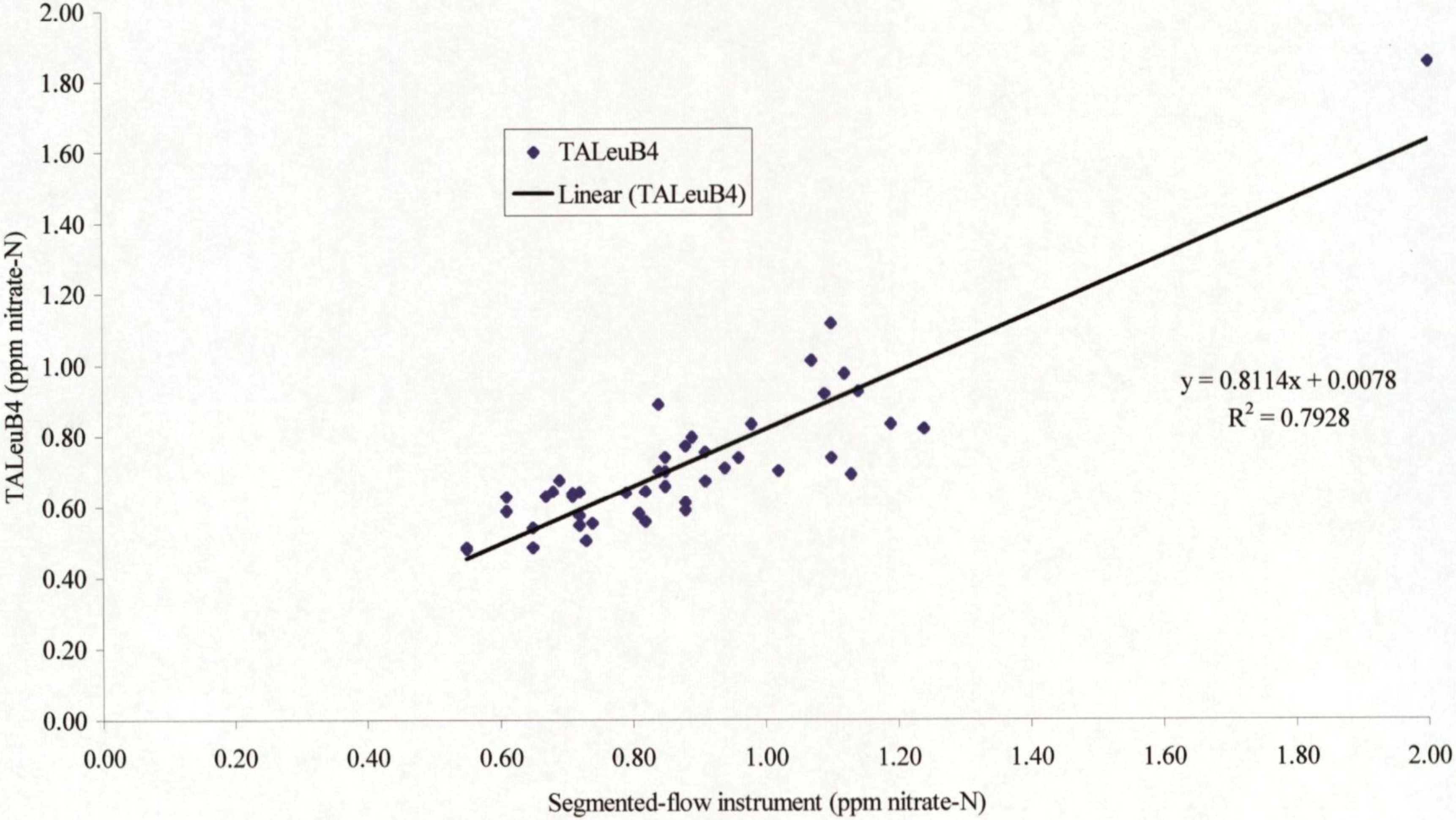


Figure 4.20 Initial calibration and subsequent checks for TALeuB3

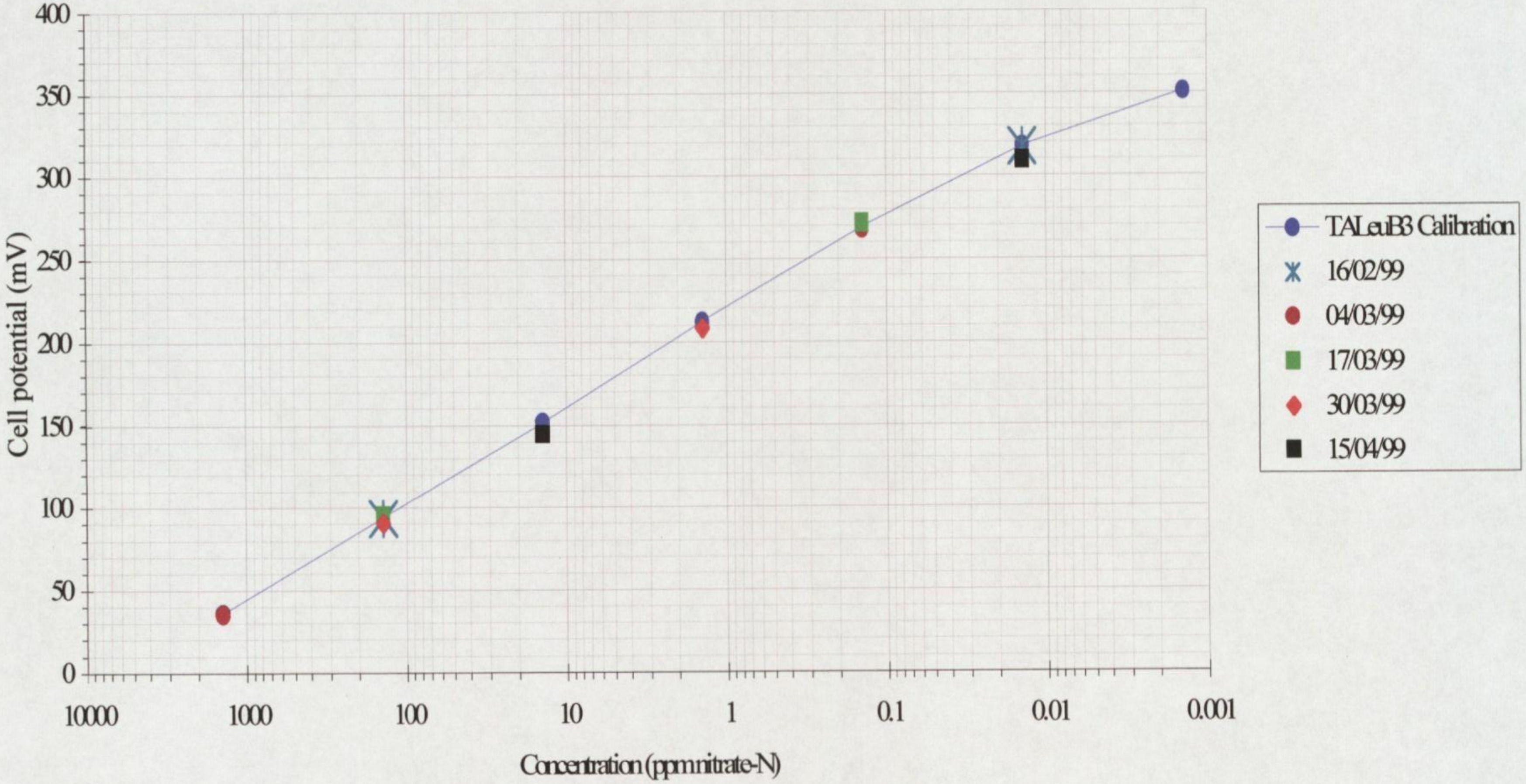
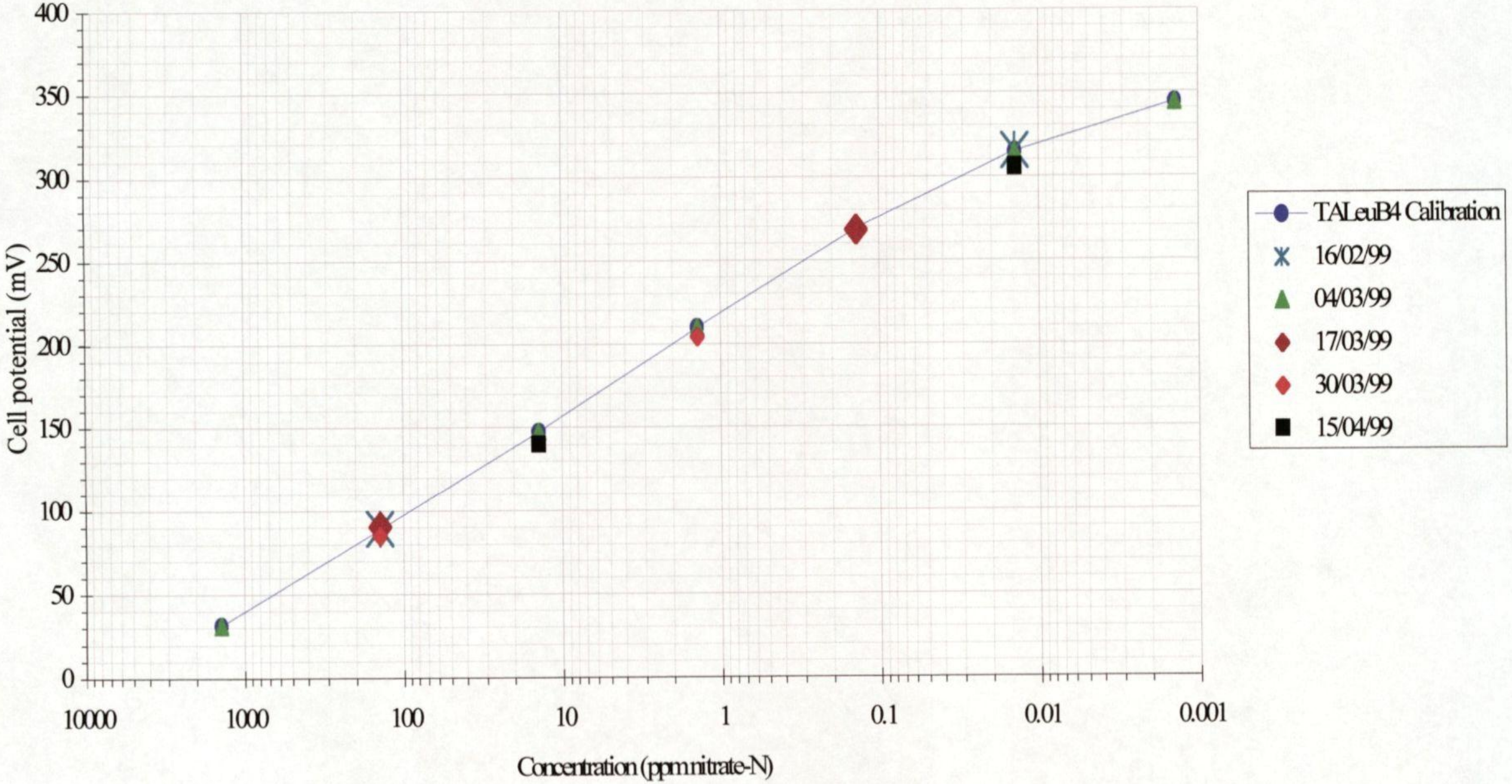


Figure 4.21 **Initial calibration and subsequent checks for TALeuB4**



The correlation between the two techniques is possibly limited by the sampling. Accurate analysis using the segmented-flow instrument requires filtration of the sample immediately after collection to avoid any errors in the nitrate analysis arising from the presence of biota. However, samples were sometimes unfortunately stored for several days prior to analysis by IGER staff. Erroneous nitrate determination for a few samples by the laboratory instrument could affect the correlation and the regression line between the two techniques.

Therefore, all the data, shown in table 4.1, used for the correlation and the regression analysis between the two nitrate ISEs and the segmented-flow instrument were analysed using 'unscrambler' (The unscrambler, 1998). This latter is a software package that gives the opportunity to use experimental design and multivariate data analysis in a very efficient way.

An outlier is a data point that for some reason looks different from the others and is not well described by the model. As a consequence, it is possible that one or more of the model components focus only on trying to describe how this sample is different from the others, even if this is irrelevant to the more important structure in the other samples.

A quick and efficient way to detect outliers is to plot Y-residuals against predicted Y values. The residual is the difference between observed Y-value and predicted Y-value which is computed for each sample by applying the model equation. If the model adequately predicts variations in Y, the residuals should be randomly distributed. If this is not the case appropriate action should be taken such as ignoring this sample in the model and checking its influence on the correlation and regression between the nitrate ISEs and the segmented-flow instrument. Using this method five outliers were detected in the data as shown in table 4.1.

Table 4.1 Data used for the correlation and the regression analysis between the two nitrate ISEs (TALeuB3 and TALeuB4) and the segmented-flow instrument

Sample Number	TALeuB3 (ppm nitrate-N)	TALeuB4 (ppm nitrate-N)	Segmented-flow instrument (ppm nitrate-N)
Sa1	0.55	0.64	0.68
Sa2	0.73	0.70	1.02
Sa3	0.60	0.64	0.71
Sa4	0.79	0.76	0.91
Sa5	0.87	0.82	1.24
Sa6	0.97	0.92	1.09
Sa7	0.94	0.93	1.14
Sa8	0.88	0.84	0.98
Sa9	0.81	0.74	0.96
Sa10	0.64	0.61	0.88
Sa11	0.60	0.63	0.67
Sa12	0.61	0.58	0.81
Sa13	0.67	0.64	0.79
Sa14	0.76	0.69	1.13
Sa15	0.81	0.74	1.10
Sa16	0.68	0.67	0.91
Sa17	0.92	0.84	1.19
Sa18	0.75	0.74	0.85
Sa19	0.81	0.71	0.94
Sa20	0.95	0.80	0.89
Sa21	0.92	0.77	0.88
Sa22	0.90	0.89	0.84
Sa23	0.74	0.70	0.85
Sa24	0.71	0.70	0.84
Sa25	0.69	0.63	0.71
Sa26	0.65	0.68	0.69
Sa27	0.58	0.56	0.74
Sa28	0.63	0.56	0.82
Sa29	0.62	0.50	0.73
Sa30	0.59	0.54	0.65
Sa31	0.60	0.49	0.65
Sa32	0.63	0.63	0.61
Sa33	0.57	0.49	0.55
Sa34	0.52	0.48	0.55
Sa35	0.64	0.59	0.61
Sa36	0.66	0.58	0.72
Sa37	0.62	0.55	0.72
Sa38	0.73	0.64	0.72

Sample Number	TALeuB3 (ppm nitrate-N)	TALeuB4 (ppm nitrate-N)	Segmented-flow instrument (ppm nitrate-N)
Sa39	1.91	1.85	2.00
Sa40	1.14	1.12	1.10
Sa41		1.01	1.07
Sa42		0.98	1.12

In yellow: outliers

The results of the Y-residuals against predicted Y values plots, regression and correlation plots are presented in figures 4.22-4.29 for the electrode TALeuB3 and figures 4.30-4.37 for the electrode TALeuB4. The rejection of the outliers was made for data points having a Y-residual out of the 95% confidence interval as shown in figure 4.22.

Figure 4.22 Y-Residuals versus predicted Y for TALeuB3 and segmented-flow instrument

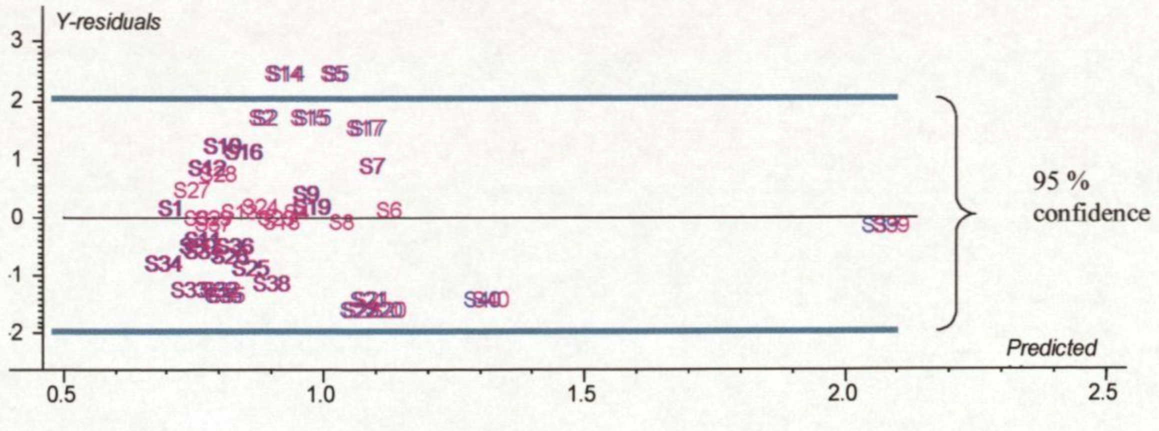


Figure 4.23 Correlation and regression between TALeuB3 and segmented-flow instrument

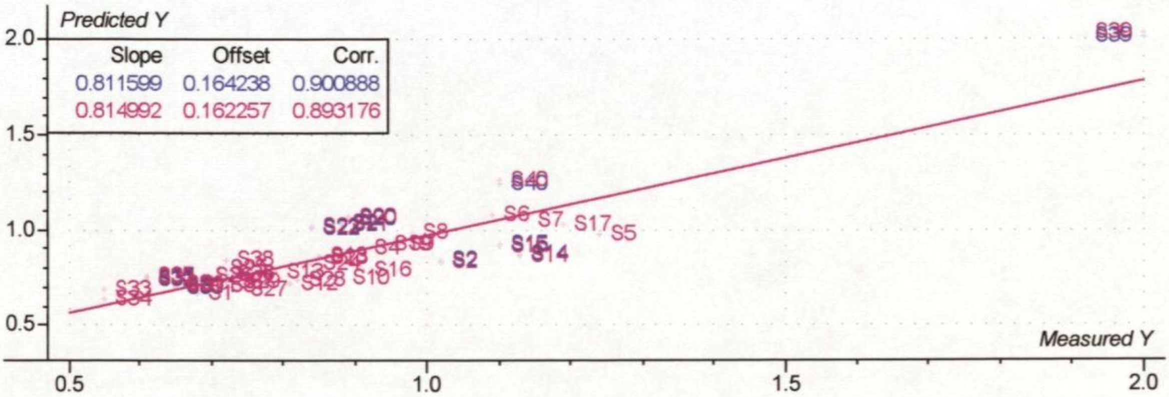


Figure 4.24 Y-Residuals versus predicted Y for TALeuB3 and segmented-flow instrument (samples 14 and 5 ignored)

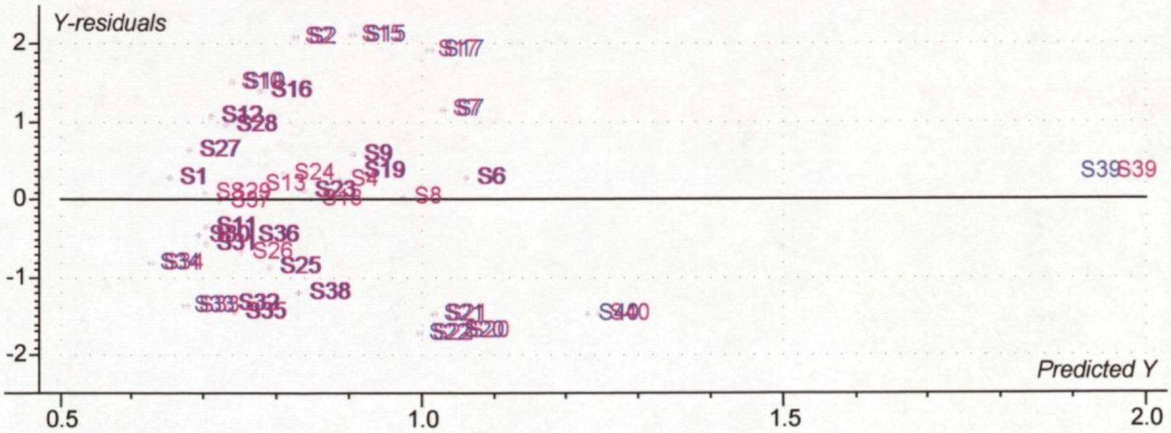


Figure 4.25 Correlation and regression between TALeuB3 and segmented-flow instrument (samples 14 and 5 ignored)

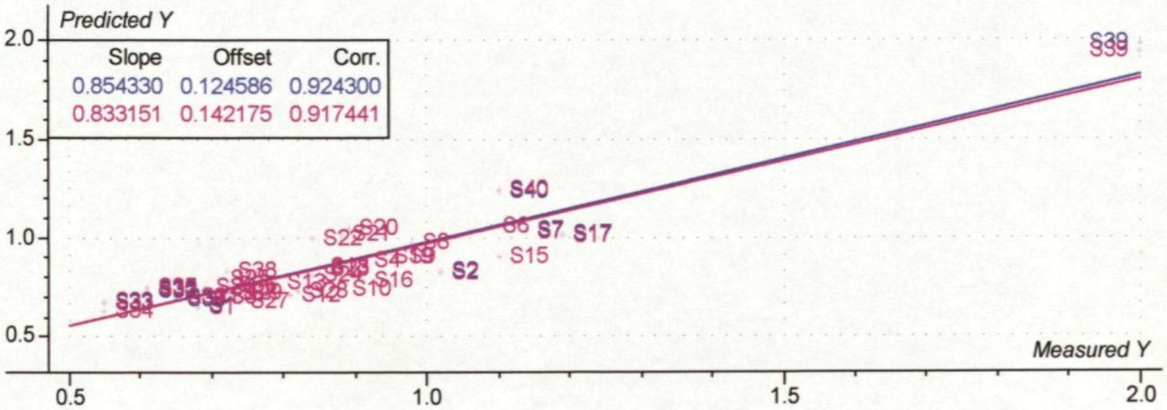


Figure 4.26 Y-Residuals versus predicted Y for TALeuB3 and segmented-flow instrument (samples 15, 5, 2 and 15 ignored)

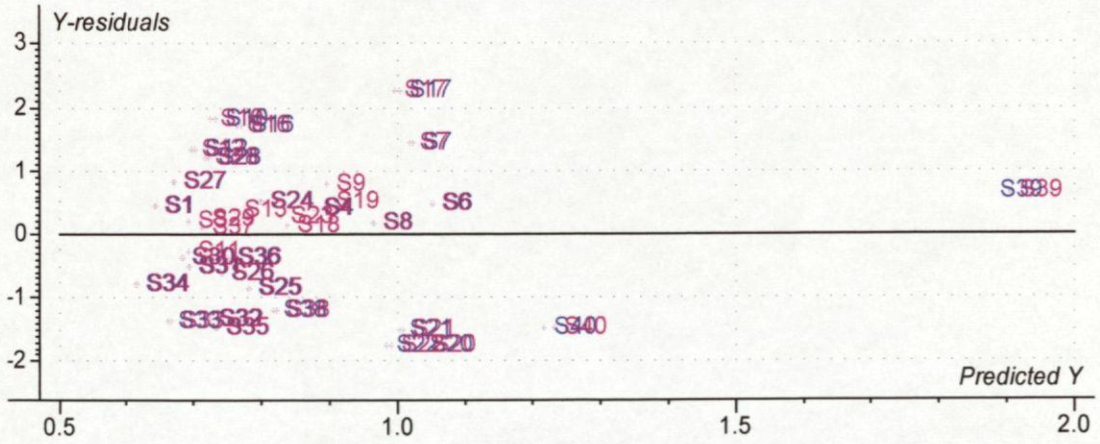


Figure 4.27 Correlation and regression between TALeuB3 and segmented-flow instrument (samples 14, 5, 2 and 15 ignored)

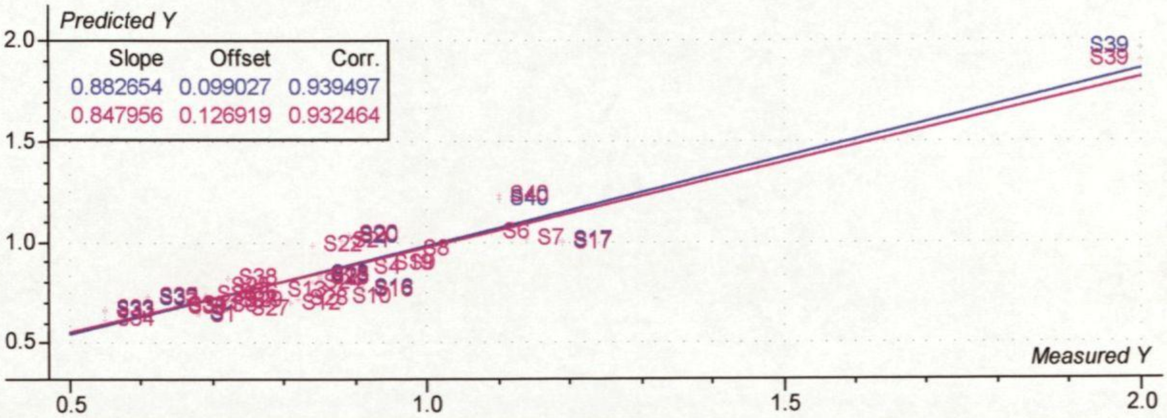


Figure 4.28 Y-Residuals versus predicted Y for TALeuB3 and segmented-flow instrument (sample 14, 5, 2, 15 and 17 ignored)

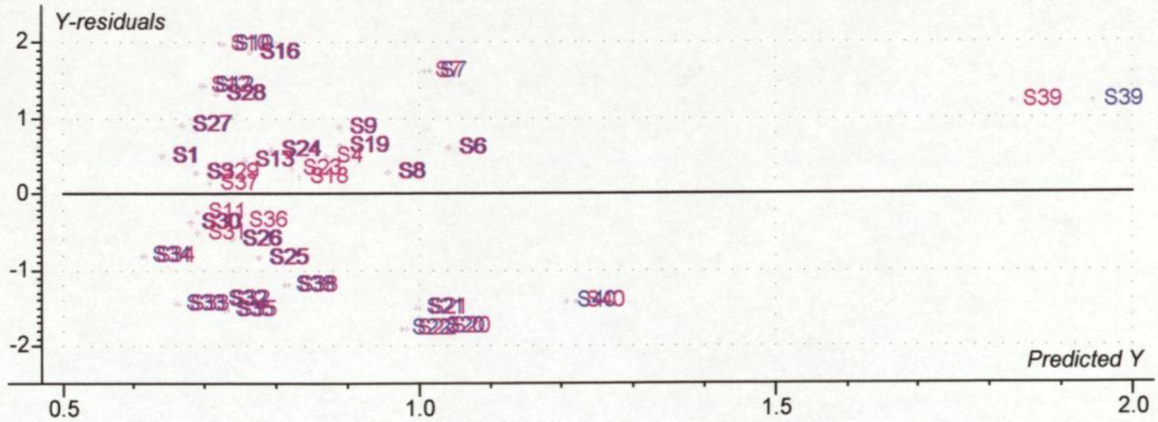


Figure 4.29 Revised correlation between the segmented-flow instrument and TALeuB3

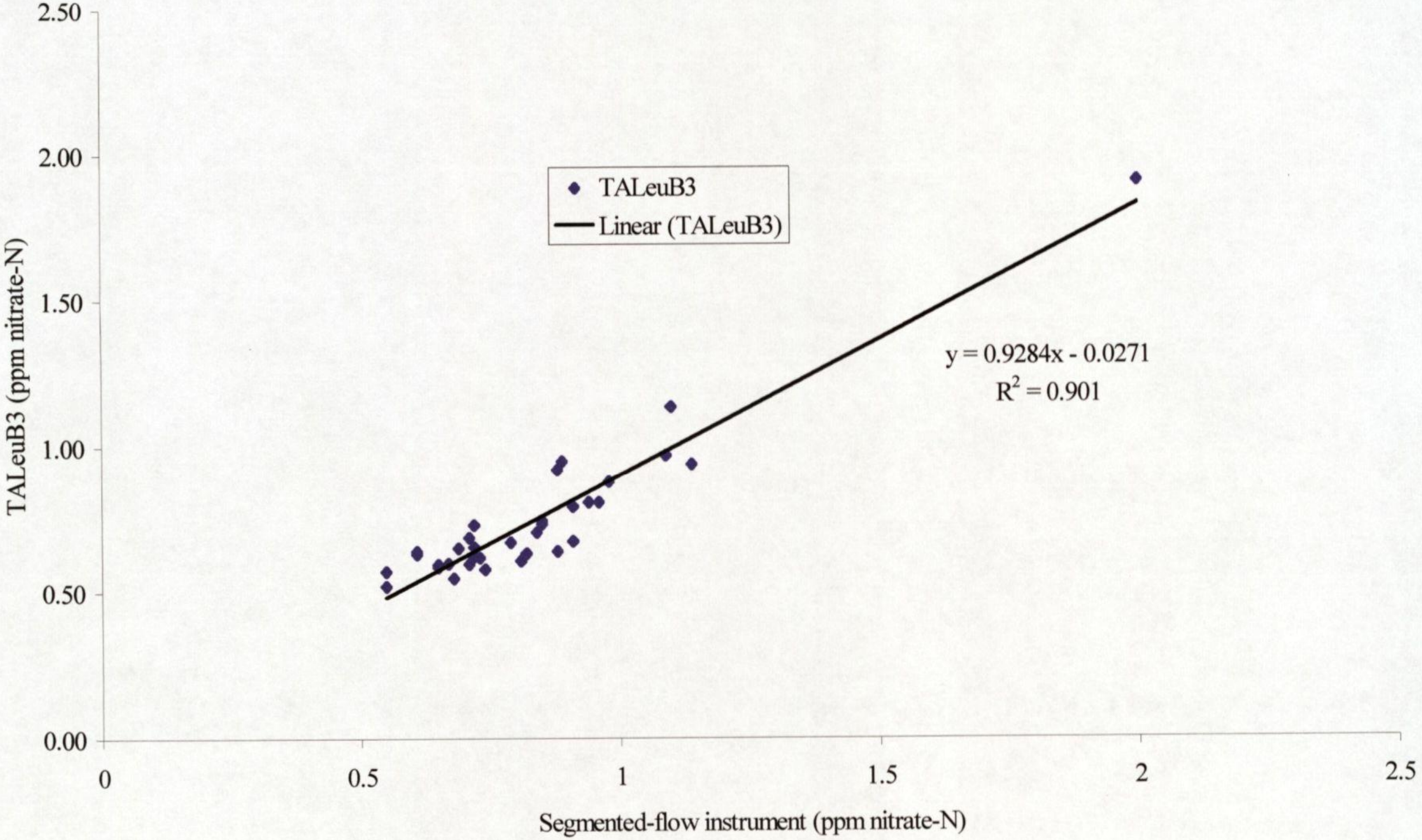


Figure 4.29 shows the final correlation and regression between TALeuB3 and the segmented flow instrument when samples numbered 14, 5, 2, 15 and 17 are ignored. The correlation coefficient (R^2) is 0.89 and the regression line now has a gradient of 0.93. The performance of the electrode in the river now matches these observed in the weir.

The data for the electrode TALeuB4were treated in the same way as for the electrode TALeuB3.

Figure 4.30 **Y-Residuals versus predicted Y for TALeuB4 and segmented-flow instrument**

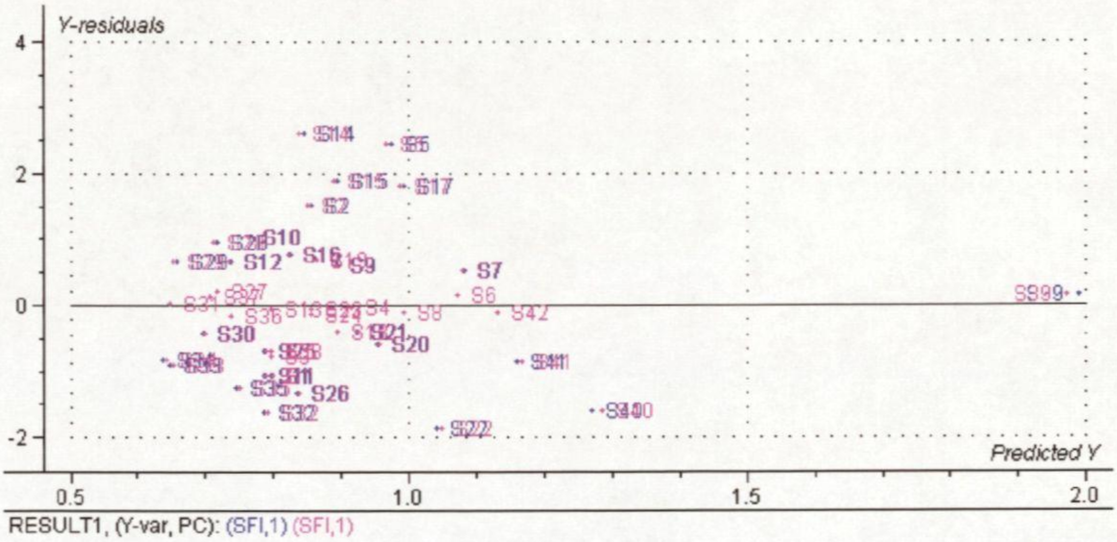


Figure 4.31 Correlation and regression between TALeuB4 and segmented-flow instrument

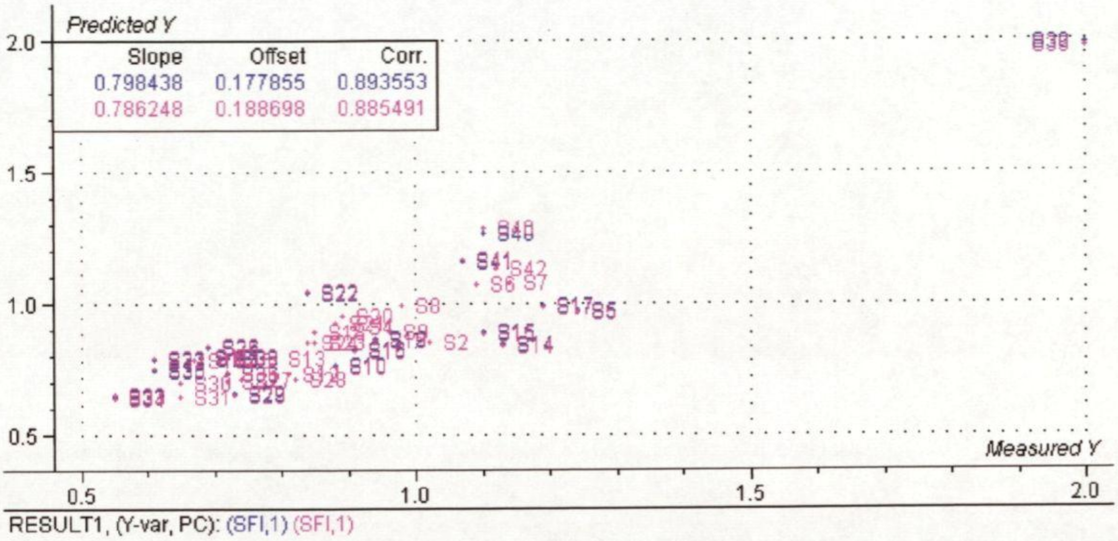


Figure 4.32 Y-Residuals versus predicted Y for TALeuB4 and segmented-flow instrument (samples 14 and 5 ignored)

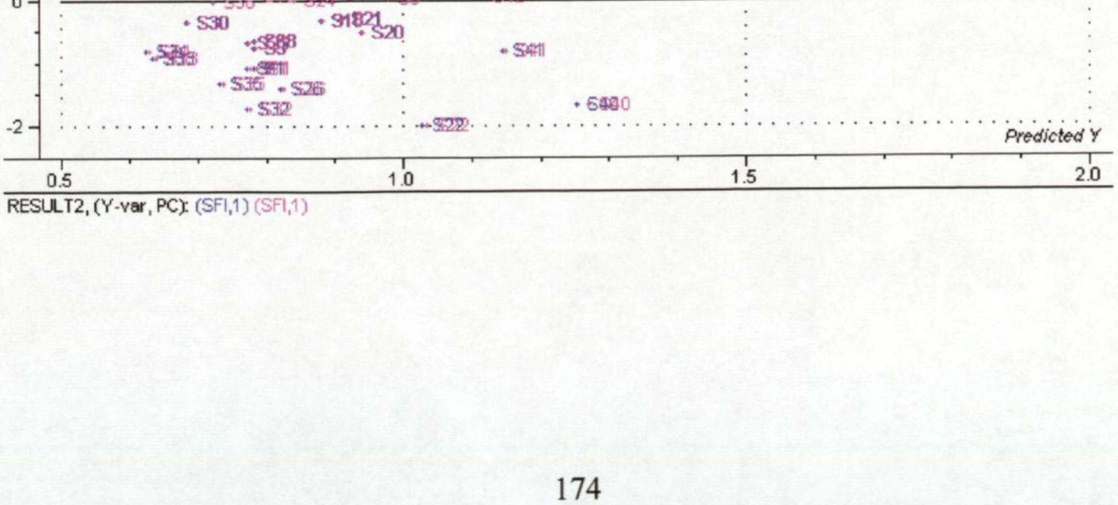


Figure 4.33 Correlation and regression between TALeuB4 and segmented-flow instrument (samples 14 and 5 ignored)

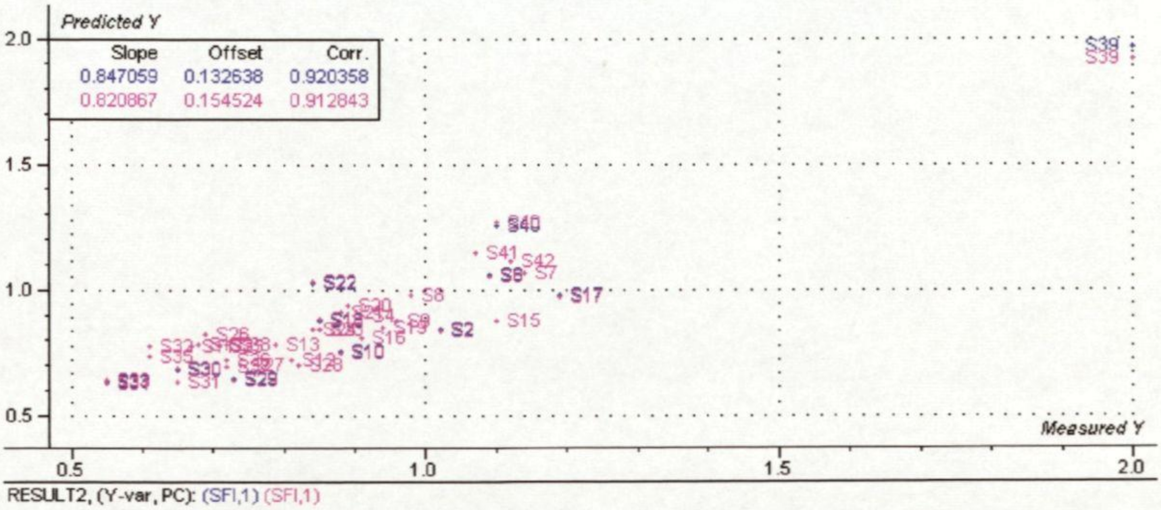


Figure 4.34 Y-Residuals versus predicted Y for TALeuB4 and segmented-flow instrument (samples 14, 5, 15 and 17 ignored)

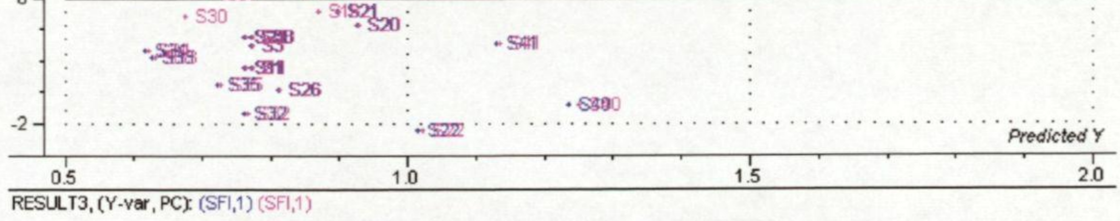


Figure 4.25 Correlation and regression between [illegible] and computed [illegible]

[illegible]

Figure 4.26 [illegible]

[illegible]

Figure 4.35 Correlation and regression between TALeuB4 and segmented-flow instrument (samples 14, 5, 15 and 17 ignored)

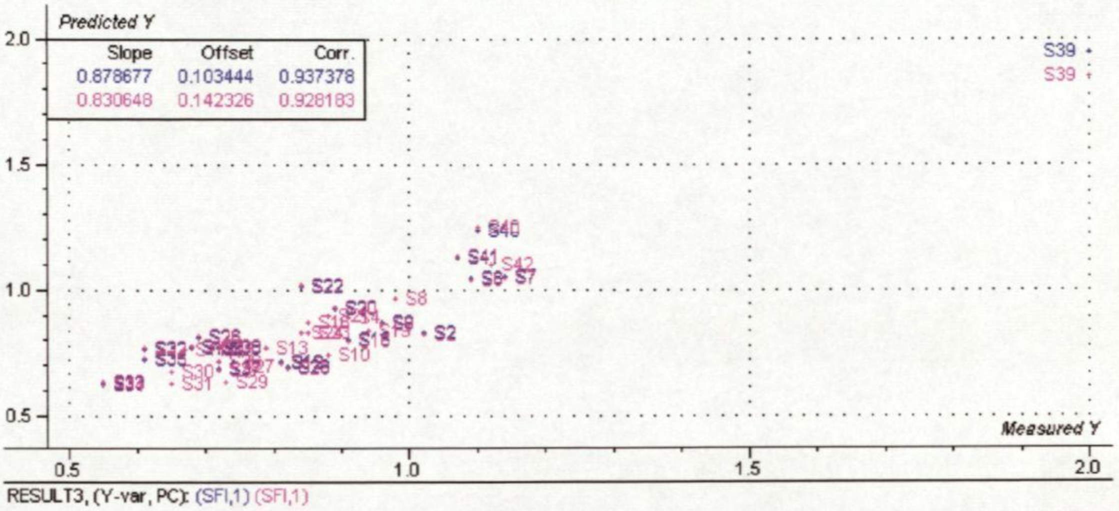


Figure 4.36 Y-Residuals versus predicted Y for TALeuB4 and segmented-flow instrument (samples 14, 5, 15, 17, 2 and 22 ignored)

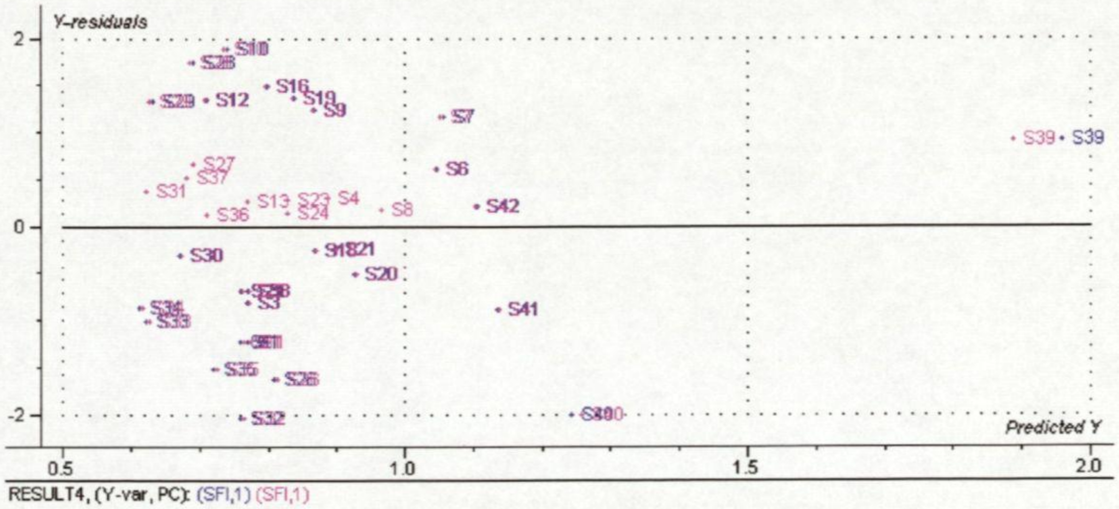


Figure 4.37 shows the final correlation and regression between TALeuB4 and the segmented-flow instrument when samples numbered 14, 15, 17, 5, 2 and 22 are ignored. The correlation between the two techniques is good with R^2 value of 0.9 and the regression line has a gradient of 0.93 that is similar than those obtained with electrode TALeuB3.

4.4 Conclusions

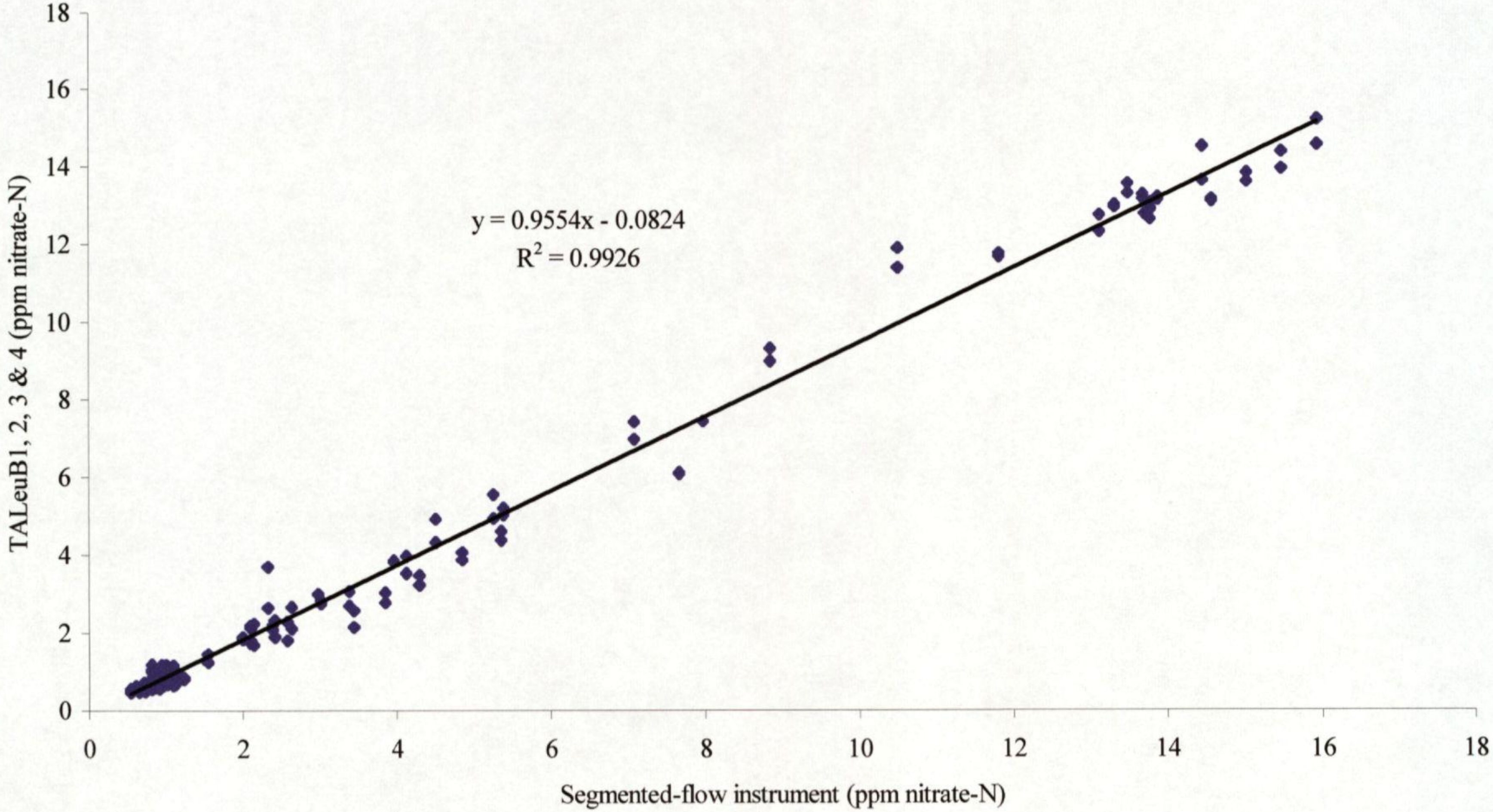
The trials using N,N,N-triallyl leucine betaine in SBS were carried out in the agricultural run-off weir for over 5 months and in river water for over 2 months without any significant drift. Potentiometric drift is a recognised limitation for the long-term use of ISEs (YSI, 1996). The same membrane was used throughout each separate study and no degradation or fouling was observed and cleaning of the surface of the membrane was not necessary. The anti-microbial nature of certain quaternary ammonium compounds has been recognised for years (Sykes, 1965). These compounds act by interfering with cellular metabolic processes, denaturing cell proteins, and damaging cytoplasmic membranes. Triallyloctyl, triallylnonyl and triallyldecyl ammonium nitrates were used as anti-microbial agents against various test organisms (Sutton, 1996) and exhibited bactericidal and fungicidal activities.

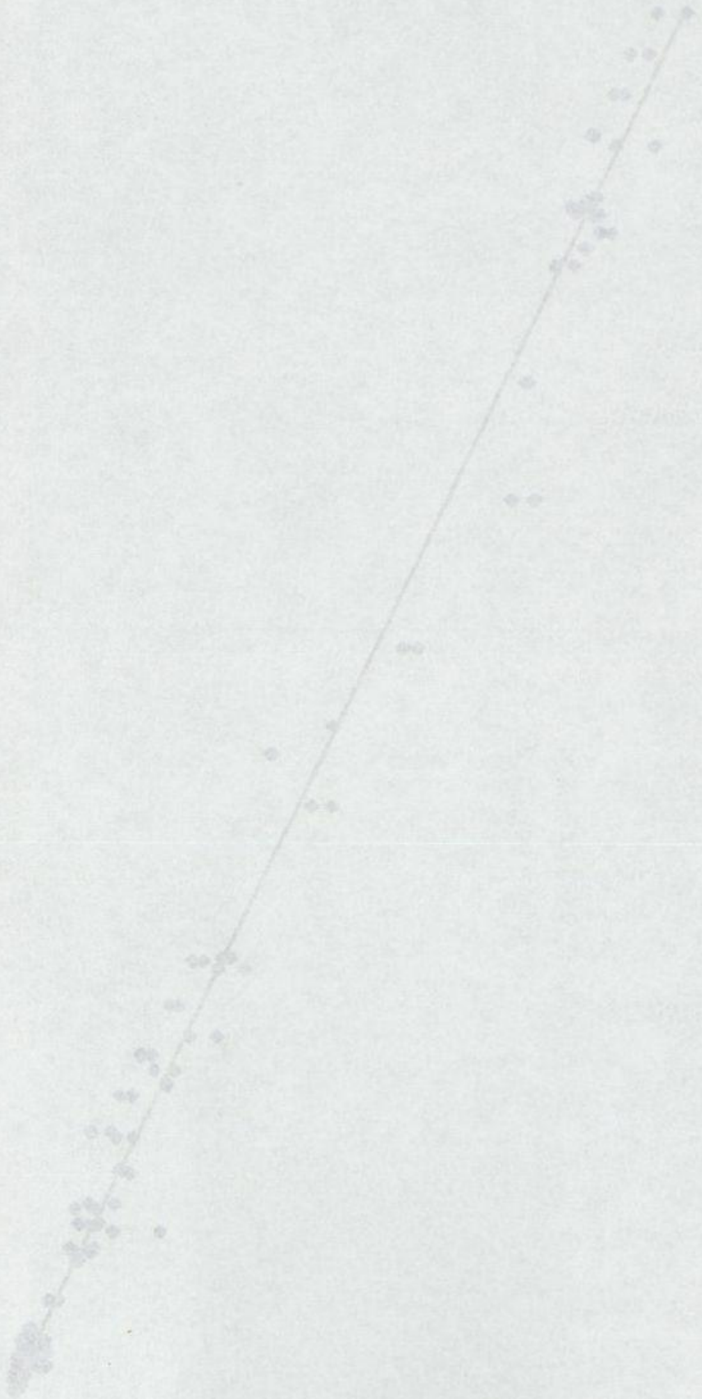
Good correlation between the levels determined by the segmented-flow instrument and the levels measured by the nitrate ISEs were obtained. In the weir experiment, correlation coefficients, R^2, of 0.99 were obtained for both nitrate ISEs. In the river experiment, correlation coefficients, R^2, of 0.89 and 0:9 were obtained for the two nitrate ISEs evaluated.

ISEs were also reproducible. In both experiments, the regression lines between the two electrode have very similar gradients. In the weir experiment regression lines with gradients of 0.96 and 0.94 were obtained. In the river experiment, regression lines with gradients of 0.93 were obtained (after removal of the outliers). Figure 4.38 shows the comparison of the nitrate levels as determined by the nitrate ISEs (TALeuB1, 2, 3 &4) and the levels determined by the segmented-flow instrument in both the weir and river experiments. Over this large range (0.47 - 16 ppm nitrate-N) there is excellent correlation ($R^2 = 0.99$) between the two techniques. The regression line has a gradient (0.95) that does not differ significantly from 1 at the 95% confidence level indicating that there is no systematic error.

The nitrate ISEs containing N,N,N-triallyl leucine betaine are the most sensitive ISEs obtained to date (LOD: 0.007 ppm nitrate-N) with good selectivity and a wide linear Nernstian range (1400 – 0.07 ppm nitrate-N). The membranes work well over an environmentally acceptable pH range (pH 2 - pH 8) with 90% of the potential reached within 30 seconds and a long working lifetime.

Figure 4.38 **Correlation between the segmented flow instrument and TALeuB 1, 2, 3 & 4 for the combined weir and river experiments**





CHAPTER 5

DEVELOPMENT OF A DIBASIC PHOSPHATE-SELECTIVE ELECTRODE

5.1 Background

As stated in the introductory section Carey and Riggan (1994) had developed a phosphate sensor based on a heterocyclic macrocycle which, when trapped in PVC gave a sensitive phosphate sensor at neutral pH. This method was adopted as starting point for our own study. The membranes composition used by Carey and Riggan was composed of PVC (37 % m/m), dibutyl sebacate (DBS) (40 % m/m) and the sensor molecule (23 % m/m).

5.2 Reagents and polymers

All the chemicals required for organic synthesis were obtained from Aldrich Ltd. (Gillingham, Dorset, UK) unless stated otherwise. Purity was routinely checked by chromatography (GC or TLC) or nuclear magnetic resonance spectroscopy. Solvents were of HPLC grade and obtained from Rathburn Chemicals Ltd. (Walkerburn, Peeblershire, Scotland). Tetrahydrofuran (THF) was freshly distilled prior to use. For the synthesis of mono- and di-substituted malonate esters and the cyclisation reactions, ethanol was dehydrated using the reaction with magnesium ethoxide (Lund and Bjerrum, 1931). Dibutyl sebacate (DBS) (Selectophore grade, Fluka Chemicals, Gillingham, Dorset, UK) were used as received.

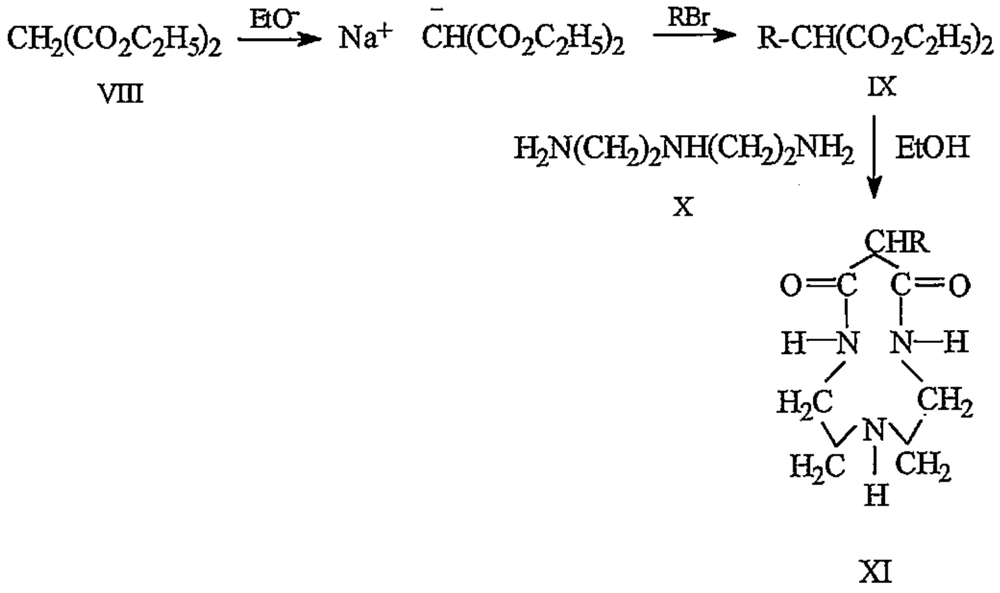
The polymeric material poly(vinyl chloride) (high molecular weight, Aldrich) was used as matrix for phosphate-selective electrode. The matrix was evaluated either without sensor or in the presence of sensor simply entrapped in the polymer.

5.3 Carey sensor

5.3.1 Synthetic route

Carey reported that the synthesis of 3-decyl-1,5,8-triazacyclodecane-2,4-dione (XI, R = $C_{10}H_{21}$) was achievable using α-monosubstituted diethyl malonate esters (IX) and diethylene triamine (X), as illustrated in figure 5.1. The reaction was carried out in high dilution to minimise the competitive polymerisation route.

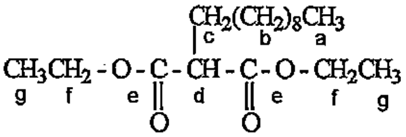
Figure 5.1 Carey synthetic route


$CH_2(CO_2C_2H_5)_2$
EtO⁻
Na^+ $\overset{-}{C}H(CO_2C_2H_5)_2$
RBr
$R\text{-}CH(CO_2C_2H_5)_2$
VIII
IX
$H_2N(CH_2)_2NH(CH_2)_2NH_2$
EtOH
X
CHR
O=C
C=O
H—N
N—H
H_2C
CH_2
N
H_2C
CH_2
H
XI

5.3.2 Synthesis of individual compounds

5.3.2.1 Synthesis of diethyl α-decyl malonate

$C_{17}H_{32}O_4$, MW = 300



$$CH_3CH_2-O-\underset{\underset{O}{\|}}{C}-\overset{\overset{CH_2(CH_2)_8CH_3}{|}}{CH}-\underset{\underset{O}{\|}}{C}-O-CH_2CH_3$$

The reaction was protected from moisture (dropping funnel and condenser were fitted with guard tubes filled with calcium chloride). Sodium (3.5 g, 0.15 mol) was added slowly to ethanol (250 ml) to form sodium ethoxide. Freshly distilled diethyl malonate (23.8 g, 0.15 mol) was added dropwise to the ethanolic sodium hydroxide at 50°C. To the resulting solution, distilled n-decyl bromide (33.2 g, 0.15 mol) was added drop-wise, and the reaction mixture was refluxed for 4 hours. Finally, the reaction mixture was evaporated to dryness. The crude product was washed with water and purified using vacuum distillation. The pure diethyl α-decyl malonate was collected as a colourless oil.

Yield : 20.5 g (45.5 %), **bp** : 143°C / 0.1 mmHg (lit. 144-146°C / 0.1 mmHg), Purity by **GC** : 99 %

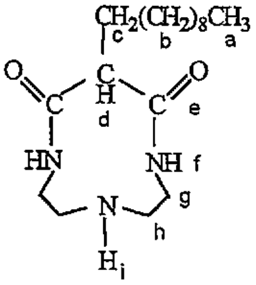
IR : $\nu_{(-C=O)} = 1737\ cm^{-1}$, $\nu_{(-COOR)} = 1033\ cm^{-1}$, $\nu_{(-O-CH2CH3)} = 1033\ cm^{-1}$, $\nu_{(-CH,\ CH2)} = 2927$, $2857\ cm^{-1}$, $\delta_{(-CH,\ CH2)} = 1465\ cm^{-1}$.

NMR ^{1}H ($CDCl_3$): δ (ppm) : 0.75 ($6H_g$, t), 1.13 ($3H_a$, t), 1.13 ($16H_b$, m), 1.76 ($2H_c$, dt), 3.31 (H_d, $4H_f$, m).

NMR ^{13}C : δ (ppm) : 14.03 (C_a), 22.59, 27.4, 29.3, 31.82 (C_b), 30.5 (C_c), 39.06 (C_f), 48.16 (C_g), 54.07 (C_d), 171.2 (C_e).

5.3.2.2 Synthesis of 3-decyl-1,5,8-triazacyclodecane-2,4-dione

$C_{17}H_{33}N_3O_2$, MW = 311



The synthesis was accomplished in dilute solution to avoid polymerisation. Diethyl α-decylmalonate (0.076mol, 22.80g) and diethylene triamine (0.076mol, 7.74g) were refluxed for 45 days in 1500 dm^{-3} of ethanol previously dried according to the method developed by Lund and Bjerrum (1931). The reaction mixture was monitored with ^1H NMR analysis every 5 days to determine the completeness of the reaction. After completion of the reflux the reaction mixture was evaporated to dryness. The residue was washed, several times with petroleum spirit (40-60°C) to remove any remaining diethyl α-decylmalonate and recrystallised several times with hot acetone to obtain pure 3-decyl-1,5,8-triazacyclodecane-2,4-dione as a white solid.

Yield : 10.5g (44.5 %), **mp** : 60-150°C (lit. 150-180°C)

IR : $\nu_{(\text{-C=O, amide})} = 1666\ cm^{-1}$, $\nu_{(\text{-CH})} = 3071, 2994\ cm^{-1}$, $\nu_{(\text{-CH, CH2})} = 2922, 2852\ cm^{-1}$, $\delta_{(\text{-CH, CH2})} = 1467\ cm^{-1}$, $\nu_{(\text{-NH})} = 3292\ cm^{-1}$, $\delta_{(\text{-NH, amide})} = 1559, 722\ cm^{-1}$.

NMR ^{1}H ($CDCl_3$): δ (ppm) : 0.87 ($3H_a$), 1.24 ($16H_b$, $2H_c$), 1.82 (H_i), 2.74 ($4H_h$), 3.31 ($4H_g$, H_d), 3.62 (H_f).

NMR ^{13}C : δ (ppm) : 14.03 (C_a), 22.59, 27.4, 29.3, 30.5, 31.82 (C_b, C_c), 39.06 (C_h), 48.16 (C_g), 54.07 (C_d), 171.2 (C_e).

5.4 Membrane fabrication and method of evaluation

5.4.1 Membrane composition

The membrane composition was the same as used by Carey and Riggan. PVC (0.74 g, 37 % m/m) was dissolved in THF (12 ml). Dibutyl sebacate (DBS) (0.80 g, 40 % m/m) were added, followed by sensor molecule (0.46 g, 23 % m/m). The mixture was shaken until homogeneous. PVC blank membanes were composed of PVC (0.96 g, 48 % m/m) and dibutylsebacate (1.04 g, 52 % m/m).

5.4.2 Membrane fabrication by solvent-casting

The mixture was carefully poured into a glass ring (i.d. 65mm, lower side ground flat) which was fixed tightly on a glass plate with a rubber band. The glass ring was protected from dust and currents of air using a large beaker upside down. After allowing the solvent to evaporate (2 days) a membrane of about 2 mm thickness was obtained. The membrane with the glass ring was carefully removed from the glass plate and discs of 7 mm diameter were punched out.

5.4.3 Technique for membrane evaluation

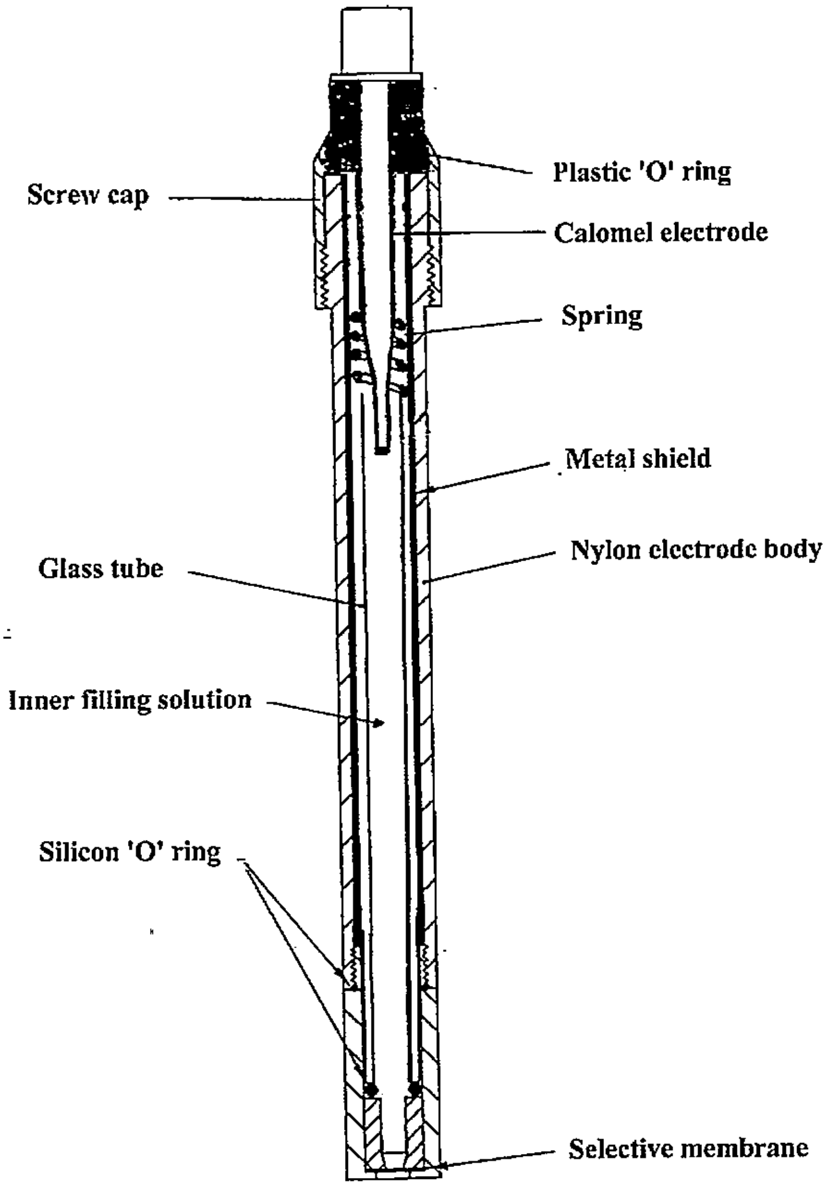
5.4.3.1 Equipment

5.4.3.1.1 Phosphate-selective electrode construction

Discs of 7 mm diameter were conditioned in 0.2 mol dm^{-3} potassium dihydrogen orthophosphate (AnalaR grade, BDH) and the solution titrated to pH 7.2 with potassium hydroxide (AnalaR grade, BDH) solution. The conditioned membrane was assembled into the tip of a modified commercially available electrode body (IS560, Philips Analytical, Cambridge, UK) as shown in figure 5.2. The inner filling solution was composed of 2 x 10^{-1} mol dm^{-3} potassium dihydrogen orthophosphate solution titrated to pH 7.2 with potassium hydroxide (AnalaR grade, BDH) solution. The internal reference of the electrode was a calomel electrode (Type K4113, Radiometer Copenhagen, Radiometer Ltd., Crawley, UK) filled with 4 mol dm^{-3} potassium chloride (AnalaR grade, BDH) solution.

A hole of 0.7 cm was drilled into the screw cap of the sensing electrode to insert the internal reference. A glass tube of 8 cm length and 0.6 cm diameter was placed inside the sensing electrode body and filled with the inner filling solution. The calomel electrode was passed through a hole (0.7 cm diameter) drilled in the screw cap and a plastic 'O' ring was placed around it. Then a spring was placed at the contact between the calomel electrode and the glass tube. Finally the cap was screwed in order to have the internal reference in contact with the inner filling solution.

Figure 5.2 Sensing electrode arrangement


Plastic 'O' ring
Screw cap
Calomel electrode
Spring
Metal shield
Nylon electrode body
Glass tube
Inner filling solution
Silicon 'O' ring
Selective membrane

5.4.3.1.2 The reference electrode

The reference electrode selected for this work was the same used by Riggan and Carey, being the Ag / AgCl internal reference of a pH electrode (Gelplas, General Purpose Combination, BDH, Lutterworth).

5.4.3.2 Experimental procedures

The experimental procedures were the same as described in section 2.2.3.2 apart from the measurement of the cell potential and the preparation of the standard solutions.

5.4.3.2.1 Measurement of the cell potential

The following arrangement was used :

Ag | AgCl | $KCl_{(sat.)}$ | Sample solution | Membrane
| 0.2 mol dm^{-3} phosphate buffer (pH 7.2) | Hg | Hg_2Cl_2 | $KCl_{(sat.)}$

The EMF measurements were done using a high impedance voltmeter (model 931402, Hanna instruments, Bedfordshire, UK) connected to a custom built pre-amplifier (Wood, Research Instrument Design, Penmoth, Ruthern Bridge, Cornwall, UK). The pH measurements were done using a pH electrode (Gelplas, General Purpose Combination, BDH, Lutterworth) and a high impedance pH meter (model 290, PYE UNICAM, Cambridge, UK).

5.4.3.2.2 Standard solutions

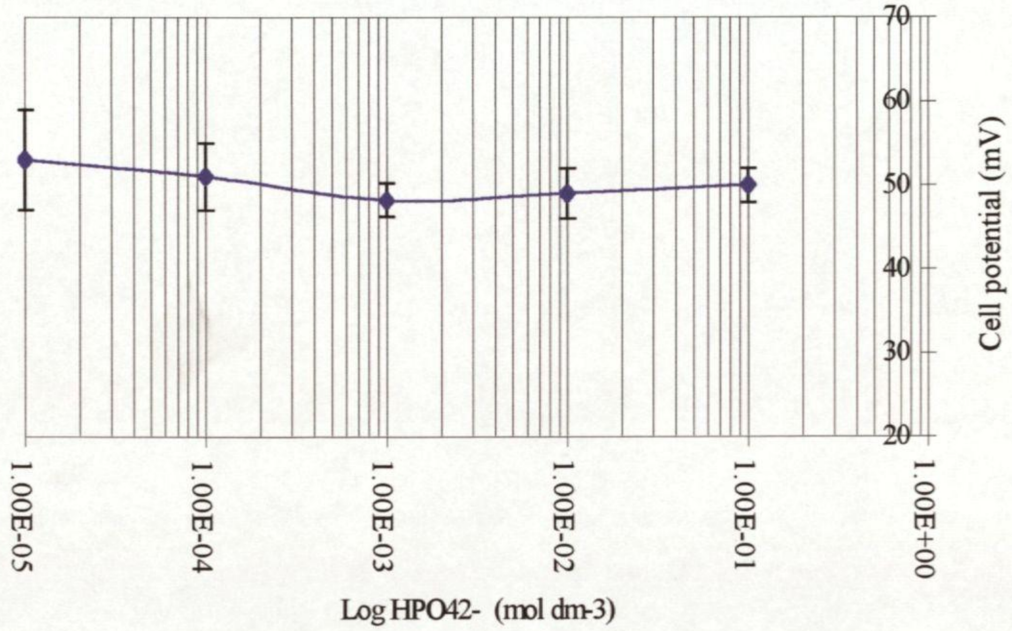
The standard solutions were prepared daily using analytical reagents of the highest purity commercially available. Standard solutions were prepared with potassium dihydrogen orthophosphate (AnalaR grade, BDH chemicals, Poole, Dorset, UK) dissolved in Milli-Q water (Milli-Q, Millipore (UK) Ltd.). A range of standards 10^{-1}-10^{-7} mol dm^{-3} were prepared by serial dilution of 10^{-1} mol dm^{-3} stock solution. The standard solutions were all titrated to pH 7.2 using potassium hydroxide solution.

5.5 Electrochemical evaluation of PVC-based membranes

5.5.1 PVC blank membranes

Blank membranes composition was as stated in section 5.4.1 and the membrane evaluation was carried out as described in section 5.4.3. Figure 5.3 shows the response to HPO_4^{2-} of the electrode made with 5 different blank membranes.

Figure 5.3 Phosphate response for PVC blank membranes



Blank membranes did not respond to dibasic phosphate and therefore the response observed with dibasic phosphate-selective electrodes containing ionophores could only be attributed to the sensor and not to the polymeric material.

5.5.2 PVC membranes containing 3-decyl-1,5,8-triazacyclodecane-2,4-dione

5.5.2.1 Phosphate response

Membrane composition and fabrication were as described in section 5.4.1. Five membranes were punched from the master membrane and evaluated using the same method decribed in section 5.4.3.

[illegible]

[illegible]

[illegible]

[illegible]

[illegible]

Figure 5.4 **Phosphate response of membranes containing 3-decyl-1,5,8-triazacyclodecane-2,4-dione**

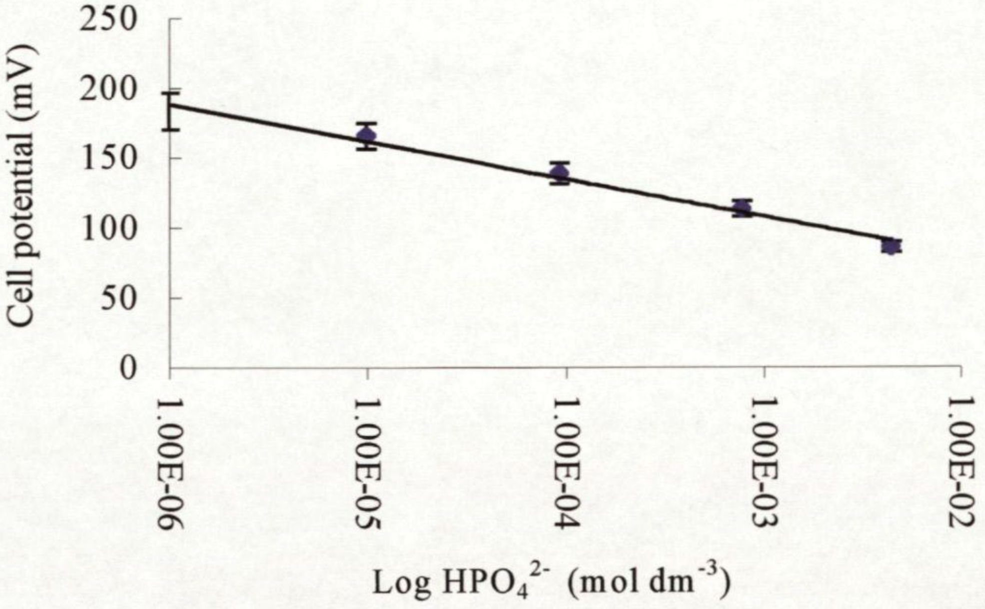


Figure 5.4 shows the response of five phosphate-selective electrodes made with 3-decyl-1,5,8-triazacyclodecane-2,4-dione to the activity of HPO_4^{2-}.

Carey and Riggan reported a linear Nernstian range between 0.1 mol dm^{-3} and 1×10^{-6} mol dm^{-3} HPO_4^{2-} activity , a Nernstian slope of –29 mV per activity decade and a limit of detection of about 1×10^{-6} mol dm^{-3} HPO_4^{2-} activity.

The results obtained in the present study were very similar to those obtained by Carey and Riggan. The phosphate-selective electrodes exhibited a Nernstian response between 5×10^{-3} and 1×10^{-6} mol dm^{-3} HPO_4^{2-} , a Nernstian slope of -27 ± 2 mV per activity decade and a limit of detection of 1×10^{-6} mol dm^{-3} HPO_4^{2-} activity.

5.5.2.2 Lifetime study

Table 5.1 Lifetime of the PVC membrane containing 3-decyl-1,5,8-triazacyclodecane-2,4-dione

Time of conditioning prior to use	Electrochemical parameters	Results
3 hours	Slope (mV/ dec)	-22.0
	Linear range (mol dm^{-3} HPO_4^{2-})	5 x 10^{-3} - 10^{-5}
10 hours	Slope(mV/ dec)	-25.0
	Limit of detection (mol dm^{-3} HPO_4^{2-})	7 x 10^{-6}
	Linear range (mol dm^{-3} HPO_4^{2-})	5 x 10^{-3} - 5 x 10^{-6}
20 hours	Nernstian slope(mV/ dec)	-27.0
	Limit of detection (mol dm^{-3} HPO_4^{2-})	1.0 x 10^{-6}
	Linear range (mol dm^{-3} HPO_4^{2-})	5 x 10^{-3} - 1x 10^{-6}
40 hours	Nernstian lope(mV/ dec)	-26.0
	Limit of detection (mol dm^{-3} HPO_4^{2-})	3.3 x 10^{-6}
	Linear range (mol dm^{-3} HPO_4^{2-})	5 x 10^{-3} - 5 x 10^{-6}
80 hours	Slope(mV/ dec)	-24.0
	Linear range (mol dm^{-3} HPO_4^{2-})	5 x 10^{-3} - 10^{-5}
120 hours	unresponsive	

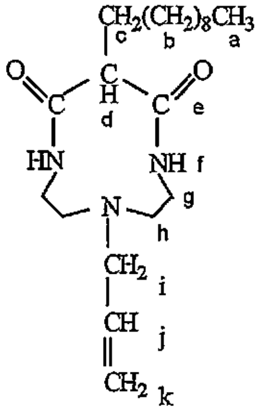
The phosphate ionophore was only trapped in PVC and therefore the lifetime was expected to be short due to the diffusion of the sensor from the membrane. For this experiment the membranes were left soaking in the conditioning solution and calibration of the electrodes were carried out until loss of the phosphate response. Table 5.1 shows that the phosphate response remains Nernstian for about 2 days but is totally lost after 5 days.

5.6 Modified Carey sensor

Having repeated the published work the next step was to attempt to produce a modified sensor, capable of being covalently bound to a polymer in the hope that the performance of the sensor molecule would not be impaired by the binding to the polymer. An obvious accessible place to introduce an allyl group on to the macrocycle was at the N_8 position (in red in section 5.6.1) by allylation of the secondary amino group (Larock, 1980). This was achieved as described in the following section.

5.6.1 Synthesis of 8-allyl-3-decyl-1,5,8-triazacyclodecane-2,4-dione

$C_{20}H_{37}N_3O_2$, MW = 351



3-Decyl-1,5,8-triazacyclodecane-2,4-dione (2.25 mmol, 700 mg) was added to water (15 ml) followed by sodium carbonate (7.875 mmol, 0.84 g), then allyl bromide was added drop-wise to the mixture. The solution was refluxed for 24 hours and a brown precipitate was obtained at the surface of the water. The precipitate was filtered, washed several times with water and identified as 8-allyl-3-decyl-1,5,8-triazacyclodecane-2,4-dione.

Yield : 250mg (20 %), **Rf** : 0.95 (7.3.1 / $CHCl_3$-EtOH-$(Et)_3N$)

IR : $\nu_{(-C=O,\ amide)}$ = 1666 cm^{-1}, $\nu_{(-CH)}$ = 3071, 2994 cm^{-1}, $\nu_{(-CH,\ CH2)}$ = 2922, 2852 cm^{-1}, $\delta_{(-CH,\ CH2)}$ = 1467 cm^{-1}, $\nu_{(-NH)}$ = 3292 cm^{-1} , $\delta_{(-CH,\ vinyl)}$ = 3083.7 cm^{-1} , $\delta_{(-CH\ out\ of\ plane,\ vinyl)}$ = 999.8 cm^{-1}, $\delta_{(-NH,\ amide)}$ = 1559, 722 cm^{-1}.

NMR ^{1}H ($CDCl_3$) : δ (ppm) : 0.87 ($3H_a$), 1.24 ($16H_b$, $2H_c$), 1.82 ($2H_f$), 2.74 ($4H_h$), 2.8 (H_d), 3.05 ($2H_i$), 3.3 (H_g), 5.1 ($2H_k$), 5.8 (H_j).

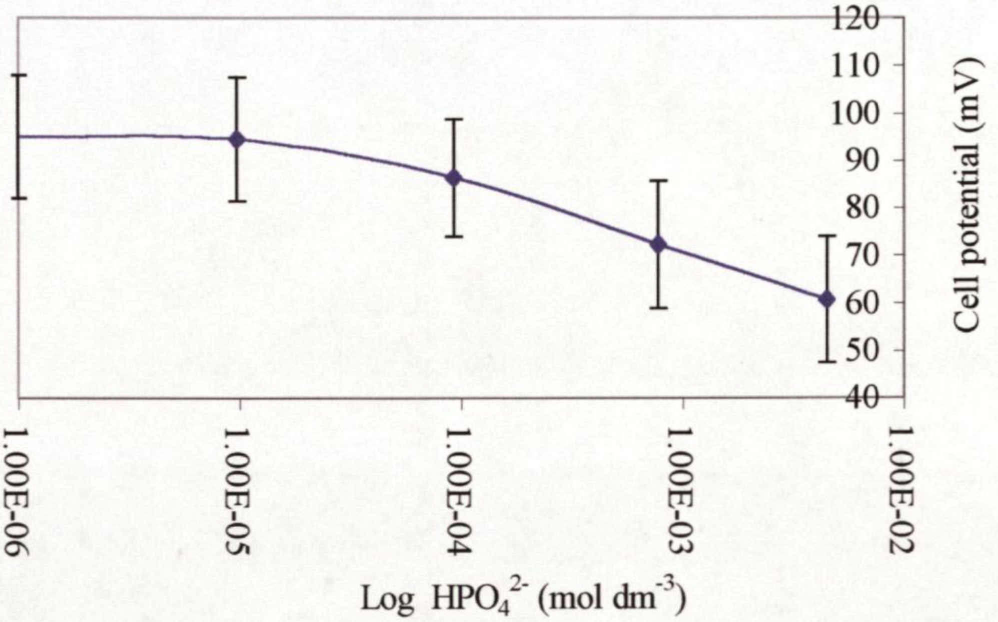
NMR ^{13}C : δ (ppm) : 14.03 (C_a), 22.59, 27.4, 29.3, 30.5, 31.82 (C_b, C_c), 37.091 (C_h), 52.11 (C_g), 47.89 (C_d), 56.11 (C_i), 117.7 (C_k), 135.04 (C_j), 171.2 (C_e).

5.6.2 Electrochemical evaluation of membranes containing 8-allyl-3-decyl-1,5,8-triazacyclodecane-2,4-dione

8-Allyl-3-decyl-1,5,8-triazacyclodecane-2,4-dione was evaluated in a PVC membrane. Membrane composition and fabrication were as described in section 5.4.1. Five membranes were punched from the master membrane and after appropriate conditioning they were evaluated using the same method decribed in section 5.4.3.

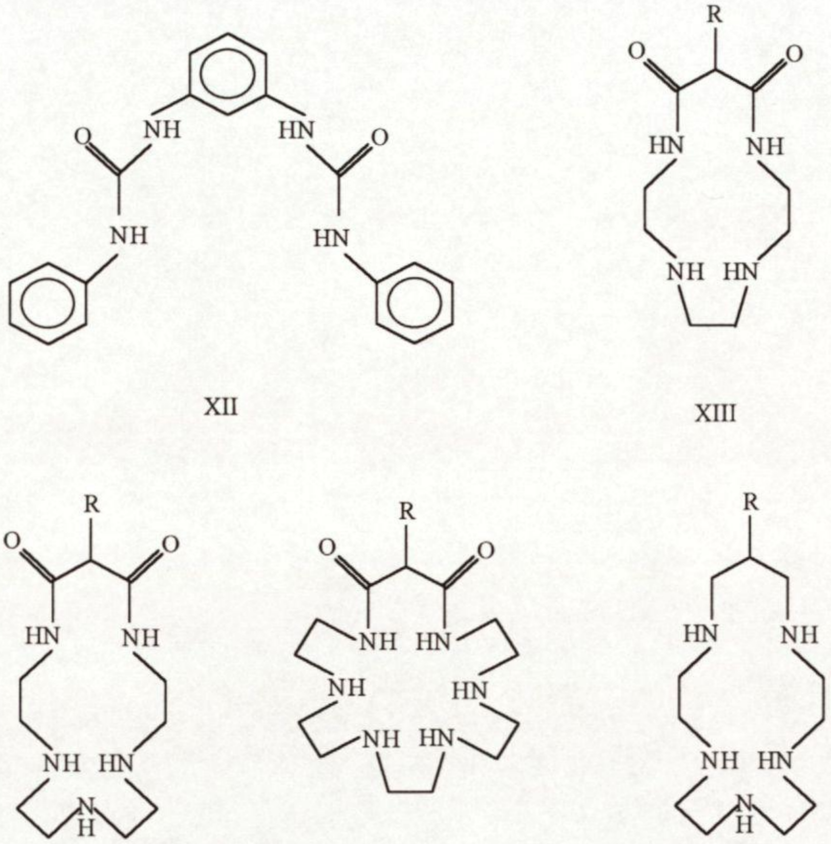
The results were very disappointing, the addition of the allyl group to the amino group in the ring resulted in considerable loss of activity. The average slope was – 12 mV per activity decade over a linear range from 5 x 10^{-3} to 1 x 10^{-4} mol dm^{-3} dibasic phosphate. The calibration graph of this sensor is presented in figure 5.5.

Figure 5.5 **Phosphate response for membranes containing N-allyl-3-decyl-1,5,8-triazacyclodecane-2,4-dione**



The first observation to be made is the importance of the secondary amino group in the phosphate response. When the hydrogen atom on N-8 was replaced by an allyl group, the sensor has a limited activity for phosphate. It is an interesting fact, from the mechanistic standpoint, that this secondary amino group seems to be necessary to get a phosphate response. Carey investigated several different sizes of macrocycle as shown in figure 5.6 (XIII, XIV and XV). the performance of these macrocycle is given in table 5.2.

Figure 5.6 Structure of various phosphate sensors cited in the literature and containing amino groups.


NH
HN
O
O
NH
HN
R
O
O
HN
NH
NH HN
R
O
O
HN
NH
NH HN
N
H
R
O
O
NH HN
NH
HN
NH HN
R
HN
NH
NH HN
N
H

XII XIII

XIV XV XVI

Table 5.2 Electrochemical performance of various phosphate sensors

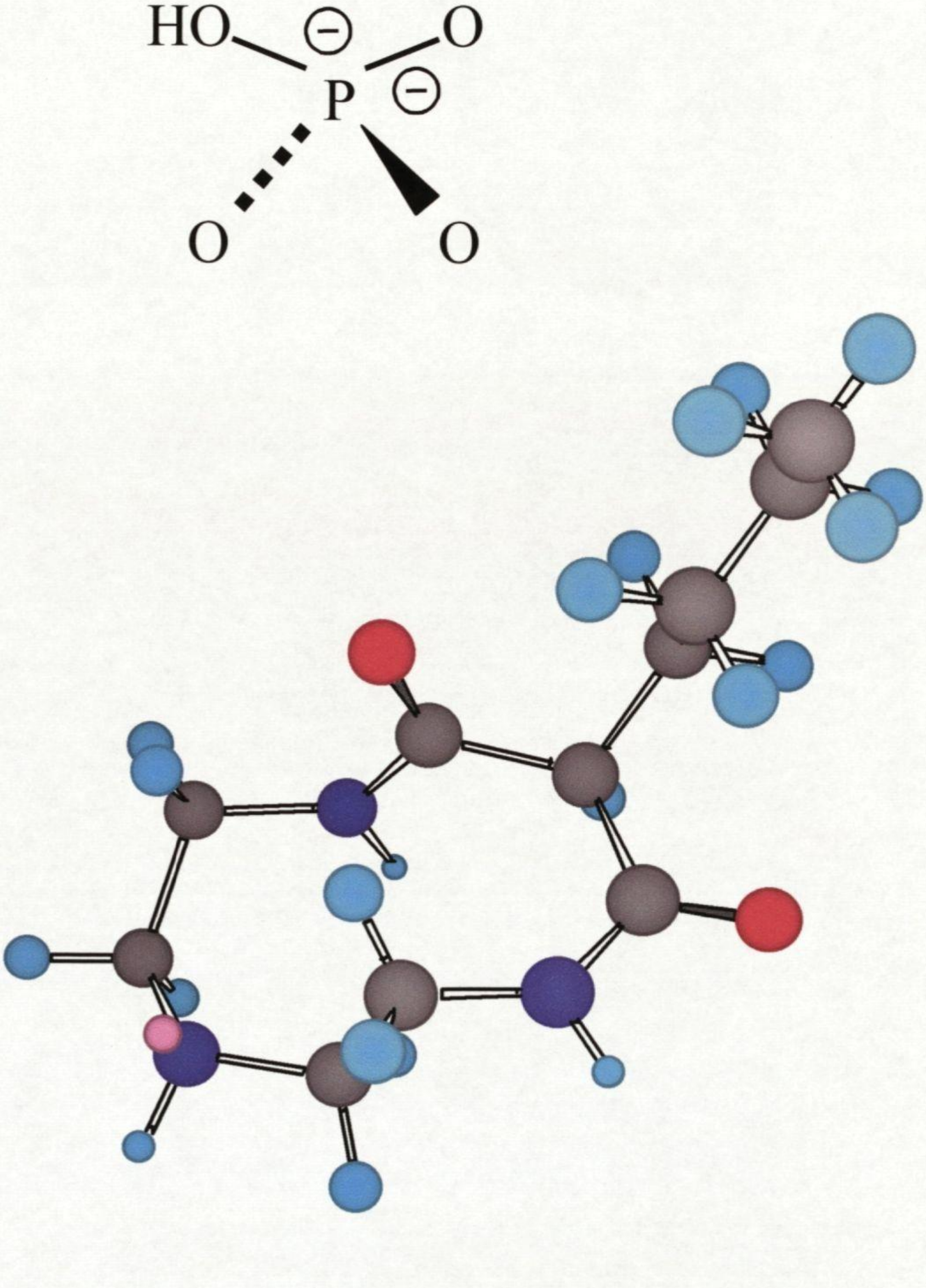
Sensor	Slope (mV dec^{-1})	Linear range (mol dm^{-3})	Reference
XII	unresponsive	unresponsive	Nishizawa *et al.*, 1998
XIII	-24.3	2×10^{-5}-9×10^{-3}	Carey and Riggan, 1994
XIV	-28.1	1×10^{-4}-1×10^{-2}	Carey and Riggan, 1994
XV	-27.4	3×10^{-4}-3×10^{-2}	Carey and Riggan, 1994
XVI	-14.5	1×10^{-4}-1×10^{-3}	Umezawa *et al.*, 1988

Table 5.2 shows the performance of various phosphate sensors and it is interesting to notice that if the size of the macrocycle increases the linear range decreases. Carey and Riggan suggested that the relationship between ring size of the ionophore and the linear range and selectivity for HPO_4^{2-} is evidence that there are steric factors which help the selection for HPO_4^{2-}. The polyamine ring (XVI) developed by Umezawa *et al.* (1988) is very similar to compoud XIV with the only exception of the two carbonyl oxygens. However, XVI gives sub-Nernstian response to HPO_4^{2-} over a very limited linear range showing the importance of these two amido groups in the ring. Carey and Riggan suggested that the amido groups are stabilizing the positively charged center of the macrocyle and direct the phosphate anion in the center of the ring where hydrogen bonds or ionic bonds may be formed between the amine and the charged oxygen atoms of the HPO_4^{2-}.

More recently, a bis-urea ionophore (XII) was developed by Nishizawa *et al.* (1998). This compound is neutral and was unresponsive to HPO_4^{2-} suggesting that the positive charge on the amino group is necessary to get a phosphate response.

In conclusion, the structural configuration of the macrocycle (XI) with two amido groups and the secondary amino group seems to be the best one to get an optimum phosphate response. A three dimensional model of the best macrocycle (XI) is shown in figure 5.7; the tetrahedral structure of HPO_4^{2-} is also presented.

Figure 5.7 Three dimensional model of macrocycle XI (R=C_4H_9 presented with HPO_4^{2-}



● Nitrogen atom, ● Hydrogen atom, ● Lone pair of electrons, ● Carbon atom,

● Oxygen atom

Figure 5.7 suggests that there is probably a ring-size effect of the macrocycle forming a 'cage' where HPO_4^{2-} can be bound. However, more structure-activity relationship investigation is needed.

5.7 Improvement of Carey's synthetic route

The long time required to make Carey macrocycle , 42 days reflux plus purification, prompted an investigation of alternative more rapid syntheses especially those which would readily accommodate one or two covalent attachment points on carbon atom where R group is attached to (structure XI in figure 5.1 p 182) of the macrocycle. The following series of syntheses were then investigated.

5.7.1 Use of sodium methoxide as reaction catalyst

The method used for this reaction is as described by previous workers (De Feoand and Strickler, 1963). Sodium methoxide has been found to catalyse the reaction between aliphatic ethyl esters and aromatic primary amines to form secondary amides. An investigation of its use in the present reaction was undertaken. The reaction was investigated with diethyl α-decyl malonate (IX, R = $C_{10}H_{21}$). A mixture of 10 g (33 mmol) of diethyl α-decyl malonate, 34.33 g (33 mmol) of diethylene triamine, 39.22 g (72.6 mmol) of sodium methoxide (72.6 mmol), and 250 ml of dry toluene was stirred under reflux for 48 hours.

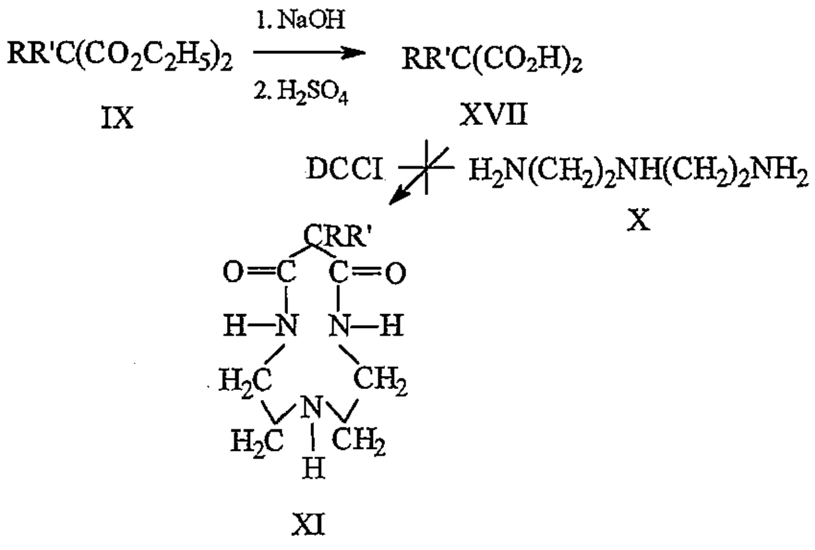
3-Decyl-1,5,8-triazacyclodecane-2,4-dione had been previously synthesised using the Carey synthetic route and it was known that this compound was insoluble in toluene. Therefore a precipitate was expected using the method described above. Unfortunately, no precipitate was obtained. Thin layer chromatography (BAW / I_2) was also used to monitor the reaction mixture and comparison between reference materials including diethylene triamine and 3-decyl-1,5,8-triazacyclodecane-2,4-dione was performed and showed no formation of the target molecule.

5.7.2 Use of dicyclohexylcarbodiimide (DCCI)

The use of DCCI in the formation of amides especially as a peptide coupling agent has been long established (Williams, 1981).

This method was therefore investigated using mono and di-substituted malonic acid (XVII, R = CH_2=CH-CH_2; R'= H or CH_2=CH-CH_2). For this method, diallyl malonic acid (0.7544 g, 4.1 mmol) and diethylenetriamine (0.42g, 0.4 ml) were dissolved in 100 ml of dry pyridine. DCCI (1.87g, 9.1 mmol) dissolved in dry pyridine (10 ml) was then added to the acid and the amine. The reaction mixture was left for 24 hours. No precipitate of N,N-dicyclohexylurea in the pyridine solution was obtained indicating no coupling occured between diethylene triamine and diallyl malonic acid.

Figure 5.8 **Synthetic route using DCCI**



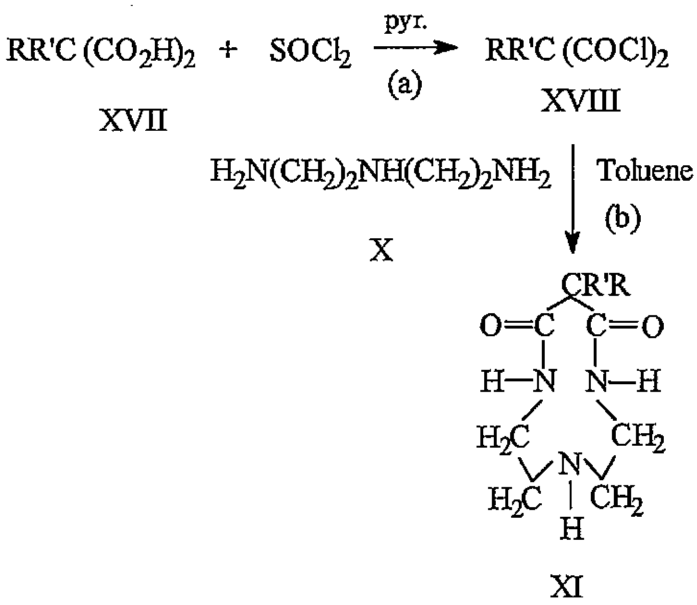
5.7.3 Use of acid halide to form the macrocycle

The reaction of acid halides with amines has been widely used in the formation of amides (Larock, 1999 and Warren, 1982).

The first step of this synthesis was the formation of the acid chloride (XVIII). The first method to be investigated, using an excess of thionyl chloride (Raha, 1963), gave a poor conversion of carboxylic acid to the acid chloride. However, another method (Dauben *et al.*, 1952) using a large excess of thionyl chloride in the presence of pyridine, as shown in figure 5.9 (a) was attempted and gave very good conversion and was used for the following step.

The second step of this reaction, described in figure 5.9 (b) consisted in the amide bond formation and was investigated for the preparation of 1,5,8-triazacyclodecane-2,4-dione (XI, R'= R= H), 3,3-diallyl-1,5,8-triazacyclodecane-2,4-dione (XI, R=R'= CH_2=CH-CH_2) and 3-butenyl-1,5,8-triazacyclodecane-2,4-dione (R=H, R'= CH_2=CH-CH_2-CH_2).

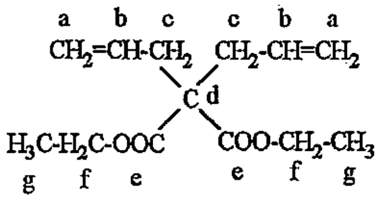
Figure 5.9 Use of acid chloride


RR'C $(CO_2H)_2$ + $SOCl_2$ $\xrightarrow[(a)]{pyr.}$ RR'C $(COCl)_2$
XVII
XVIII
$H_2N(CH_2)_2NH(CH_2)_2NH_2$
X
Toluene
(b)
CR'R
O=C C=O
H—N N—H
H_2C CH_2
N
H_2C CH_2
H
XI

5.7.4 Synthesis of individual compounds

5.7.4.1 Synthesis of diethyl α-diallyl malonate

$C_{13}H_{20}O_4$, MW = 240



The reaction was protected from moisture. Sodium (8.897 g , 0.387 mol) and ethanol (500 ml) were refluxed. When the sodium ethoxide reached 50°C, freshly distilled diethyl malonate (31 g , 0.1935 mol) was added slowly to the reaction mixture. To this, distilled allyl bromide (46.8 g , 0.387 mol) was added drop-wise. The reaction mixture was refluxed for 2 days. Then the remaining ethanol was removed under reduced pressure. The crude product was washed with water and purified using fractional vacuum distillation. The pure diethyl α-diallyl malonate was collected as a colourless oil.

Yield : 20 g (43 %), **bp** : 110°C / 10 mm Hg, **GC** (99%)

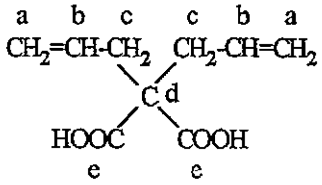
IR : $\nu_{(\text{-C=O})} = 1733\ \text{cm}^{-1}$, $\nu_{(\text{-O-CH2CH3})} = 1036.9\ \text{cm}^{-1}$, $\nu_{(\text{-CH, CH2})} = 2936.5, 2875\ \text{cm}^{-1}$, $\delta_{(\text{-CH , CH2})} = 1442\ \text{cm}^{-1}$, $\nu_{(\text{C=C})} = 3080.4\ \text{cm}^{-1}$, $\nu_{(\text{C=C})} = 1642.3\ \text{cm}^{-1}$, $\delta_{(\text{CH})} = 997.5\ \text{cm}^{-1}$.

NMR ^{1}H ($CDCl_3$): δ (ppm) : 1.25 ($6H_g$, t), 2.64 ($4H_c$, d), 4.18 ($4H_f$, q), 5.10 ($4H_a$, dd), 5.65 ($2H_b$, ddt).

NMR ^{13}C : δ (ppm) : 14.054 (C_g), 36.623 (C_c), 57.126 (C_d), 61.169 (C_f), 119.12 (C_b), 132.219 (C_a), 170.69 (C_e).

5.7.4.2 Synthesis of diallyl malonic acid

$C_9H_{12}O_4$, MW = 183



Potassium hydroxide (4.80 g, 0.085 mol) was dissolved in 10 ml of water and 30 ml of ethanol was added to get a homogeneous solution. To this diethyl α-diallyl malonate (8.4 g , 0.035 mol) was slowly introduced with shaking. The resulting mixture was refluxed for 4 hours; hydrolysis was then complete, i.e. a test portion dissolves completely in excess of water. Ethanol was removed under reduced pressure and the residue was dissolved in 25 ml of water. The solution was cooled at 0°C in a conical flask surrounded by ice; 10% sulphuric acid was slowly added to it, whilst stirring vigorously, until the solution was acid. The solution was extracted with three 75 ml portions of diethyl ether; the portions were combined and dried with magnesium sulphate. The mixture was filtered and evaporated. The solid residue was recrystallised from hot toluene to yield pure diallyl malonic acid as needle-shaped white crystals.

Yield : 5.36 g (71 %), **mp**: 134-135°C, **Rf** : 0.76 (BAW).

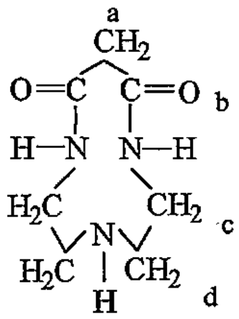
IR : $\nu_{(-C=O)} = 1703\ cm^{-1}$, $\nu_{(-CH,\ CH2)} = 2930.5,\ 2875\ cm^{-1}$, $\delta_{(-CH\ ,\ CH2)} = 1453\ cm^{-1}$, $\nu_{(C=C)} = 3085\ cm^{-1}$, $\nu_{(C=C)} = 1645\ cm^{-1}$, $\delta_{(CH)} = 1001\ cm^{-1}$.

NMR ^{1}H (D_2O): δ (ppm) : 2.37 ($4H_c$, d), 4.96 ($4H_a$, dd), 5.58 ($2H_b$, ddt).

NMR ^{13}C : δ (ppm) : 37.51 (C_c), 58.66 (C_d), 119.95 (C_a), 132.55 (C_b), 175.80 (C_e).

5.7.4.3 Synthesis of 1,5,8-triazacyclodecane-2,4-dione

$C_7H_{11}O_2N_2$, MW = 155



This reaction was performed, as a preliminary test, to check if the acid halide route was a possible candidate for the formation of 3-alkenyl-1,5,8-triazacyclodecane-2,4-dione. A solution of diethylene triamine (10.317g, 0.1 mol) in 100 ml of dry toluene was prepared and poured into a 250 ml round-bottom flask fitted with a double surface condenser and a 50 ml dropping funnel (both fitted with guard tubes containing calcium chloride). Malonyl dichloride (7.0475g, 0.05 mol) was dissolved in 25 ml and carefully poured into the dropping funnel and added drop-wise to the solution of diethylene triamine. An orange precipitate was formed instantaneously. The reaction was stirred for 48 hours at room temperature. The solution was filtered and a yellow solid was isolated. Comparison between spectrosopic analysis of 3-decyl-1,5,8-triazacyclodecane-2,4-dione and the solid was promising and showed the disappearance of the acid chloride.

mp : 101°C

IR : $\nu_{(\text{-C=O, amide})}$ = 1671 cm^{-1}, $\delta_{(\text{-CH2})}$ = 1478 cm^{-1}, $\nu_{(\text{-NH})}$ = 3269 cm^{-1}, $\delta_{(\text{-NH, amide})}$ = 1566 cm^{-1}.

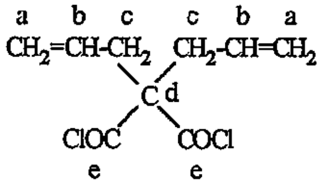
NMR ^{1}H (D$_2$O): δ (ppm) : 3.25 (2 H$_a$), 2.77 (4H$_d$), 2.92 (4H$_c$).

NMR ^{13}C: δ (ppm) : 170.385 (C_b), 47.607 (C_a), 46.362 (C_c), 39.462 (C_d).

This reaction was very fast (48 hours) and made the acid chloride route 5.7.3 look promising for the synthesis mono- or di-substituted-1,5,8-triazacyclodecane-2,4-dione (XI).

5.7.4.4 Synthesis of diallyl malonyl chloride

$C_9H_{10}O_2Cl_2$, MW = 221



Diallyl malonic acid (1.472 g, 8 mmol) was placed into 25 ml conical flask, thionyl chloride (7.5ml, 100 mmol) was slowly added to the carboxylic acid followed by 50µl of pyridine. The reaction was very vigourous after the addition of the thionyl chloride. The mixture was heated at 40°C for 3 days. The excess of thionyl chloride then was removed under reduce pressure to yield diallyl malonyl chloride as a pale yellow oil. Shorter times of reaction did not give full conversion of carboxylic acid to acid chloride.

Yield : 1.4 g (78 %).

IR : $\nu_{(Cl\text{-}C=O)}$ = 1794.1 cm^{-1}, $\nu_{(\text{-}CH,\ CH2)}$ = 2930.5, 2875 cm^{-1}, $\delta_{(\text{-}CH,\ CH2)}$ = 1440 cm^{-1}, $\nu_{(C=C)}$ = 3083.8 cm^{-1}, $\nu_{(C=C)}$ = 1642.2 cm^{-1}, $\delta_{(CH)}$ = 995.1 cm^{-1}.

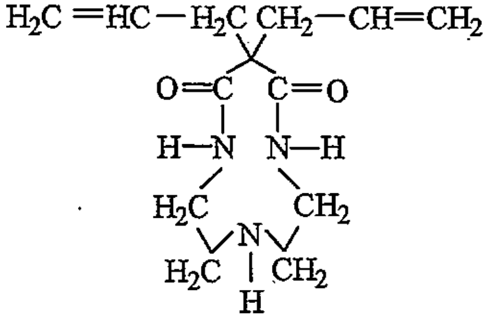
NMR ^{1}H ($CDCl_3$): δ (ppm) : 2.86 ($4H_c$, d), 5.30 ($4H_a$, dd), 5.62 ($2H_b$, ddt).

NMR ^{13}C : δ (ppm) : 36.31 (C_c), 56.33 (C_d), 122.049 (C_a), 128.895 (C_b), 169.86 (C_e).

The acid chloride was used, immediatelly, in the next step of the reaction.

5.7.4.5 Synthesis of 3,3-diallyl-1,5,8-triazacyclodecane-2,4-dione

$C_{13}H_{21}O_2N_3$, MW = 251



The synthesis of 3,3-diallyl-1,5,8-triazacyclodecane-2,4-dione was attempted using the 4 synthetic routes respectively described in sections 5.3.1 (esters), 5.7.1 (sodium methoxide), 5.7.2 (DCCI) and 5.7.3 (acid chloride). The first three failed completely and did not yield any product. The last one was performed many times according to the method described in section 5.7.4.3 and under different conditions. This reaction was performed in higher dilution (5, 10 and 15 mmol dm^{-3} of starting materials in toluene), with a base (sodium carbonate and triethylamine) and under nitrogen atmosphere to avoid oxidation of the acid chloride. Each time the reaction produced a mixture of several compounds, in very low yields, possibly containing the target molecule. The separation of these compounds using flash chromatography was considered to be too difficult in view of small amount of product likely to be obtained.

Previous work (Russel, 1950) showed that the conversion of disubstituted malonic esters to malondiamides was very difficult compared to monosubstituted malonic esters. Therefore the problems encountered, using the different synthetic routes, were may be due the use of 3,3-diallyl-1,5,8-triazacyclodecane-2,4-dione. The synthesis of 3-monosubstituted-1,5,8-triazacyclodecane-2,4-dione appeared to be the solution to this problem.

5.7.4.6 Diethyl α-butenyl malonate

$C_{11}H_{18}O_4$, MW = 214

$$\underset{h}{CH_3}\underset{g}{CH_2}\underset{f}{O_2C}\text{-}\underset{e}{CH}(\underset{d}{CH_2}\underset{c}{CH_2}\underset{b}{CH}{=}\underset{a}{CH_2})\underset{f}{CO_2}\underset{g}{CH_2}\underset{h}{CH_3}$$

The reaction was carried out as described in section 5.3.2.1. Pure diethyl α-butenyl malonate was obtained as a colourless oil.

Yield : 40 %, **bp** : 112°C / 6mm Hg, **Rf** : 0.9 (BAW).

IR : $\nu_{(\text{-C=O})}$ = 1734.3 cm^{-1}, $\nu_{(\text{-O-CH2CH3})}$ = 1033.6 cm^{-1}, $\delta_{(\text{C=C})}$ = 3077.9 cm^{-1}, $\nu_{(\text{-CH, CH2})}$ = 2938.5, 2857 cm^{-1}, $\delta_{(\text{-CH , CH2})}$ = 1448.7 cm^{-1}, $\nu_{(\text{C=C})}$ = 1641.5 cm^{-1}.

NMR ^{1}H ($CDCl_3$): δ (ppm) : 1.28 ($6H_h$, t), 2.0 ($2H_c$, $2H_d$, m), 3.38 (H_e, t), 4.18 ($4H_g$, q), 5.0 ($2H_a$, dd), 5.76 (H_b, ddt).

NMR ^{13}C : δ (ppm) : 14.01(C_h), 27.78 (C_d), 31.25 (C_c), 51.14 (C_e), 61.25 (C_g), 115.9 (C_a), 136.3 (C_b), 169.38 (C_f).

5.7.4.7 Synthesis of α-butenyl malonic acid

$C_7H_{10}O_4$, MW = 158

$HO_2C\text{-}CH(CH_2CH_2CH{=}CH_2)CO_2H$

f e d c b a f

The reaction was carried out using the method described in section 5.7.4.2. Pure α-butenyl malonic acid was obtained as needle-shaped white crystals.

Yield : 82 %, **mp** : 84 °C , **Rf** : 0.72 (EA).

IR : $\nu_{(-C=O)}$ = 1715.3 cm^{-1}, $\delta_{(C=C)}$ = 3077.9 cm^{-1}, $\nu_{(OH\ bonded)}$ = 2662.4, 2613.6 cm^{-1}, $\nu_{(-CH,\ CH2)}$ = 2938.5, 2857 cm^{-1}, $\delta_{(-CH\ ,\ CH2)}$ = 1417.7 cm^{-1}, $\nu_{(C=C)}$ = 1641.1 cm^{-1}.

NMR ^{1}H ($CDCl_3$): δ (ppm) : 2.0 (2H_c, 2H_d, m), 3.36 (H_e, t), 4.95 (2H_a, dd), 5.68 (H_b, ddt), 9.71 (H_f, s).

NMR ^{13}C : δ (ppm) : 14.01(C_h), 27.62 (C_d), 31.07 (C_c), 50.69 (C_e), 61.25 (C_g), 116.56 (C_a), 136.26 (C_b), 175.0 (C_f).

5.7.4.8 Synthesis of α-butenyl malonyl chloride

$C_7H_{10}O_2Cl_2$, MW = 195

$ClOC\text{-}CH(CH_2CH_2CH{=}CH_2)COCl$

f e d c b a f

The synthesis of α-butenyl malonyl chloride was performed using the method described in section 5.7.4.4 and was obtained as a pale yellow oil. The completeness of the reaction was monitored using ^{1}H NMR analysis showing the disapearance of the proton absorption due to the OH of the carboxylic acid.

Yield : 35 %.

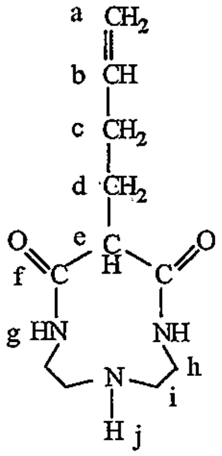
IR : $\nu_{(Cl\text{-}C=O)}$ = 1787.6 cm^{-1}, $\delta_{(C=C)}$ = 3081.7 cm^{-1}, $\nu_{(\text{-}CH,\ CH2)}$ = 2932.1, 2864 cm^{-1}, $\delta_{(\text{-}CH\ ,\ CH2)}$ = 1448.2 cm^{-1}, $\nu_{(C=C)}$ = 1643.5 cm^{-1}.

NMR ^{1}H (CDCl$_3$): δ (ppm) : 2.19 (2H$_c$, 2H$_d$, m), 4.22 (H$_e$, t), 5.09 (2H$_a$, dd), 5.69 (H$_b$, ddt).

NMR ^{13}C : δ (ppm) : , 28.57 (C$_d$), 30.20 (C$_c$), 71.37 (C$_e$), 117.73 (C$_a$), 134.93 (C$_b$), 167.65 (C$_f$).

5.7.4.9 Synthesis of 3-butenyl-1,5,8-triazacyclodecane-2,4-dione

$C_{11}H_{19}N_3O_2$, MW = 225



This reaction was carried out using the method described in section 5.7.4.3. 3-Butenyl-1,5,8-triazacyclodecane-2,4-dione was obtained as a waxy yellow solid.

Yield : 10 %, **mp** : 85-95°C

IR : $\nu_{(\text{-C=O, amide})} = 1671.7$ cm^{-1}, $\nu_{(\text{-CH})} = 3071, 2994$ cm^{-1}, $\nu_{(\text{-CH, CH2})} = 2933, 2854.8$ cm^{-1}, $\delta_{(\text{-CH, CH2})} = 1458$ cm^{-1}, $\nu_{(\text{-NH})} = 3269$ cm^{-1}, $\delta_{(\text{-NH, amide})} = 1558.5$ cm^{-1}, $\nu_{(\text{C=C})} = 3081.7$ cm^{-1}, $\nu_{(\text{-CH, CH2})} = 2932.1, 2864$, $\delta_{(\text{-CH, CH2})} = 1457.7$ cm^{-1}.

NMR ^{1}H (D_2O): δ (ppm) : 1.803 (2H$_c$, 2H$_d$, m), 3.23 (4H$_h$, 4H$_i$, H$_e$, m), 4.90 (2H$_a$, dd), 5.70 (H$_b$, ddt).

NMR ^{13}C : δ (ppm) : 28.83 (C$_c$), 31.42 (C$_d$), 36.16 (C$_i$), 45.07 (C$_h$), 48.4 (C$_e$), 115.98 (C$_a$), 138.39 (C$_b$), 177.2 (C$_f$).

5.8 Conclusion

The work done by Carey and Riggan has been duplicated successfully. The mechanism of action of the heterocyclic macrocycle (XI) is not fully understood and requires more study. It has been shown that the synthesis of 3-butenyl-1,5,8-triazacyclodecane-2,4-dione (section 5.7.4.9) was possible offering the prospect of a covalent attachment of the heterocyclic macrocycle to a polymeric material such as polystyrene-*block*-polybutadiene-*block*-polystyrene. Unfortunately, due to a lack of time this step has not been finished but will be a part of a future project by the University of Plymouth and the Environment Agency (R & D programme).

CHAPTER 6

CONCLUSIONS

6.1 Summary

The aim of this study was the development of a device for the determination of nitrate and phosphate in natural waters. In the water industry, air segmented continuous flow analysers for the determination of nitrate are commonly used. Such devices are costly to buy and to run (technical staff and chemicals required). Potentiometric sensors appeared to be an interesting alemative method of analysis. However, ion-selective electrodes based on PVC membranes containing trapped sensors suffered from drift and require regular re-calibration. To overcome this problem the sensor has to be immobilised in the matrix. In this study, several N,N,N-triallyl α-amino-acid betaine salts have been synthesised and successfully immobilised into rubbery membranes. The best performing nitrate-selective electrode contained N,N,N-triallyl leucine betaine chloride ($(CH_2{=}CH{-}CH_2)_3N^+CH(CH_2CH(CH_3)_2)CO_2H,\ Cl^-$) covalently bound to polystyrene-*block*-polybutadiene-*block*-polystyrene (SBS) with 2-nitrophenyloctyl ether (2-NPOE) as solvent mediator. This membrane after 48 hours conditioning in 1 x 10^{-1} mol dm^{-1} nitrate performed very well in the laboratory with a Nernstian slope of -59.1 mV per decade over a linear range of 1 x 10^{-1}-5 x 10^{-6} mol dm^{-3} nitrate, a limit of detection of 0.34 μmol dm^{-3} nitrate and a selectivity coefficient for nitrate against chloride ($k^{pot}_{NO_3^-,\ Cl^-}$) of 3.4 x 10^{-3}. These characteristics are better than those obtained usually with commercial electrodes (Phillips or Elite used throughout the study). A typical limit of detection for such nitrate sensors is 7 μmol dm^{-3} with a linear response over a range from 0.1 to 1 x 10^{-5} mol dm^{-3} nitrate and a$k^{pot}_{NO3-,\ Cl-}$ of 5.5 x 10^{-3}. The other advantage of this immobilised sensor was the long lifetime achieved in the laboratory (more than 5 months in constant use). The speed of response was also satisfactory with less than 1 minute over the linear range and the pH range (2-8) is sufficient for the monitoring of most natural waters. The reproducibility of the membranes punched from the same master membrane was excellent, the RSD of the potential recorded at the limit of detection was 1% (n=5) and the reproducibility of the master membrane fabrication was also good with a RSD of the potential recorded at 1 μmol dm^{-3} nitrate of less than 2% (n=12).

A long field evaluation (over 2 months in river water and over 5 months in agricultural drainage water) with continuous immersion was undertaken. This kind of experiment using nitrate-selective electrodes has never been reported in the literature before and represent a novelty for nitrate monitoring. The nitrate levels were recorded every hour using a datalogger, and the system was able to be left unattended for 2 weeks without any maintenance or re-calibration. The validation of the results obtained by the nitrate-selective electrodes was carried out using a segmented-flow nitrate analyser and showed excellent correlation between the two techniques ($R^2 = 0.99$) over a large range from 0.47 to 16 ppm nitrate-N with no systematic errors. These characteristics exceeded those of any known commercial nitrate sensors. To conclude the nitrate-selective electrode developed during this work offered stability, sensitivity and selectivity and is currently being commercialised.

A preliminary investigation of a phosphate ionophore based upon a heterocyclic macrocycle was also undertaken. This ionophore had a linear Nernstian range from 5 x

10^{-3} to 1×10^{-6} HPO_4^{2-} with a limit of detection of 1×10^{-6} HPO_4^{2-}. However, this ionophore was only trapped and therefore had a short lifetime due to leaching of the sensor from the membrane. Preliminary studies showed the importance of the secondary amino group in the phosphate response. When the hydrogen atom on N-8 (XI, p182) was replaced by an allyl group (necessary to the covalent attachment), the performance of the electrode dropped dramatically.

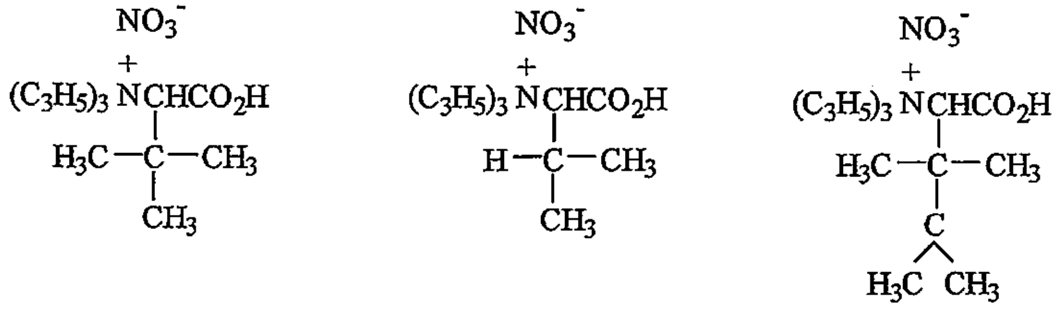
6.2 Further work

6.2.1 Nitrate front

The best nitrate sensor is based on N,N,N-triallyl leucine betaine. The synthesis of this is achieved using a method based on previous literature (Chen and Benoiton, 1976). This method has been extensively used in the formation of betaine compounds and proved to be a reliable synthetic route. However, the yield of reaction for leucine betaine remains relatively low (28%). This is probably due to the low solubility of the α-aminoacid in the solvent and some competition reactions forming by-products, such as allyl esters, which have been found to be relatively poor sensor molecules. In order to improve the yield of reaction, two different approaches could be investigated. The first is to optimise the paramaters influencing the formation of product and by-products, such as temperature and possibly solvent and base, using the Chen and Benoiton method. The second one is to use a different method. Some different synthetic routes to form betaine compounds have been identified in the literature, such as the synthesis of N,N-diallyl α-aminoacid as precursor and its quaternisation to form N,N,N-triallyl α-aminoacid betaine using an established method (Frampton, 1992) and could be alternative ways of making betaine compounds.

Different N,N,N-triallyl α-amino-acid betaine salts should be synthesised such as those showed in figure 6.1 in order to investigate the influence of the substituents on the β carbon.

Figure 6.1 Proposed N,N,N-triallyl α-amino-acid betaine salts to be synthesised and evaluated



The composition of SBS membranes is based upon four components (see 6.1 for full details) dissolved in THF. Alternative methods of making these membranes should be investigated to reduce the use of THF because of its toxicity. SBS is a tri-block polymer and is supplied in chunks which is not easy to mix with the other components of the membrane at room temperature without the use of a solvent. Both low temperature and high temperature approaches could be used to ease its incorporation in the membrane composition with milling. Polymer companies use fillers such as clay in the fabrication of various polymeric materials. The clay could be used to ease the incorporation of the solvent mediator (oil) to the polymer in a mixer for example. The alternative procedure based on a low temperature ball mill should be investigated. Nitrate forms of betaines decompose with heat and alternative cross-linking methods should be be investigated.

Multiparameter probes for *in-situ* deployement are commercially available for monitoring physical and chemical parameters such as temperature, pH, turbidity, dissolved oxygen or a variety of cations and anions (Yellow Spring Instrument, 1996). Such multiparameter probe could be converted to accept our membrane.

Research using nitrate-selective microelectrodes could be an interesting area to investigate. At the moment intracellular nitrate concentrations are measured using ion-selective microelectrodes based upon hydrophobic quaternary ammonium salts trapped in PVC membranes (Zhen *et al.*, 1992). Unfortunately, such sensor suffered from a lack of sensitivity (limit of detection = 20 μmol dm^{-3} nitrate) and N,N,N-triallyl leucine betaine seems to be an ideal candidate for being used in such electrode arrangement to overcone this lack of sensitivity.

6.2.2 Phosphate front

The plan for the future research should be to modify the structure of this ionophore (XI p182) to enable the covalent attachment of the ionophore to the polymer chain of the membrane by cross-linking. Preliminary structural work showed that modifications should be orientated on the side chain outside the ring of the ionophore. One approach would be to replace the decyl side chain, used by Carey and Riggan, by one allyl group and to cross-link onto an SBS polymer in a similar way to that used for our nitrate sensors.

Alternatively, another ionophore has been identified in the literature. A study by Chaniotakis led to the development of a phosphate ISE based on a multidentate-tin(IV) carrier incorporated into liquid polymeric membrane (Chaniotakis *et al.*, 1993). Three tin compounds were examined and the best performing one was tris(3-chlorodimethylstannyl-propyl) chlorostannane. The membrane composition was 32% m/m PVC, 66% m/m dioctyl sebacate and 2% m/m sensor molecule. This electrode exhibited a linear response to $H_2PO_4^-$ over a range between 10^{-2} and 10^{-4} mol dm^{-3} with a sub-Nernstian slope of - 40 mV per decade and a limit of detection of 1 x 10^{-5} mol dm^{-3}. This appears to be less promissing that Careys' ionophore in term of limit of detection but nevertheless the synthesis of the sensor appears to be easier suggesting it should be investigated. Unsaturation could be incorporated onto the hydrocarbon groups allowing covalent attachment to the polymer membrane.

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