

# High-Performance Nitrate-Selective Electrodes Containing Immobilized Amino Acid Betaines as Sensors

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**Ten nitrate-selective electrodes based upon rubbery membranes containing various betaine salts as sensors covalently bound to a cross-linked polystyrene-*block*-polybutadiene-*block*-polystyrene (SBS) polymer have been produced. The membranes were robust, highly selective, and effective over a pH range of 2–8. The best nitrate-selective electrode fabricated contained 10% m/m di-cumyl peroxide, 40% 2-nitrophenyloctyl ether, 6.5% triallyl leucine betaine chloride and 43.5% SBS. The characteristics of this electrode were a linear Nernstian range of  $1 \times 10^{-1}$  to  $5 \times 10^{-6}$  mol dm<sup>-3</sup> NO<sub>3</sub><sup>-</sup>, a limit of detection of  $3.4 \times 10^{-7}$  mol dm<sup>-3</sup> NO<sub>3</sub><sup>-</sup>, and a selectivity coefficient for nitrate against chloride,  $K_{\text{NO}_3, \text{Cl}^-}^{\text{pot}}$ , of  $3.4 \times 10^{-3}$ . These figures represent a significant improvement on current commercial nitrate sensors. Response times were 1 min or less; stability of response and electrode lifetime in continuous use was also very satisfactory.**

Numerous nitrate potentiometric sensors have been reported in the literature. Most of them are quaternary ammonium salts,<sup>1–3</sup> quaternary phosphonium salts,<sup>4,5</sup> or nickel(II) complexes.<sup>6,7</sup> They exhibit acceptable performance under laboratory conditions. The best nitrate-selective electrode reported<sup>2</sup> had a slope of  $-57$  mV dec<sup>-1</sup> over a linear range from  $10^{-2}$  to  $5 \times 10^{-5}$  mol dm<sup>-3</sup> nitrate with a limit of detection of  $1.5 \mu\text{mol dm}^{-3}$  nitrate. Stability remains, however, the main problem with the need for regular recalibration in monitoring situations. The electrode described here is, however, suitable for long term, in situ, nitrate monitoring.

Most commercially available nitrate-selective electrodes are based upon a membrane having a quaternary ammonium salt sensor trapped in an inert polymer matrix such as poly(vinyl chloride). Trapped sensor molecules can diffuse out of the polymer matrix, a problem that reduces the lifetime of the electrode so that they are unsuitable for environmental use.

A variation on this principle has involved immobilizing the quaternary ammonium salt sensor by covalent attachment to a polymer.<sup>8,9</sup> The value of covalent sensor attachment has recently been demonstrated by the development of a nitrate-selective electrode suitable for environmental measurements in nonsaline waters (limit of detection  $8.8 \times 10^{-6}$  mol dm<sup>-3</sup> nitrate).<sup>10</sup>

In all the above cases, the quaternary ammonium salt sensor molecules have been very hydrophobic in character as a result of the presence of long ( $>C_{10}$ ) *n*-alkyl chains. An exception to this apparently necessary condition of sensor hydrophobicity was indicated in a recent communication,<sup>11</sup> which showed that a very promising nitrate-selective electrode could be obtained by trapping a glycine betaine salt ( $\text{CH}_3)_3\text{N} + \text{CH}_2\text{COOH,Cl}^-$ ) in a polymer matrix. As expected, the electrode had a short lifetime as a result of the loss of sensor by diffusion. It was thought that the use of amino acid betaines as possible sensor molecules in nitrate electrodes suitable for environmental determinations should be investigated. The present communication will present the results obtained from electrodes containing membranes having a variety of betaine molecules covalently bound to the polymer matrix and the influence of structural variation in the betaine on electrode performance.

## EXPERIMENTAL SECTION

**Chemical Reagents.** All of the chemicals required for organic synthesis and the polymers polystyrene-*block*-polybutadiene-*block*-polystyrene containing phenolic-based antioxidant (SBSa), polystyrene-*block*-polybutadiene-*block*-polystyrene with low content of this antioxidant (SBS) and poly(acrylonitrile butadiene) copolymer (Krynac 50.75) were obtained from Aldrich (Gillingham, Dorset, U.K.), unless indicated otherwise. Purity of chemicals was routinely checked by chromatography (GC or TLC) or nuclear magnetic resonance spectroscopy. Solvents were of HPLC grade and were obtained from Rathburn Chemicals Ltd. (Walkerburn, Peebleshire, Scotland). Tetrahydrofuran (THF) was refluxed over potassium metal and freshly distilled prior to use. SBS was purified by dissolving 15 g in 75 mL of THF and reprecipitating it in 200 mL of cold methanol.

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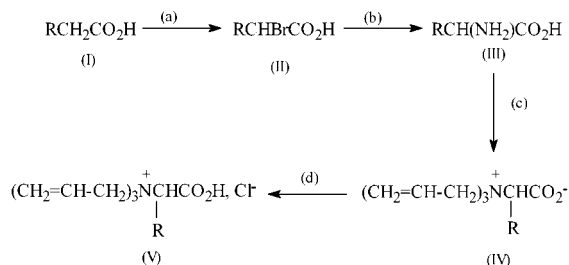
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Dicumyl peroxide (DCP) and the solvent mediator 2-nitrophenyloctyl ether (2-NPOE) (Selectophore grade, Fluka, Glossop, Derbyshire, U.K.) were used as received. Results were compared with those obtained by commercially available nitrate-selective electrodes using either an IS560 nitrate-selective electrode (Philips Analytical, Cambridge, U.K.) containing a PVC supported liquid membrane or an Elit 021 nitrate-selective electrode (BPS, Nico, supplied by Fluka Ltd., Gillingham, Dorset, U.K.) containing a solid-state membrane.

**Synthesis of Betaine Salts.** *N,N,N*-triallyl  $\alpha$ -amino acid betaine salts (V) were synthesized by the following route:



[a, Br<sub>2</sub>, PCl<sub>3</sub> (Ref 12); b, NH<sub>3</sub> (Ref 13); c, C<sub>3</sub>H<sub>5</sub>Br, MeOH, KHCO<sub>3</sub>, or Na<sub>2</sub>CO<sub>3</sub> (Ref 14); d, HCl].

The number of steps taken to synthesize any individual betaine varied according to the commercial availability of appropriate starting materials I, II, or III. All synthesized compounds were examined for purity by TLC, following purification by flash chromatography on silica gel (particle size 40–63  $\mu\text{m}$ ) (BDH, silica gel, Poole, Dorset, U.K.). Compounds were fully characterized by IR and NMR (<sup>1</sup>H, <sup>13</sup>C, CDEPT, and COSY) spectroscopy.

**Membrane Fabrication.** Purified SBS (0.565 g, 43.5% m/m) was dissolved in freshly distilled THF (6 mL). Free-radical initiator, DCP (0.13 g, 10% m/m) and solvent mediator, 2-NPOE (0.52 g, 40%) were added, followed by the sensor molecule (0.085 g, 6.5% m/m). The suspension was then shaken until dissolution was complete. The solvent was then evaporated by drying to constant weight in a vacuum oven at room temperature (5 days) over phosphorus pentoxide.

Solvent-free membrane lyophilisates were placed between two sheets of biaxially orientated film (Melanex film, ICI Films Division, Dumfries, U.K.) and compressed at 220 kN pressure for 7 min at 150  $\pm$  3  $^\circ\text{C}$  using an industrial press (BYTEC Ltd., London, U.K.) and a steel die to produce cross-linked membranes 0.3 mm thick. The pressure was increased steadily for the first minute to expel any air pockets. The die temperature was closely monitored using a thermocouple (Conway microprocessor thermometer, Conway Ltd., U.K.). The hot pressing process was used to ensure covalent binding of the sensor moiety into a cross-linked copolymer.

**Membrane Evaluation.** Disks of 7 mm diameter were punched from hot pressed master membranes and conditioned in 0.1 mol dm<sup>-3</sup> potassium nitrate solution. Membranes fabricated using triallyl  $\alpha$ -amino acid betaine chlorides required approximately 48 h to replace the chloride by nitrate. The conditioned membrane was assembled into the tip of a commercially available electrode body IS560 (Philips Analytical, Cambridge, U.K.) having an inner filling solution composed of 0.01 mol dm<sup>-3</sup> potassium nitrate and potassium chloride solutions (1 + 1). The electro-

chemical cell was completed by a double-junction reference electrode (model 90-02, Orion Research, Cambridge, MA) filled with 0.04 mol dm<sup>-3</sup> ammonium sulfate as the outer filling solution. The EMF measurements were made using a high-impedance voltmeter (HI 931402 Microprocessor, Hanna Instruments Ltd., Beds, U.K.) interfaced to a high-impedance custom-built connected preamplifier.<sup>15</sup> Potassium nitrate standards were prepared using Milli-Q water (Milli-Q, Millipore (U.K.) Ltd., Watford, U.K.) and contained 0.01 mol dm<sup>-3</sup> potassium dihydrogen orthophosphate solution as ionic strength adjustment buffer. The EMF measurements were made at 25  $\pm$  0.5  $^\circ\text{C}$  in stirred solutions.

The slope of the calibration curve is a linear function of the logarithm of the nitrate ion concentration and was measured as the gradient of the observed linear range. The range of linear response was measured over the region of the calibration curve exhibiting a Nernstian slope.<sup>16</sup>

The limit of detection, LOD, was measured from the experimental data as the point of the intersection between a linear extrapolation of the Nernstian slope, and the horizontal part of the upper curve where the EMF is a constant value.<sup>16</sup>

Selectivity coefficients ( $K_{\text{NO}_3^-, \text{Cl}^-}^{\text{pot}}$ ) were determined according to IUPAC recommendations<sup>17,18</sup> using the fixed interferent method (FIM) with a constant level of interfering anion, generally 0.01 mol dm<sup>-3</sup> unless stated otherwise. The 18 mV method,<sup>19</sup> in which the two linear portions of the calibration curve in the presence of an interfering agent are extrapolated and the point of intersection should be 18 mV above the measured potential, was also used for comparison purposes.

pH dependency experiments were carried out on blank cross-linked membranes fabricated from 50% m/m polymeric material, 43% m/m 2-NPOE, and 7% DCP and on membranes containing the sensor molecules. The pH was adjusted using 0.05 mol dm<sup>-3</sup> citric acid/trisodium citrate/sodium hydroxide buffer solutions containing 1 mmol dm<sup>-3</sup> potassium nitrate. The pH was monitored with a pH electrode (Gelplas, General Purpose Combination, BDH, Lutterworth, U.K.) and a high-impedance pH meter (model 290, Pye Unicam, Cambridge, U.K.). Three polymeric materials were examined: SBSa containing a phenolic-based antioxidant, SBS with a low content of phenolic-based antioxidant and poly-(acrylonitrile butadiene)copolymer (Krynac 50.75).

Stability of the response was determined using an autologger (CR10X, Campbell Scientific, Shepshed, Leics., U.K.). Potentials were recorded automatically every 30 min in 1 mmol dm<sup>-3</sup> potassium nitrate solution for 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.

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Table 1. Electrode Response of Nitrate-Selective Membranes

membrane number	electrode sensor (R)	linear Nernstian range (mol dm <sup>-3</sup> NO <sub>3</sub> <sup>-</sup> )	Nernstian slope (mV/dec)	limit of detection (μmol dm <sup>-3</sup> NO <sub>3</sub> <sup>-</sup> )	$K_{\text{NO}_3, \text{Cl}^-}^{\text{pot}}$ (10 <sup>-2</sup> mol dm <sup>-3</sup> Cl <sup>-</sup> )
	commercial	1 × 10 <sup>-1</sup> to 1 × 10 <sup>-5</sup>	-59.0	7.0	5.50 × 10 <sup>-3</sup>
S1	H	1 × 10 <sup>-1</sup> to 1 × 10 <sup>-4</sup>	-55.3 ± 0.4	30 ± 3	12.0 × 10 <sup>-3</sup>
S2	CH <sub>3</sub> CH <sub>2</sub>	1 × 10 <sup>-1</sup> to 5 × 10 <sup>-4</sup>	-50.5 ± 0.5	25 ± 7	20.0 × 10 <sup>-3</sup>
S3	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>2</sub>	1 × 10 <sup>-1</sup> to 5 × 10 <sup>-5</sup>	-53.4 ± 0.5	6.6 ± 0.7	6.0 × 10 <sup>-3</sup>
S4	(CH <sub>3</sub> ) <sub>2</sub> CH	1 × 10 <sup>-1</sup> to 1 × 10 <sup>-5</sup>	-58.1 ± 0.2	1.5 ± 0.2	6.0 × 10 <sup>-3</sup>
S5	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>3</sub>	1 × 10 <sup>-1</sup> to 5 × 10 <sup>-5</sup>	-57.6 ± 0.6	3.4 ± 0.5	4.0 × 10 <sup>-3</sup>
S6	(CH <sub>3</sub> ) <sub>2</sub> CHCH <sub>2</sub>	1 × 10 <sup>-1</sup> to 5 × 10 <sup>-6</sup>	-59.1 ± 0.2	0.34 ± 0.05	3.4 × 10 <sup>-3</sup>
S7	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>4</sub>	1 × 10 <sup>-1</sup> to 5 × 10 <sup>-5</sup>	-55.0 ± 0.5	8 ± 1	6.0 × 10 <sup>-3</sup>
S8	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>5</sub>	1 × 10 <sup>-1</sup> to 5 × 10 <sup>-5</sup>	-57.0 ± 0.5	7.4 ± 0.6	6.0 × 10 <sup>-3</sup>
S9	CH <sub>3</sub> (CH <sub>2</sub> ) <sub>9</sub>	1 × 10 <sup>-1</sup> to 5 × 10 <sup>-5</sup>	-56.6 ± 0.6	11.2 ± 0.8	8.0 × 10 <sup>-3</sup>
					(5 × 10 <sup>-3</sup> ) <sup>a</sup>
S10	(VI)	1 × 10 <sup>-1</sup> to 5 × 10 <sup>-5</sup>	-57.5 ± 0.1	2.9 ± 0.6	4.5 × 10 <sup>-3</sup>

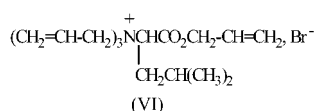
<sup>a</sup> -18 mV method.

## RESULTS AND DISCUSSION

**Synthesis.** The following betaine salts (V) were synthesized and used in the study:

R= H, CH<sub>3</sub>CH<sub>2</sub>, CH<sub>3</sub>(CH<sub>2</sub>)<sub>2</sub>, (CH<sub>3</sub>)<sub>2</sub>CH, CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>, (CH<sub>3</sub>)<sub>2</sub>CHCH<sub>2</sub>, CH<sub>3</sub>(CH<sub>2</sub>)<sub>4</sub>, CH<sub>3</sub>(CH<sub>2</sub>)<sub>5</sub>, CH<sub>3</sub>(CH<sub>2</sub>)<sub>9</sub>.

In addition (VI), *N,N,N*-triallyl leucine allyl ester bromide salt



was synthesized and investigated. All compounds were analyzed and found to be >99% pure by TLC, IR, and NMR spectroscopic analysis. DL-isomers were used throughout. The betaine salts (V) and the ester (VI) were viscous oils at room temperature except for R= H and CH<sub>3</sub>CH<sub>2</sub>. These latter two were crystalline solids, mp 154 °C and 120 °C, respectively.

**Studies on Blank Membranes.** Previous work<sup>8,9</sup> on covalently bound sensors had used a block copolymer of butadiene and acrylonitrile, Krynac 50.75. Previous work<sup>10</sup> has shown a pH dependence in membranes made from this polymer manifesting itself as a rapid loss in sensitivity at pH values greater than 5.5, which limited their use in environmental waters. Krynac-based electrodes had performed well in buffered solutions, with the best reported to date<sup>10</sup> having a limit of detection of 8.8 × 10<sup>-6</sup> mol dm<sup>-3</sup>. In an attempt to improve overall electrode performance, the SBS copolymers were investigated. Blank membranes of Krynac and SBS, with and without a low content of phenolic-based antioxidant, were prepared as described in the Experimental Section.

Neither the SBSa nor the SBS blank membranes exhibited the short-lived nitrate response previously reported for Krynac.<sup>10</sup> In addition, the EMF measurements using blank membranes made from SBS lacked the pH dependency observed for the Krynac and SBSa membranes. From this, it seemed most likely that the pH dependency of both Krynac and SBSa was due to the phenolic-based antioxidant present in the polymeric material. Accordingly, SBS was chosen as the polymer for the present study.

**Electrode Evaluation.** Table 1 shows the comparison of the electrode performances of the membranes studied. It is clear that the most satisfactory performance is given by the betaine having

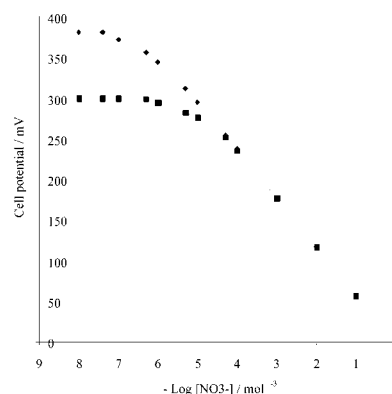


Figure 1. Calibration curve for commercial nitrate-selective electrode (■) compared with *N,N,N*-triallyl leucine betaine (◆).

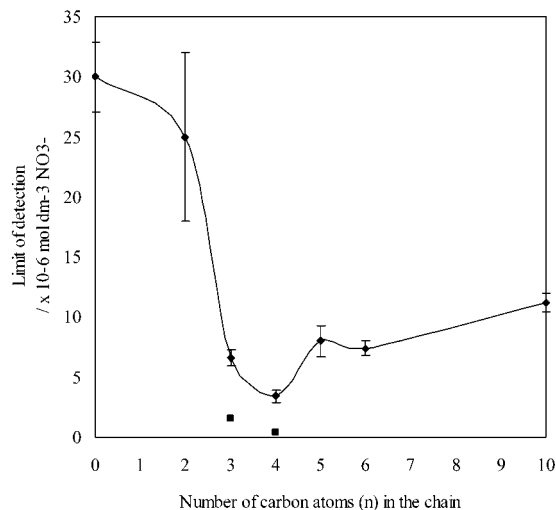


Figure 2. Limit of detection versus the number of carbon atoms in the betaine side chain (*n*); (◆) straight chain and (■) branched chain.

the isobutyl side chain (triallyl leucine betaine, S6). A graphical comparison of the leucine betaine-based electrode with that of the commercial nitrate electrode is presented in Figure 1. Figures 2 and 3 show how the electrochemical parameters LOD and  $K_{\text{NO}_3, \text{Cl}^-}^{\text{pot}}$  vary with the number of carbon atoms in the side chain. It appears that hydrophobicity of the sensor molecule is not a dominant factor in determining electrode performance in the case of betaine sensors. This contrasts with established understanding

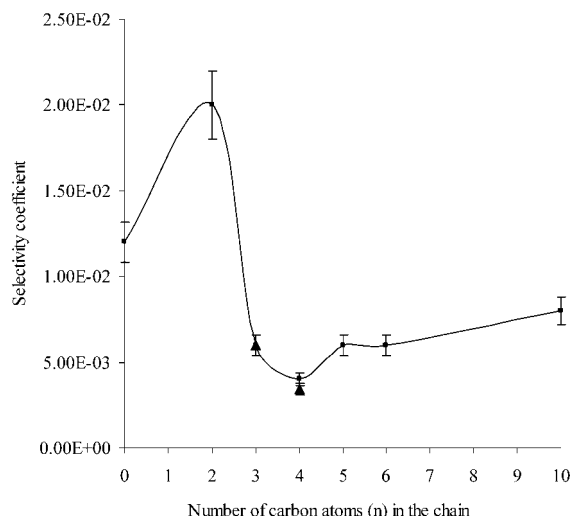


Figure 3. Selectivity coefficient  $K_{\text{NO}_3^-, \text{Cl}^-}^{\text{pot}}$  versus the number of carbon atoms in the betaine side chain ( $n$ ); (—■—) straight chain and (—▲—) branched chain.

for commercial sensors that are long chain *N*-alkyl quaternary ammonium salts trapped in PVC<sup>20</sup> and our own previous findings<sup>8,9</sup> of covalently bound *N*-alkyl quaternary ammonium compounds. The optimum performance was observed with the leucine betaine  $\text{R} = \text{CH}_2\text{CH}(\text{CH}_3)_2$  which with a LOD of  $3.4 \times 10^{-7} \text{ mol dm}^{-3} \text{ NO}_3^-$  was a significant improvement upon the norleucine betaine betaine, S5,  $\text{R} = (\text{CH}_2)_3\text{CH}_3$ , ( $3.4 \times 10^{-6} \text{ mol dm}^{-3}$ ). Both compounds have four carbon atoms in the side chain; the leucine, however, has a branched, as opposed to a straight, chain. It would appear that a steric factor was contributing to the performance of the sensor, since a comparison of structures shows that leucine is the only molecule to show a branched group,  $(\text{CH}_3)_2\text{CH}$ ,  $\beta$  to the positively charged N atom. The allyl ester of leucine betaine, S10, showed a marked decrease in LOD ( $2.9 \times 10^{-6} \text{ mol dm}^{-3}$ ), as compared with the parent free carboxylic acid. The ester showed a narrower pH working range (2–7 instead of 2–8 pH units). This difference lies well above the  $\text{pK}_a$  for  $\text{CO}_2\text{H}$  groups; thus, a polar influence seems unlikely. There remains the possibility of a spatial effect, however, since covalent bonding of the ester to the polymer may also take place through the *O*-allyl groups.

The performance of the best membrane (S11 containing *N,N,N*-triallyl leucine betaine hydrochloride) which gives a limit of detection of  $3.4 \times 10^{-7} \text{ mol dm}^{-3}$ , is better than the best commercially available electrodes that offer limits of detection of  $\sim 7 \times 10^{-6} \text{ mol dm}^{-3}$  for Elite 021 and IS560. The performance of S11 also is an improvement on our previous sensors, immobilized quaternary ammonium salts offering a LOD of  $8.8 \times 10^{-6} \text{ mol dm}^{-3}$  nitrate.<sup>10</sup> Lowering the LOD from  $0.12 \text{ mg L}^{-1} \text{ NO}_3^-$  as N to  $0.005 \text{ mg L}^{-1} \text{ NO}_3^-$  as N enables the monitoring of pristine natural waters. Thus, the improvement offered by this new membrane, S11, is significant in the context of environmental monitoring. The robustness and long lifetime of the new electrodes, arising from the covalent cross-linking of the sensing moiety into the membrane, together with this improvement in sensitivity and selectivity for nitrate against chloride, has enabled

us to use the electrodes to monitor moorland streams and other natural waters in experiments over a 4-month period.<sup>21</sup>

**Homogeneity of Membranes.** Five electrodes having *N,N,N*-triallyl leucine betaine hydrochloride as sensor (originated from the same master membrane) were evaluated after conditioning for 48 h. The results are shown in Table 2. The relative standard deviation, RSD, of the potential reading of these five membranes at the limit of detection is 1.05%, showing a homogeneous membrane fabrication technique.

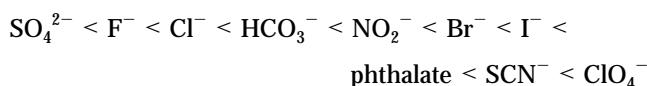
**Reproducibility of the Membrane Fabrication.** For this experiment, 12 master membranes were fabricated using the *N,N,N*-triallyl leucine betaine chloride as sensor. One membrane of 7 mm diameter was punched out of each one and evaluated. The RSD of the cell potential along the linear range is  $<1\%$  and at  $1.0 \times 10^{-6} \text{ mol dm}^{-3}$  nitrate concentration is only 2%. This confirms the reproducibility of the method of membrane fabrication and the advantages of the hot pressing technique.

**pH Dependence of the Electrode.** pH dependency studies were carried out on membranes having sensor molecules bound to all three of the polymers that were considered. The SBS with a low content of phenolic-based antioxidant-based gave the best performance. Figure 4 shows that the most sensitive betaine electrode obtained to date, viz that of leucine, has a range from pH 2 to 8 over which performance is independent of pH change. Both Krynac and SBSa with the antioxidant gave a less satisfactory pH range (2–6), as observed for the blank membranes. A working pH range of 2–8 would indicate that the electrode should be suitable for most nonsaline environmental waters. Attempts to purify Krynac in order to remove the antioxidant were unsuccessful and led to a brittle, unsuitable polymeric product.

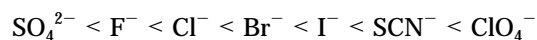
**Lifetime of the Electrodes.** Table 3 shows that the Nernstian slope, LOD, and  $K_{\text{NO}_3^-, \text{Cl}^-}^{\text{pot}}$  values of an electrode are independent of the polymer material for a substantial period of time. Krynac-based electrodes proved most satisfactory from this standpoint, but as previously mentioned, they have a limited pH working range. Krynac-based electrodes perform well in solutions buffered in the pH range 4–6.

A membrane lifetime of at least 6 months continuous use, as shown by the SBS, would prove acceptable for most environmental monitoring applications, however.

**Selectivity.** The selectivity coefficients were also determined for a variety of anions usually given in the Hofmeister series and other anions of environmental importance. Table 4 shows that the interferences from the anions increase in the following order:



This selectivity pattern is consistent with the Hofmeister series<sup>22</sup> shown below.



Selectivity for nitrate over chloride is improved in membrane S11

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Table 2. Reproducibility of Membrane Performance for Five Membranes Containing *N,N,N*-Triallyl Leucine Betaine Sensor

[NO <sub>3</sub> <sup>-</sup> ] (mol dm <sup>-3</sup> )	cell potential (mV)					av potential (mV)	std dev	RSD %
	(1)	(2)	(3)	(4)	(5)			
1.00 × 10 <sup>-1</sup>	57.0	57.0	57.0	57.0	57.0	57.0	0.00	0.00
1.00 × 10 <sup>-2</sup>	117.0	117.0	117.0	117.0	117.0	117.0	0.00	0.00
1.00 × 10 <sup>-3</sup>	177.9	177.9	176.3	177.8	177.6	177.5	0.68	0.38
1.00 × 10 <sup>-4</sup>	237.5	237.4	235.3	236.8	236.0	236.6	0.94	0.40
5.00 × 10 <sup>-5</sup>	255.3	255.3	252.7	255.2	254.5	254.6	1.11	0.44
1.00 × 10 <sup>-5</sup>	295.6	294.8	292.6	294.6	293.9	294.3	1.13	0.38
5.00 × 10 <sup>-6</sup>	312.9	312.1	309.6	312.1	310.7	311.5	1.32	0.42
1.00 × 10 <sup>-6</sup>	346.8	343.8	341.7	345.5	342.4	344.0	2.12	0.62
5.00 × 10 <sup>-7</sup>	361.5	356.8	352.6	356.5	352.3	355.9	3.75	1.05
1.00 × 10 <sup>-7</sup>	378.1	370.8	367.7	375.0	367.2	371.8	4.71	1.27
4.00 × 10 <sup>-8</sup>	388.0	379.6	376.5	382.2	374.9	380.2	5.17	1.36
1.00 × 10 <sup>-8</sup>	388.0	379.6	376.5	382.2	375.0	380.3	5.15	1.35

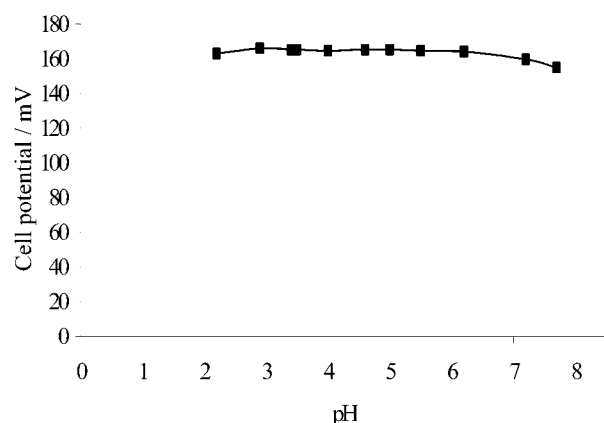


Figure 4. pH versus cell potential in 10<sup>-3</sup> mol dm<sup>-3</sup> NO<sub>3</sub><sup>-</sup> solution for membranes containing triallyl leucine betaine as sensor in SBS.

as compared to the commercial electrode evaluated. There is also a significant improvement in selectivity over sulfate using the betaine membrane S11. These improvements enhance the performance of the new sensor in environmental applications.

**Stability of Electrode Response.** The stability of the cell potential was measured using the method described in the Experimental Section. Over 3 days, the cell potential varied only by  $\pm 1\%$ .

**Structure–Activity Relationships.** The purpose of this section is to understand how the structure of *N,N,N*-triallyl  $\alpha$ -amino acid betaine chlorides influence the electrochemical parameters of the nitrate-selective membrane. Table 1 can be visualized graphically by plotting LOD and  $K_{NO_3, Cl}^{pot}$  versus the number of carbons in the betaine side chain and by making the difference between straight and branched alkyl chains.

Figures 2 and 3 are very similar, showing an optimum performance with the C<sub>4</sub>-branched side chain of leucine. Figure 5 shows the close relationship between selectivity and sensitivity. Membrane S2 has not been considered because of its sub-Nernstian response (slope,  $-50.5$  mV dec<sup>-1</sup>). Generally, for all of the membranes studied, an improved selectivity results in an improved sensitivity.

Often commercial nitrate sensors are hydrophobic *N*-alkyl quaternary ammonium salts, e.g., tridodecylmethylammonium nitrate,<sup>23</sup> trapped in PVC or quaternary ammonium salts, such as triallyl decylammonium nitrate covalently bound to polymers.<sup>10</sup> In the case of the betaines, the performance slowly declined with

increasing chain length after C<sub>4</sub>. It has generally been assumed that the main factor of influence has been the hydrophobicity of the sensor molecules.<sup>24</sup> This does not seem to be the case with the betaine molecule. There were two isomers with a C<sub>4</sub> side chain examined. Membrane S5 contained the C<sub>4</sub> straight chain and had a LOD of  $3.4 \times 10^{-6}$  mol dm<sup>-3</sup> nitrate; the C<sub>4</sub> branched chain of S6 had a LOD of  $3.4 \times 10^{-7}$  mol dm<sup>-3</sup> nitrate, an improvement of 10-fold. A similar increase in improvement with branching was observed between the two C<sub>3</sub> side-chain isomers S3 and S4. It would be noted that the sensor moieties in this study were covalently cross-linked into a polymer matrix. Thus, not only are they not available for dissolution in the test solution but also the effective carbon chain length is very long.

It has recently been shown using an immobilized quaternary ammonium salt that the mechanism of nitrate response is due to an ion-exchange<sup>25</sup> 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 S6 and S10 shows that converting the free carboxylic acid group to a carboxylate ester resulted in a decrease in LOD from  $0.34 \times 10^{-6}$  to  $2.9 \times 10^{-6}$  mol dm<sup>-3</sup> nitrate. The difference in inductive effect between a free carboxyl group and an ester  $\beta$  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<sub>a</sub> for CO<sub>2</sub>H 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<sub>2</sub>H group present in betaines could possibly improve the hydration or hydrogen bonding capacity near to the positively charged nitrogen atom.

Because 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 charges).<sup>26</sup> The strongest inductive effect will be that from the

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(24) Nielsen, H. J.; Hansen, E. H. *Anal. Chim. Acta*, **1976**, *98*, 151–155.

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(26) Ingold, C. K. *Structure and Mechanism in Organic Chemistry*, 2nd ed; G. Bell Sons Ltd.: London, 1969; pp 63–64.

Table 3. Lifetime for Triallyl Norleucine Betaine (TANB) in Krynac 50.75, SBSa, and SBS as Compared with Triallyl Leucine Betaine (TALeuB)

	lifetime studies	Krynac 50.75 TANB	SBS TANB	SBSa TANB	SBS TALeuB
day 7	slope, mV dec <sup>-1</sup>	-57.0	-57.6	-57.0	-59.1
	limit of detection × 10 <sup>-6</sup> mol dm <sup>-3</sup> NO <sub>3</sub> <sup>-</sup>	3.0	3.4	3.0	0.34
	$K_{\text{NO}_3, \text{Cl}^-}^{\text{pot}}$	$3.0 \times 10^{-3}$	$4.0 \times 10^{-3}$	$3.0 \times 10^{-3}$	$3.4 \times 10^{-3}$
day 48	slope, mV dec <sup>-1</sup>	-57.0	-57.0	-56.5	-56.9
	limit of detection × 10 <sup>-6</sup> mol dm <sup>-3</sup> NO <sub>3</sub> <sup>-</sup>	3.0	3.3	3.2	0.7
	$K_{\text{NO}_3, \text{Cl}^-}^{\text{pot}}$	$3.0 \times 10^{-3}$	$3.0 \times 10^{-3}$	$3.0 \times 10^{-3}$	$3.0 \times 10^{-3}$
day 100	slope, mV dec <sup>-1</sup>	-57.0	-56.0	-55.0	-56.7
	limit of detection × 10 <sup>-6</sup> mol dm <sup>-3</sup> NO <sub>3</sub> <sup>-</sup>	4.0	5.0	3.5	1.0
	$K_{\text{NO}_3, \text{Cl}^-}^{\text{pot}}$	$4.0 \times 10^{-3}$	$4.0 \times 10^{-3}$	$3.3 \times 10^{-3}$	$3.5 \times 10^{-3}$
day 147	slope, mV dec <sup>-1</sup>	ND <sup>a</sup>	ND	-45.0	-55.6
	limit of detection × 10 <sup>-6</sup> mol dm <sup>-3</sup> NO <sub>3</sub> <sup>-</sup>	ND	ND	3.5	1.5
	$K_{\text{NO}_3, \text{Cl}^-}^{\text{pot}}$	ND	ND	$3.3 \times 10^{-3}$	$4 \times 10^{-3}$
day 240	slope, mV dec <sup>-1</sup>	-57.0	-53.0		-55.0
	limit of detection × 10 <sup>-6</sup> mol dm <sup>-3</sup> NO <sub>3</sub> <sup>-</sup>	5.5	13.0	<i>b</i>	1.2
	$K_{\text{NO}_3, \text{Cl}^-}^{\text{pot}}$	$5.0 \times 10^{-3}$	N.D	<i>b</i>	$6.0 \times 10^{-3}$

<sup>a</sup> No data. <sup>b</sup> Unresponsive.

Table 4. Selectivity Data for a Variety of Anions Using a SBS Membrane Containing Triallyl Leucine Betaine (TALeuB)

[J], mol dm <sup>-3</sup>	S11	$K_{\text{NO}_3, \text{J}}^{\text{pot}}$ commercial <sup>a</sup>
F <sup>-</sup> (10 <sup>-2</sup> )	$3.0 \times 10^{-4}$	(10 <sup>-4</sup> )
Cl <sup>-</sup> (10 <sup>-2</sup> )	$3.4 \times 10^{-3}$	$5.5 \times 10^{-3}$ ( $5.6 \times 10^{-3}$ )
Br <sup>-</sup> (10 <sup>-2</sup> )	$7.3 \times 10^{-2}$	<i>b</i>
I <sup>-</sup> (10 <sup>-4</sup> )	14	<i>b</i>
SCN <sup>-</sup> (10 <sup>-5</sup> )	37	<i>b</i>
ClO <sub>4</sub> <sup>-</sup> (10 <sup>-5</sup> )	400	<i>b</i>
HCO <sub>3</sub> <sup>-</sup> (10 <sup>-2</sup> )	$3.0 \times 10^{-2}$	( $5 \times 10^{-3}$ )
NO <sub>2</sub> <sup>-</sup> (10 <sup>-2</sup> )	$3.9 \times 10^{-2}$	<i>b</i>
SO <sub>4</sub> <sup>2-</sup> (10 <sup>-2</sup> )	$4.6 \times 10^{-5}$	$1.6 \times 10^{-4}$ (10 <sup>-4</sup> )
phthalate (10 <sup>-5</sup> )	15	<i>b</i>

<sup>a</sup> Values in parentheses represent manufacturer selectivity coefficient. <sup>b</sup>No data.

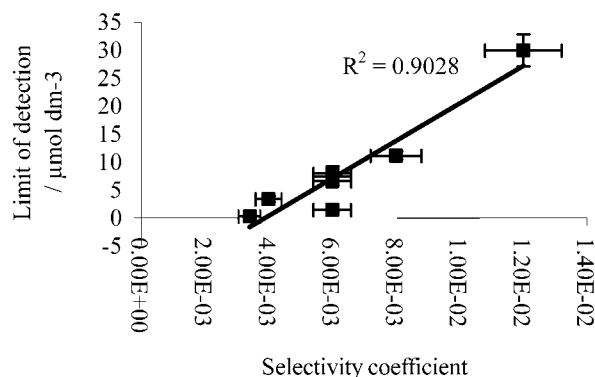
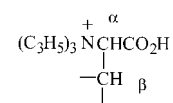


Figure 5. Limit of detection against selectivity coefficient for nitrate against chloride for membranes S6–S14 (except S7).

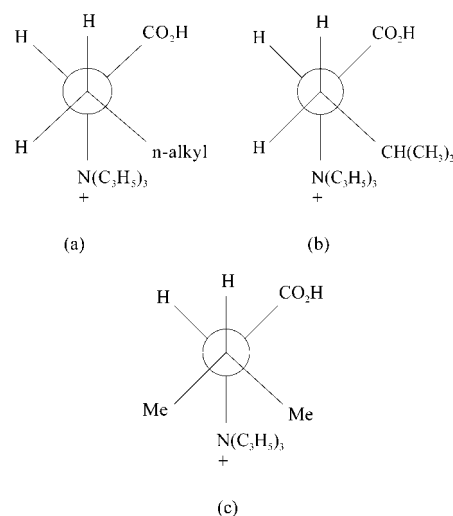
side chain of S4 (isopropyl), not that of the isobutyl of S6, which gives the best membrane. An examination of Table 1 does not suggest that the inductive effect of the side chain is a primary contribution to the performance.

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 (S1), have the following common features:



There does, however, seem to be a steric influence from the group occupying the  $\beta$  position. The following three projection structures of the various betaines will serve to illustrate the point.



(a) shows a projection diagram of betaines S2 to S9, with the exception of S4; (b) shows the projection diagram for S6; and (c) shows the projection diagram for S4 arising from the  $\beta$  carbon atom. From a  $\beta$  position, the two structures (b) S6 and (c) S4 have a possibly greater steric effect on the vicinity of the cationic nitrogen atom than on the structure of the type (a), which has a primary alkyl group. It is not easy to predict which of structures (b) and (c) will have the greatest influence. S4 and S6 are the best performing membranes, however.

Table 5. Performances of *N,N,N*-Trialkenyl  $\alpha$ -Amino Acid Betaine

alkenyl group	LOD ( $\mu\text{mol dm}^{-3}$ )	$K_{\text{NO}_3, \text{Cl}^-}^{\text{pot}}$
$\text{CH}_2=\text{CH}-\text{CH}_2-\text{CH}_2-$	3.1	$4 \times 10^{-3}$
$\text{CH}_2=\text{CH}-\text{CH}(\text{CH}_3)-$	7.0	$6 \times 10^{-3}$
$\text{CH}_2=\text{C}(\text{CH}_3)-\text{CH}_2-$	4.0	$5 \times 10^{-3}$

A separate study investigated the influence of modification of the allyl group on membrane performance, as shown in Table 5. 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 that dominates the relationship between structure and activity.

#### CONCLUSION

Amino acid betaines covalently bound via *N*-allyl groups to an SBS polymer with a low level of antioxidant make extremely efficient sensors for nitrate-selective electrodes. *N,N,N*-Triallyl leucine betaine yields an electrode having a LOD of  $3.4 \times 10^{-7}$  mol  $\text{dm}^{-3}$  and  $K_{\text{NO}_3, \text{Cl}^-}^{\text{pot}}$  of  $3.4 \times 10^{-3}$ . The low limit of detection, good stability, rapid response, acceptable lifetimes, and a working pH range of 2–8 make these electrodes the most suitable ever

reported for use in environmental nitrate monitoring as well as general sensitive laboratory use.

Field tests have already been conducted with excellent results and will form the basis of a separate publication. The unusual structure of betaines compared with the currently used very hydrophobic quaternary ammonium salt sensors raises questions about their mode of action. The data seem to point to the significance of a steric or field effect from the position  $\beta$  to the positively charged nitrogen atom. Studies are currently taking place on this topic.

The great sensitivity of betaines toward nitrate now demonstrated combined with the selectivity against chloride ions makes the question of the significance of betaine molecules in nitrate recognition in the plant kingdom, already discussed by Braven et al.,<sup>10,11</sup> yet more significant.

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