Problems in the Application of Ion-selective Electrodes to Serum Lithium Analysis

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Three types of lithium ion-selective electrode (ISE) have been studied in microconduit flow injection analysis for their response to lithium ions in serum samples containing between 0.21 and 2.00 mM lithium. The effects of possible interferents, such as proteins and sodium chloride, in samples have been studied with regard to the poor quality of data.

Adjustment of the serum sodium chloride level to 153.9 mM improved the ISE lithium data to match more closely those obtained by flame photometry. Nevertheless, even the best of the three lithium ISEs studied, namely that based on an electroactive component of barium propoxylate, produced data that matched flame photometric data for only five serum samples out of the ten examined.

The other two electrode types studied were based on PVC matrix membranes of a commercial lithium ISE (Philips Cat. No. 561-LI) and dodecylmethyl-14-crown-4

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The development of lithium ion-selective electrodes (ISEs) for monitoring lithium ions in blood serum has recently received considerable attention, as lithium salts are prescribed for the treatment of manic depression.

Several crown ethers,[1–5] lipophilic diamides[6–8] and other diverse materials[9–11] have been investigated as sensors for lithium ISEs. Most of these sensors lack satisfactory sodium selectivities for the determination of lithium in blood serum where the sodium level is typically over 1400 times higher than the lowest lithium levels of clinical interest. So far, lithium ISEs reported to have good selectivity, *e.g.*, $k^{pot}_{Li,Na} \approx 0.002$, have been restricted to lithium analysis in artificial sera,[5,6] which is unrealistic as proteins and most other organic materials found in blood serum have been excluded.

A new PVC lithium ISE based on the tetraphenylborate of a barium polypropoxylate, $Ba(PPG\text{-}1025)_{0.69}TPB_2$, and dioctylphenyl phosphonate (DOPP) solvent mediator has a linear slope of 57 mV decade^{-1} down to 10^{-4} M lithium.[12] The qualities of this inexpensive PVC electrode motivated this investigation of its behaviour for the determination of lithium in sera.

Experimental

Apparatus and Materials

Microconduits made from PVC blocks were used as a sample cell in the manner described previously.[12] Compressed air was used to propel the carrier solution through the microconduit at 1.0 cm^3 min^{-1}. A Radiometer PHM64 millivoltmeter was used for e.m.f. measurements and the response was recorded on a Linear Instrument Corp. Model 0555-0000 chart recorder.

Reagents and Chemicals

The barium polypropoxylate, $Ba(PPG\text{-}1025)_{0.69}TPB_2$, was synthesised as reported by Jaber *et al.*[13] Dioctylphenyl phosphonate was obtained from Lancaster Synthesis, Morecambe, UK, polypropylene glycol (PPG-1025) from BDH Chemicals, Poole, UK, sodium tetraphenylborate and dialysis membranes from Aldrich, Gillingham, Dorset, UK, polycarbonate and cellulose acetate protein exclusion membrane from Millipore, Molsheim, France, α-globulin, β-globulin, albumin (bovine) and glucose from Sigma, Poole, UK, and a lyophilised powder mixture of cholesterol (free and total), triglyceride, phospholipids and β-lipoproteins from Boehringer Mannheim, East Sussex, UK.

The commerical lithium PVC membrane (Type No. 561-LI) was obtained from Philips Scientific and Analytical Equipment, Cambridge, UK, and dodecylmethyl-14-crown-4 was a gift from Professor T. Shono, Osaka University, Japan.

All solutions were prepared from analytical-reagent grade salts using distilled, de-ionised water.

Electrode Fabrication

Three different lithium cocktails were utilised in the fabrication of otherwise identical PVC lithium ISEs.

The barium complex, $Ba(PPG\text{-}1025)_{0.69}TPB_2$, (0.04 g) was dissolved in dioctylphenyl phosphonate (0.36 g) and the sensor cocktail completed by adding PVC (0.17 g) in tetrahydrofuran (6 cm^3). Electrodes (Type 1) were fabricated as reported earlier.[12]

One Philips lithium PVC membrane disc (7.0 mm) was dissolved in tetrahydrofuran (1 cm^3) and the cocktail used to fabricate Type 2 electrodes in an analogous fashion to the above PVC barium model.[12] Type 3 electrodes were similarly fabricated from a cocktail consisting of dodecylmethyl-14-crown-4 (3.6 mg), *o*-nitrooctylphenyl ether (0.25 g), PVC (0.1 g), potassium tetra(4-chlorophenyl)borate (2.3 mg) and trioctylphosphine oxide (3.6 mg) in tetrahydrofuran (3 cm^3).

Each exclusion membrane was carefully attached to the surface of the particular lithium PVC membrane and sealed around the edges to the microconduit channel with PVC glue.

Serum Analysis Procedure

Analyses were carried out by flow injection analysis employing microconduits.[11] Serum samples (1–10) were obtained from the University Hospital of Wales. Of these, samples 1 and 10 were diafiltrated serum with added lithium carbonate, and samples 2–9 were mixes of 1 and 10 to give a graded lithium content. Each sample (1–10) was previously analysed for lithium and other cations (Table 1). Serum samples from patients, containing between 0.1 and 2.8 mM lithium (analysed by flame photometry), were also briefly studied. Aliquots (100 mm^3) of the serum samples were injected into the carrier stream of artificial serum electrolyte A, specified below, and the change in potential was recorded. Calibrations were made using lithium standards in artificial serum electrolyte A.

Table 1. Cation concentrations in serum samples determined by previous analysis at the University Hospital of Wales

	Cation concentration/mM			
Serum sample No.	Li	Na	K	Ca
1	0.21	105.3	1.77	1.62
2	0.40	110.7	2.36	1.78
3	0.61	116.1	2.95	1.97
4	0.79	122.0	3.57	2.16
5	1.00	126.9	4.17	2.35
6	1.20	132.3	4.78	2.53
7	1.40	137.7	5.38	2.72
8	1.58	143.5	6.03	2.90
9	1.75	148.7	6.60	3.09
10	2.00	153.9	7.18	3.27
Analytical technique	Flame photometry	ISE	ISE	Spectrophotometry

The artificial serum electrolyte, A, consisted of sodium chloride (140 mM), potassium chloride (2.8 mM), potassium dihydrogen phosphate (1.3 mM), calcium chloride (2.5 mM) and magnesium sulphate (2.3 mM). Its pH was adjusted to *ca.* 7.3 with potassium hydroxide solution (1.0 M).

A modified artificial serum electrolyte B, used as an alternative carrier for the pre-treated real sera, differed only in that it comprised 153.9 mM sodium chloride (to match that in sample 10 of Table 1) instead of the 140 mM in the case of serum A.

Investigation of Interference effects

Albumin bovine (0.2889 g), α-globulin (0.0771 g), β-globulin (0.0705 g), γ-globulin (0.12 g) and glucose (0.0067 g) were each dissolved in 5 cm^3 of 0.1 M lithium chloride solution made up in artificial serum electrolyte A. Another solution consisting of all five compounds in 0.1 M LiCl artificial serum electrolyte A (5 cm^3) was also prepared.

The Boehringer Mannheim powder, consisting of cholesterol, triglyceride, phospholipids and β-lipoproteins, was dissolved in 10 cm^3 of 0.1 M lithium chloride/artificial serum electrolyte A.

Each of these protein- or protein/fat-based sera was also injected into the carrier stream and the response was compared with that from 0.1 M lithium chloride in solution A.

Results and Discussion

Clearly, the Type 1 electrode responds reproducibly to lithium, even at 0.1 mM in artificial serum A (Fig. 1). The results from the serum samples (confirmed by flame photometry to contain between 0.21 and 2.0 mM lithium; see Table 1) were unexpected in that either negative or positive FIA electrode responses were obtained for some samples whereas others (Nos. 4, 5 and 7) gave both negative and positive peaks (Fig. 2). Moreover, most responses were not reproducible. Similar erratic responses were obtained for patients' serum samples shown by flame photometry to contain up to 2.8 mM lithium. However, as a wider range of electrolyte data were available for samples 1–10, the remainder of this study was focused on these.

The response patterns mentioned above could relate to effects such as pH, anions, proteins, lipoproteins, the nature of the electrode itself and/or the varying electrolyte concentrations of the serum samples (Table 1). The pH of the serum samples analysed ranged from 7.1 to 7.8, but the PVC barium electrode suffers little hydrogen ion interference.[12] The negative response is also unlikely to be due to chloride and phosphate anions (the predominant anions in serum), as similar concentrations of these anions were present in the carrier solution A.

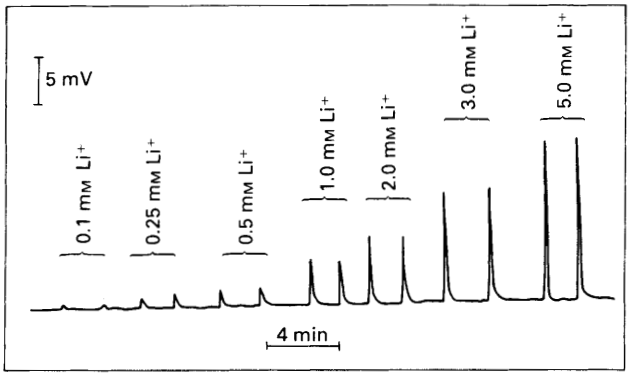

Fig. 1. Flow injection profiles of the Type 1 electrode to lithium standards in artificial serum electrolyte A. Carrier: artificial serum electrolyte A at pH 7.1

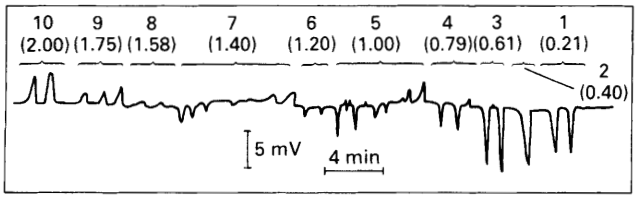

Fig. 2. Flow injection analysis profiles of the Type 1 electrode for ten serum samples. Carrier: artificial serum electrolyte A at pH 7.1. Numbers in parentheses are lithium levels (mM) found by flame photometry

Effect of Natural Products on the Electrode

The electrode response was unaffected by α-, β- and γ-globulin, albumin (bovine) and glucose, both individually and all five together. The same applied to cholesterol (free and total), triglyceride, phospholipids and β-lipoproteins, both individually and in admixture in artificial serum A. Of course, many other organic materials in serum (not studied here) could affect the electrode response.

In clinical applications of commercial ISEs protein (or high relative molecular mass) exclusion membranes are often applied to the sensor surfaces to offset large molecule effects. Thus, such exclusion membranes were attached to the Type 1 electrode surface as described. Three different exclusion membranes were used, namely a dialysis membrane capable of excluding molecules with relative molecular mass >2000, a polycarbonate protein exclusion membrane with a pore size of 0.4 μm and cellulose acetate with a pore size of 0.22 μm. The respective exclusion membranes resulted in 50, 0 and 26% reduction in response when 0.1 M lithium chloride in artificial

serum electrolyte A (*i.e.*, no protein or large molecule components were present) were injected into the carrier stream. The reductions of 50 and 26% are most unfavourable. Indeed, even at the 5 mM lithium chloride level, the reduction of 59% observed for cellulose acetate was even worse than the 50% noted for 0.1 M lithium chloride.

With the polycarbonate protein exclusion membrane, where no reduction of artificial serum lithium response was observed, the response to serum lithium was still unsatisfactory, *i.e.*, the peaks were irreproducible and often showed negative profiles. This suggests that either the polycarbonate protein exclusion membrane was inefficient in excluding interfering large molecules from the electrode, or the effect on the electrode is due to one or more unidentified low relative molecular mass organic component(s) of the serum. Fortunately, this anomalous response is temporary, as the FIA base line is quickly restored after passage of sample and the PVC electrodes showed their usual Nernstian response in lithium standards.

Nature of Sensor

To establish that these FIA electrode behaviour patterns are not due to the nature of the sensor in the electrode, the responses of the other two lithium ISEs were similarly investigated. Their respective responses to real serum lithium were similar to those illustrated in Fig. 1. Therefore, these strange FIA patterns seem to relate to component(s) in serum rather than to the sensors.

Effect of Varying Electrolyte Levels in Serum Samples

The sodium content of the serum samples studied varied from 105.3 to 153.9 mM (Table 1). It is frequently assumed that the average concentration of sodium in serum is about 140 mM and the carrier artificial serum electrolyte A was accordingly set at 140 mM. Serum samples containing less sodium than the carrier solution A generally resulted in negative peaks and serum samples containing more sodium than the carrier solution A usually resulted in positive peaks (Fig. 2). This was investigated by injecting mock serum samples (free of organic components) containing lithium, sodium, potassium and calcium ions in amounts equivalent to real serum samples Nos. 2, 6 and 10 (Table 1) into the carrier electrolyte stream A.

As suspected, the low-sodium system gave negative peaks (Fig. 3), supporting the idea that the variable sodium in the serum samples is partly responsible for the strange response of the electrodes. It should be noted, however, that the responses obtained using the mock serum samples are reproducible, unlike the results when real serum samples were used. In addition to the varying sodium effect in the serum, there must be other parameters in the serum that affect the electrode response.

Serum Electrolyte Correction

To attempt a correction for the effect of varying sodium concentration on the lithium response (see Fig. 2), serum samples (1 cm^3) were spiked with appropriate volumes of sodium chloride solution (1.0 M) to bring the concentration of sodium in each serum to the same level as that in the carrier solution B, namely 153.9 mM. These spiked samples were then injected into the non-protein based artificial serum electrolyte B. The results obtained using the three different electrodes showed improvement over the previous analysis. The results, however, are not statistically satisfactory. For the serum samples analysed with each electrode, only three results out of ten samples examined showed any agreement with the actual flame photometric (Table 1) lithium values obtained using the Type 3 electrode (Fig. 4). No response was obtained for samples 2, 3 and 4 in Table 1. Sample 10 (Table 1) containing 2 mM lithium as determined by flame photometry is to be compared with the value of 2.3 mM (standard deviation 0.49 mM; $n = 4$) obtained with the Type 3 electrode. For the Type 2 electrode only one result out of five samples examined showed some agreement. However, only five serum samples were analysed with the Type 2 electrode owing to a lack of sample.

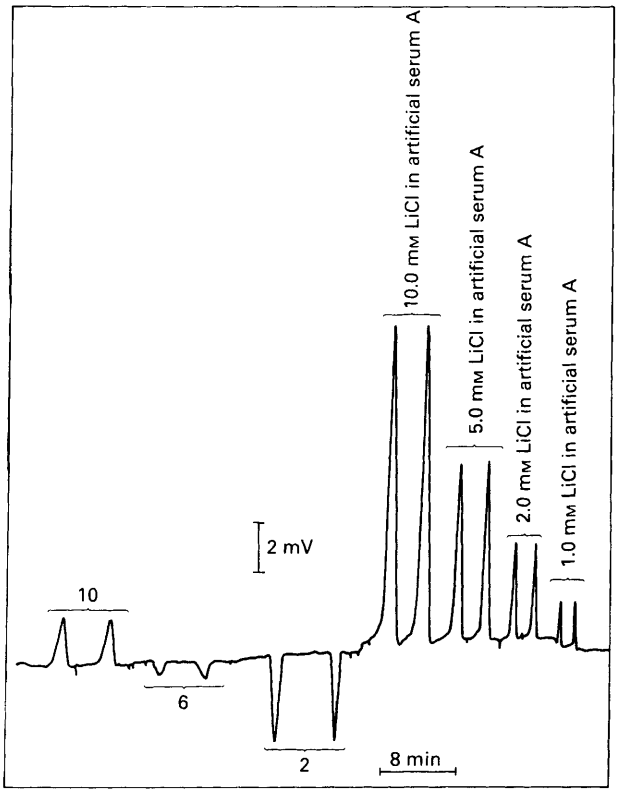

Fig. 3. Flow injection analysis profiles of Type 2 electrode to three mock serum samples. Carrier: artificial serum electrolyte A at pH 7.1

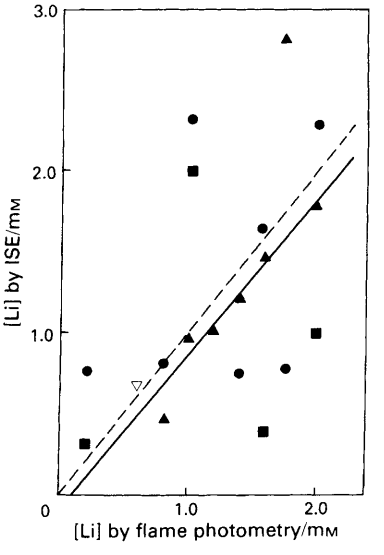

Fig. 4. Comparison of lithium ISE data with flame photometric data for serum samples. Samples for ISE data were adjusted to 153.9 mM sodium chloride, except for Type 1 (special case), which was used as it was. Carrier for ISE data: artificial serum electrolyte B at pH 7.1 except for Type 1 (special case), where the carrier solution was 116.1 mM sodium chloride, 2.95 mM potassium chloride, 1.97 mM calcium chloride and 1.01 mM magnesium chloride. Broken line: theoretical line for $[Li^+]_{ISE} = [Li]_{flame\ photometry}$. Electrodes: ▲, Type 1; ■, Type 2; ●, Type 3; and ▽, Type 1 (special case)

For the Type 1 electrode, six results out of ten samples examined showed relatively close agreement (Fig. 4). No response was obtained for samples 1, 2 and 3.

The best six results for the Type 1 electrode gave the line (full line in Fig. 4) $[Li^+]_{Type\ 1\ electrode} = 0.93[Li^+]_{flame\ photometry} - 3.77 \times 10^{-5}$, with a correlation coefficient of 0.992 and a standard error of the estimate of 5.89×10^{-5}.

This modified procedure for serum lithium analysis requires a prior knowledge of the sodium level in the serum samples in order to increase the sodium concentration to that of the carrier B before injection. Nevertheless, as seen above, the quality of the results obtained, even for the Type 1 electrode, is still poor but mainly because no response is obtained for the samples low in lithium. The high sodium level in the carrier solution (153.9 mM) can interfere with the electrode response at low lithium concentrations and is one reason for the inability to obtain data for certain serum samples. To offset such problems, a different carrier solution containing the same amount of sodium, potassium and calcium as the serum sample was tried for the analysis of serum sample No. 3 using the Type 1 electrode. Thus, the lithium standards were prepared in a protein-free carrier solution containing 116.1 mM sodium chloride, 2.95 mM potassium chloride, 1.97 mM calcium chloride and 1.01 mM magnesium chloride. A value of 0.68 mM lithium was obtained (Fig. 4), compared with 0.61 mM by flame photometry. However, this method of different carrier solutions for each sample, even though it allows low levels of lithium to be measured, is too laborious and not practicable.

From Fig. 4, it appears that there is a greater serum component effect on the Type 2 and 3 electrodes than on the Type 1 electrode.

Conclusion

It can be concluded that potentiometric analysis for serum lithium requires further research, firstly regarding a lithium ISE with $k^{pot}_{Li,Na} < 10^{-3}$, which can be used without sample pre-treatment for adjusting sodium levels. Here, the respective separate solution selectivity coefficients for Type 1, 2 and 3 electrodes[12] at 4.4×10^{-2}, 1.0×10^{-2} and 9.4×10^{-3} are higher. However, a recently described[7] lithium ion sensor based on *cis*-cyclohexane-1,2-dicarboxamide (ETH 1810) with a selectivity coefficient of 5.0×10^{-3} is a step towards the ideal value of $<10^{-3}$.

This study has shown that lithium ISEs are subject to serious interferences from other serum components, which have not been identified despite eliminating the more obvious proteins and lipids as the source of error. The different sensors were affected by serum to different extents, and this aspect of performance may need as much attention as the selectivity over sodium.

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