

## **The Behaviour of Potassium Sensors Based on NO<sub>2</sub>-Substituted Benzo-15-Crown-5 in Non-Plasticised Methacrylic-Acrylic Membranes.**

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**Abstract :** The potentiometric response of lipophilic NO<sub>2</sub>-substituted bis and mono benzo-15-crown-5 (BME44 and B143) in an unplasticised methacrylic-acrylic polymer was investigated. The unplasticised membranes with the bis- or mono-crown ethers and lipophilic anionic sites were used to construct solid-state ISEs with Ag/AgCl as a substrate and hydrated poly(2-hydroxyl ethyl methacrylate) as the inner layer. The potentiometric slopes and selectivity patterns of these ISEs were similar to those of conventional ISEs based on plasticised PVC membranes. This showed that with the methacrylic-acrylic polymers, even in the absence of plasticisers, these ionophores were able to execute their complexation characteristics. Deterioration of the lower detection limits of the electrodes was observed when the lipophilic anionic sites in the membranes increased. Tests with artificial human blood serum indicated satisfactory sensor selectivity and reproducibility. Thus, the solid-state sensor based on BME44 ionophore/non-plasticised methacrylic-acrylic membrane may be suitable for potassium ion determination in body fluids.

**Keywords :** Methacrylic-acrylic polymer, unplasticised, benzo-15-crown-5, BME44, solid-state ISE, potassium ion-selective electrode.

**Abstrak :** Sifat potensiometri bis- dan mono- eter benzo-15-makhota-5 lipofilik yang terganti -NO<sub>2</sub> (BME44 dan B143) dalam membran polimer metakrilat-akrilat yang tanpa pemplastik telah dikaji. Membran yang mengandungi bis- atau mono- eter makhota dan anion lipofilik digunakan untuk membina elektrod pemilih ion (ISE) jenis keadaan-pepejal dengan Ag/AgCl sebagai substrat dan poli(2-hidroksi etil metakrilat) sebagai lapisan dalaman. Kecerunan potensiometri dan corak keselektifan ISE yang dibina adalah sama dengan ISE konvensional yang terdiri daripada membrane polivinil klorida (PVC) dan mengandungi pemplastik. Kajian ini menunjukkan walaupun dalam keadaan tanpa pemplastik, polimer jenis metakrilat-akrilat masih membolehkan ionofor seperti eter makhota menunjukkan sifat pengkompleksan. Kerosotan had pengesanan rendah berlaku apabila amalan anion lipofilik dalam membran bertambah. Analisis ion kalium serum darah manusia buatan dengan sensor menunjukkan keselektifan dan kebolehulangan analisis yang memuaskan. Dengan itu, sensor jenis keadaan pepejal yang berdasarkan ionofor BME 44/membran metakrilat-akrilat tanpa pemplastik adalah sesuai untuk analisis ion kalium dalam bendalir badan manusia.

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### **Introduction**

Among the available ionophores (neutral carriers), crown ethers have been used successfully in liquid-membrane based ion-selective electrodes (ISE) for the analysis of alkali and alkaline earth metal ions. The complexing property of crown ethers and their derivatives is influenced by the number and variety of heteroatoms, the topology of the crown ethers and also depends on the relative sizes of ions and hole of the crown ether ring [1]. In general, increase selectivity in ionophores can be achieved by maintaining a flexible ionophore structure to allow rapid ion-exchange but the

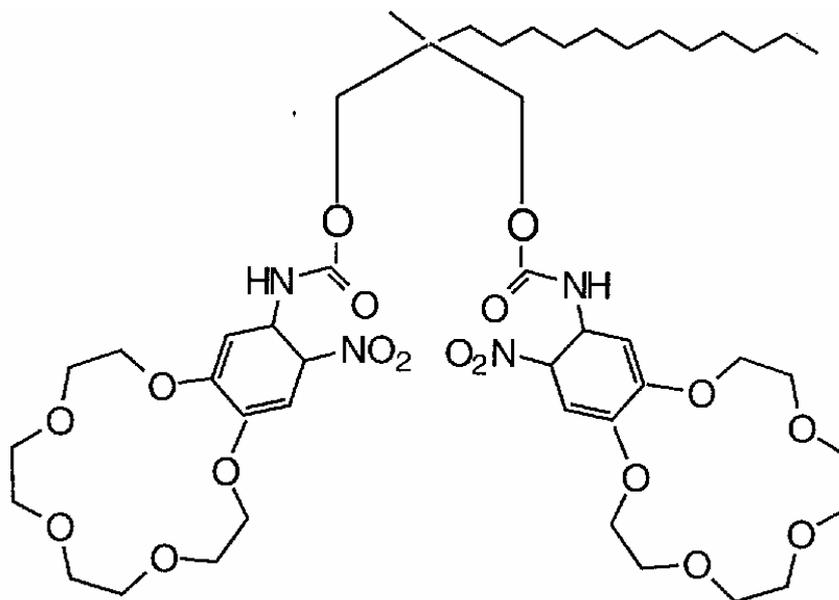
structure of the ionophore-ion complex must be rigid. It is well known that selectivity of some crown ethers towards a particular cation could be enhanced when there is formation of a 2:1 complex between the crown ether and the ion. This was demonstrated by Kimura et al. using bis(benzo-15-crown-5) for the complexation of potassium ion [1]. Enhancement of potentiometric ion-selectivity could also be achieved by introducing substituted NO<sub>2</sub> groups [2]. This was observed when NO<sub>2</sub> substituted bis(benzo-15-crown-5) rings exhibited improved selectivity towards potassium ions. The

enhanced potassium ion selectivity of the NO<sub>2</sub> substituted bis(benzo-15-crown-5) ionophores was attributed to the formation of hydrogen bonds between the NH and NO<sub>2</sub> moieties which imposed rigidity to the crown ether-potassium ion complex [2].

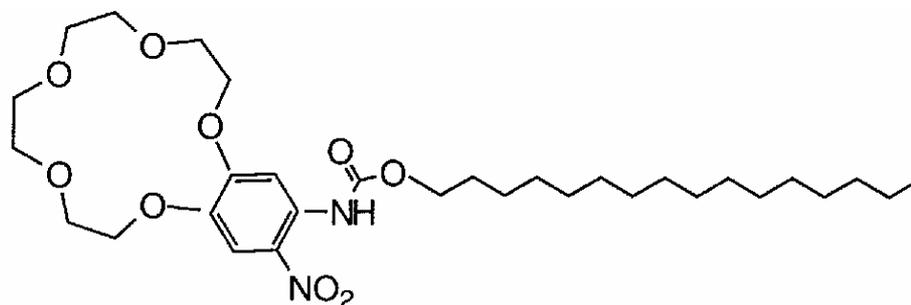
In plasticised membrane, the role of the plasticiser is normally assumed to be a solvent which enables the mutual compatibility between the polymeric membrane and the ionophore [3]. The properties of the plasticiser have great influence on the behaviour of the ionophore where the dielectric constant of the plasticiser seems to be the major factor [4]. The presence of plasticiser in an ISE membrane not only allows the dissociation of ionic species within the membrane [5] but also facilitates the transport of ionophore and ion-ionophore complexes through the membrane [6]. Dependence of selectivity of ionophores on plasticisers has been observed for both nitrate sensor [7] and calcium ISE [8]. However important is plasticisers in the proper functioning of ISE membranes, it is increasingly recognised that its usage poses some ISE fabrication and application problems, particularly when biomedical applications is concerned. In this respect, the use of ionophores in unplasticised

membranes should be of interest for ion detection with ISEs.

How the NO<sub>2</sub> substituted bis(benzo-15-crown-5) ionophores behaved when incorporated to a unplasticised membranes is of both academic and application interests. It is interesting to know the difference in the behaviour of these crown ethers in an To that end, we have carried out potentiometric studies on the behaviour of BME44 in a non-plasticised based polymer membrane. The study was performed by incorporating the ionophore into an non-plasticised methacrylic-acrylic polymer to fabricate membranes for solid-state mini ISEs and then their potentiometric responses evaluated. To investigate further the complex forming behaviour, the dependence of the selectivity on anionic sites was examined. This was carried out by varying the amount of anionic sites in the membrane and observed the changes of the potassium selectivity [10,11]. A lipophilic mono-benzo-15-crown-5 substituted with NO<sub>2</sub> groups (B143) (Figure 2) was also included in the study as a comparison. Finally the performance of the solid-state ISEs was assessed for possible application in blood electrolyte assay by using artificial serum electrolytes.



**Figure 1 :** The structure of BME44, 2-dodecyl-2-methyl-1,3-propanediyl-bis[N-(5'-nitro(benzo-15-crown-5)-4'-yl] carbamate (BME44)



**Figure 2 :** The structure of B143, hexadecyl-[N-(5'-nitro(benzo-15-crown-5)-4'-yl)] carbamate (B143).

### Experimental

**Materials :** Hexadecyl-[N-(5'-nitro(benzo-15-crown-5)-4'-yl)] carbamate (B143) and 2-dodecyl-2-methyl-1,3-propanediyl-bis[N-(5'-nitro(benzo-15-crown-5)-4'-yl)] carbamate (BME44) (Both were gifts of Technical University, Budapest) were used as potassium selective ionophores and the lipophilic salt used was potassium tetrakis(p-chlorophenyl borate) (KTCIPB) (Aldrich). The non-plasticised polymer used for the ion-selective film was a methacrylate-acrylate copolymer with the ratio of methyl methacrylate monomer to n-butyl acrylate monomer of 1:10.7. This copolymer has a molecular weight distribution (Mw) of 154,694 Da and glass transition temperature of approximately -30°C. The detail method of preparation of this copolymer was described in our previous papers [12,13]. Polymer used for the intermediate layer of the solid-state ISE was poly(hydroxyl ethyl methacrylate)(pHEMA) purchased from Aldrich. The substrate of the solid-state ISE is a Ag/AgCl disc obtained from EOS. Solvents employed for membrane casting were 1,4-dioxane and dichloromethane (Aldrich). Salts for the preparation of standard solutions were chlorides of potassium, sodium, calcium, magnesium, lithium and ammonium, all were analytical grade chemicals (Aldrich). All standard solutions were prepared in doubly deionised water purified by an Elgastat UHP water deioniser.

### Preparation of solid-state potassium ISEs

**Deposition of the inner layer.** Fabrication of the inner layer of the ISE was performed by drop coating an appropriate amount of a solution of 6 % (w/v) of pHEMA in a mixture of water-dioxane (2:3 v/v) onto a Ag/AgCl disc using a Gilson pipette. It was then left for 24 h at approximately 4°C to allow the slow evaporation of the solvent. After evaporation was completed, it was then conditioned in 0.1M KCl for 2 h before the ion-selective film was deposited.

**Deposition of ion-selective film.** Ion-selective membrane cocktails were prepared by weighing 50 mg of the polymer and the required amount of other membrane components (BME44 or B143 as ionophores and KTCIPB as lipophilic salt, Table 1). The mixture was dissolved in 1.0 ml of dichloromethane to yield a solution with suitable viscosity for drop coating. Before the ion-selective film was deposited onto the pHEMA layer, excess liquid on the pHEMA layer was removed. To ensure that the thickness of the film deposited is approximately the same, a fixed volume of the polymer solution was deposited each time. Evaporation of the membrane solvent from ion-selective layer was carried out at approximately 4°C for 24 h. Once dried, the solid-state ISE was ready to be used after 2 h conditioning in 0.1 M KCl solution.

**Table 1 :** The composition of ISE membranes used for the potentiometric studies of BME and B143 ionophores

**Membrane components	Electrode No.					
	1	2	3	4	5	6
KTCIPB (mol%)	20	50	73	15	27	70
BME44 (% w/w)	2.0	1.9	2.2			
B143 (% w/w)				2.6	2.8	2.6

\*\*Copolymer methacrylate-acrylate : Approximately 97(% w/w)

**Potentiometric testing of solid-state ISEs**

A solid-state ISE ready for testing was mounted onto a Perspex cell containing a test solution chamber. The electrode was sealed by an O-ring located in the Perspex cell. To make contact with a shielded cable, conductive epoxy (RS) was applied to the back of the electrode. This is then connected to a Jenway 3345 Ion Meter. The reference electrode used was a double junction reference with 0.1M TrisHCl (pH 7.0) as internal reference solution and 1.0 M lithium acetate as bridge electrolyte. The detail experimental setup has been published elsewhere [12, 13]. The difference in potential (mV) of the ISE and the reference electrode was then recorded on a Gould BS273 strip chart recorder.

Standard solutions of potassium were prepared in the concentration range of 10<sup>-7</sup>-10<sup>-1</sup> M. After exposing each electrode to these standard solutions, the potentiometric response was noted when the EMF changed by < 1.0 mV/min. Six to seven EMF values were obtained from the range 10<sup>-7</sup> to 10<sup>-1</sup> M and used in plotting the EMF (mV) versus logarithmic activities plot. Corrections for ion activities were made with the Debye-Huckel equation. Interference studies were carried out by the separate solution method (SSM) with a concentration of 0.1M for all interference cation solutions<sup>12</sup>. Selectivity coefficient ( $K_{K_j}^{Pot}$ ) was calculated based on Nernstian responses (59.16 mV/decade at 25 °C) [14-16]. The reproducibility of the ISEs was tested by exposing the electrodes alternatively in 10<sup>-2</sup> and 10<sup>-3</sup> M standard KCl solutions [15]. The electrodes were rinsed several times with doubly deionised water between tests. Three to five EMF readings were recorded for each standard solution. All experiments were conducted at room temperature (25±2°C).

Test with artificial blood serum electrolyte. For the testing of solid-state potassium ISEs with artificial

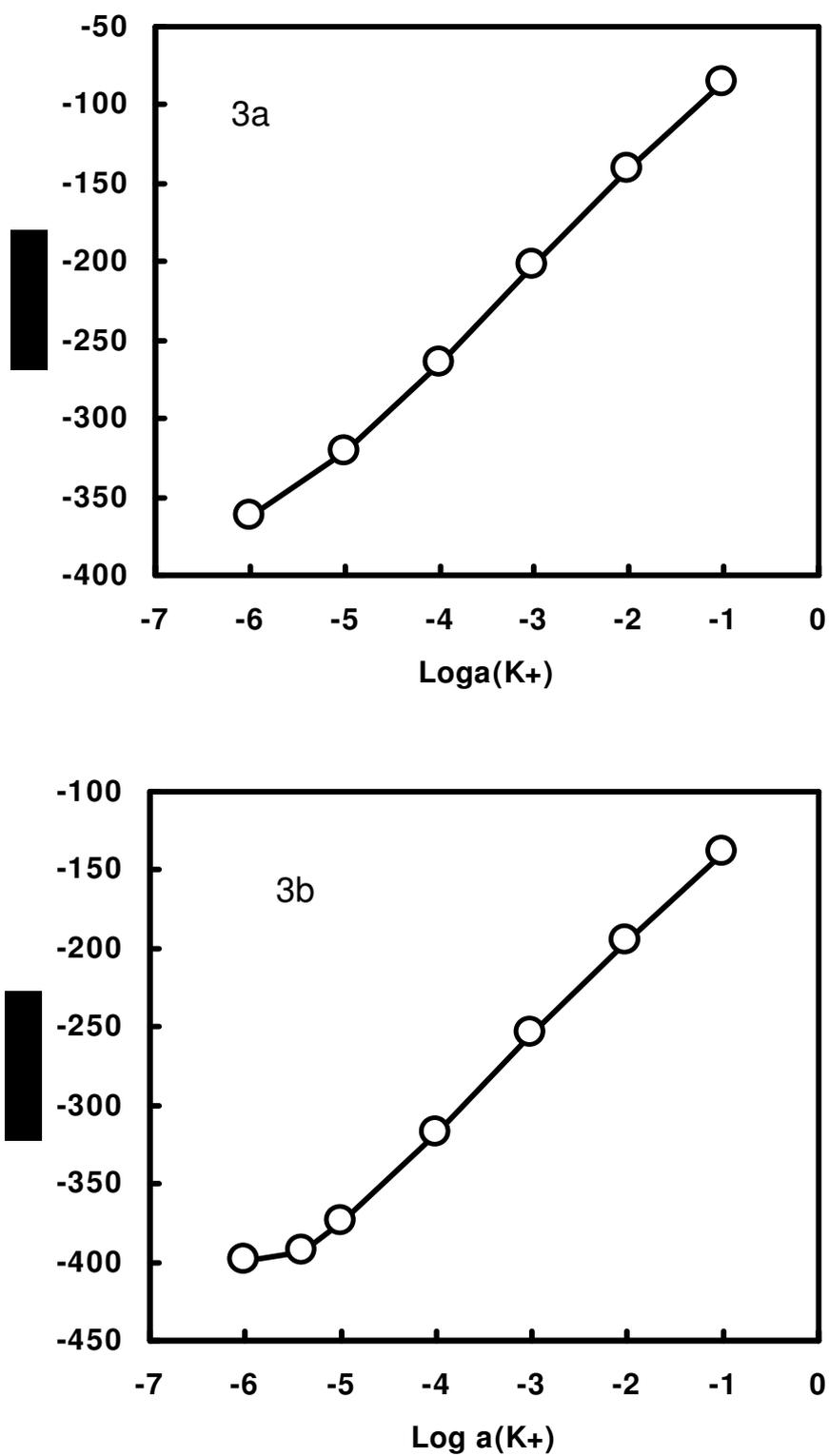
blood serum electrolyte, a test solution contained physiologically important ions of concentrations similar to those found in blood serum was prepared. The ionic concentration of the artificial serum electrolyte was sodium chloride 140mM, sodium hydrogen carbonate (24mM), magnesium chloride (0.6mM) and calcium chloride (1.1 mM) [9]. Standard KCl with concentrations from 10<sup>-7</sup>-10<sup>-1</sup> M was prepared using the artificial serum electrolyte as background. The ISEs were then tested using these standard solutions by the procedure mentioned above.

**Results and Discussion****Potentiometric responses of ISEs and variation in KTCIPB content**

The changes in KTCIPB content from 20 to 70 mol% relative to BME44 have little effect on the potentiometric response slope of the solid-state ISEs. All the slope values are near to theoretical with similar linear response ranges (Table 2). An example of potentiometric response of one such solid-state ISE is shown in Figure3 (a). For the solid-state electrodes with B143 as ionophore, larger variation of the response slope and a decrease in the linear range were observed as the KTCIPB content increased. Near theoretical response slope was obtained when KTCIPB concentration in the membrane was 15 mol% relative to B143. The potentiometric response of B143 solid-state ISE with 15 mol% KTCIPB towards potassium concentration changes is shown in Figure 3(b). As the KTCIPB content increased to 70 mol%, the lower detection limits of both type of electrodes deteriorated. Mathison and Bakker [17] have predicted that maximum lower detection limit is reached when complete saturation of ionophore at the membrane-inner electrolyte interface occurred. This is related to the free ionophore concentration.

**Table 2 :** The potentiometric response of solid-state ISEs with BME44 and B143 as potassium sensing ionophores.

Sensor	Slope (mV/decade)	Linear range (- log C) ( R2 = 0.999)	Detection limit (-logC)
<b>(BME44)</b>			
1	57.9±0.3	6.0-1.0	6.1
2	59.5±0.6	5.0-1.0	5.8
3	57.2±0.6	5.0-1.0	5.4
<b>(B143)</b>			
4	58.7±0.7	5.4-1.0	<5.4
5	52.5±0.6	4.0-1.0	<5.5
6	56.9±0.3	4.0-1.0	4.6

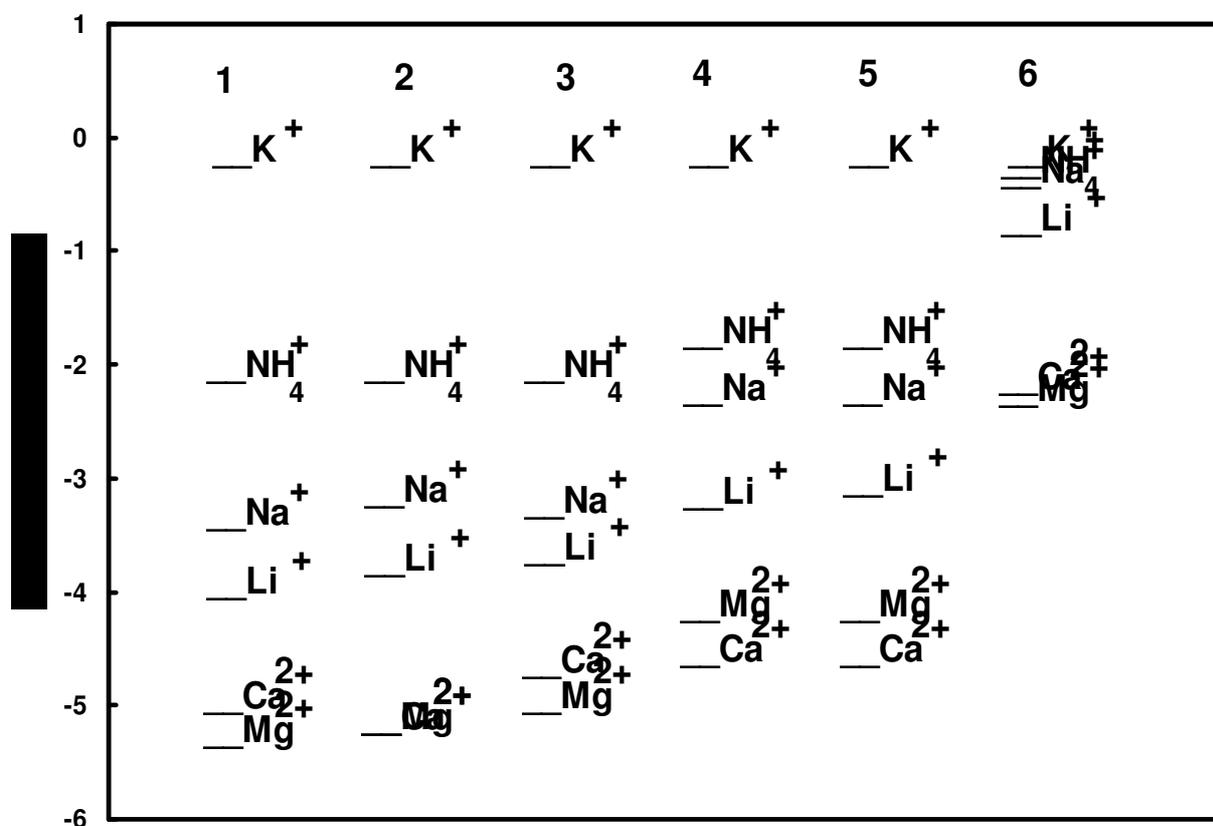


**Figure 3 :** The potentiometric responses of sensor 1 (BME44 ionophore, 3a ) and sensor 4 (B143 ionophore, 3b).

Under this limiting condition, a maximum concentration gradient across the ion-selective membrane exists which leads to the leaching of cations into the sample side, hence resulted in higher detection limit of the electrode. As the lipophilic site increases, the free ionophore concentration should decrease and hence the lower detection limit decreases. But the observation shown in Table 2 is contradicted to Mathison and Bakker's prediction. Because their prediction was based on the ability of the ions moving across the plasticised membrane from the inner solution-membrane interface to the outer membrane-sample interface, it is not sure whether such mechanism could occur in the present study where the inner layer is a hydrated solid and the membrane is non-plasticised. Perhaps some other mechanism may be required to explain the behaviour observed here.

In terms of selectivity, the selectivity pattern of BME44 ISEs did not appear to be affected by changes in KTCIPB content but in contrast, the selectivity of the B143 ISEs towards potassium ion was lost when the KTCIPB content reached 70 mol% (Figure 4). It has been shown that in plasticised PVC membranes, for an ionophore forming 2:1 ionophore-ion complexes with

the primary ion, a deterioration in selectivity of the ionophore towards the primary ion will occur when the lipophilic anions content approaches 50 mol% relative to the ionophore [10,11]. This is because at this lipophilic anion-ionophore concentration ratio, almost all ionophores are bound as complexes, and the absent of free ionophores leads to the lost of selectivity. The changes of selectivity pattern of the B143 electrodes with the increase in the KTCIPB contents are consistent with this. On the contrary, the selectivity behaviour for BME44 electrodes with the increase in KTCIPB contents did not follow that of the formation of 2:1 ionophore-ion complexes. A good selectivity to potassium ions still observed even the KTCIPB exceeded 50 mol%. However, BME44 is a bis-crown, the presence of two benzo-15-crown-5 in each molecule allowed intramolecule complexation with potassium ions as compared with the intermolecular complexation in the case of the mono-crown B143 (Figure 2). Thus, it is envisaged that no loss of selectivity of potassium over other cations will occur for the BME44 electrodes when the KTCIPB content relative to the bis-crown ether molecule until the concentration attains approximately 100 mol%.



**Figure 4 :** The selectivity behaviour of potassium sensors with BME44 (sensors 1-3) and B143 (sensors 4-6) ionophores.

**Table 3 :** Comparison of potentiometric behaviour of solid-state ISE with BME44 or B143 ionophores entrapped in non-plasticised methacrylate-acrylate (Met-Acrylate) membranes with conventional ISEs based on plasticised PVC membranes.

Polymer/ Plasticiser/ Ionophore	Slope (mV/ decade)	Linear concentration range (-logC) (R2 = 0.999)	Selectivity coefficient (Log $K_{K,j}^{Pot}$ )				
			j = NH <sub>4</sub> <sup>+</sup>	Li <sup>+</sup>	Na <sup>+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>
(a)PVC/NPOE/BME44	57.9	1-4	-2.1	-3.8	-3.2	-4.5	-5.0
(a)PVC/DNA/BME44	58.1	1-4	-2.1	-3.8	-3.3	-4.5	-4.3
Met-Acrylate/BME44	57.9	1-5	-2.0	-3.8	-3.2	-4.8	-5.1
(b)PVC/NPOE/B143	52.6	1-4	-1.8		-2.6	-4.4	-4.0
Met-Acrylate/B143	58.6	1-5.4	-1.7	-3.0	-2.1	-4.4	-4.0

(a) Ref. 9 (b) Ref. 2

NPOE = 2-nitrophenyl octyl ether; DNA = bis-(1-butylpentyl)adipate.

Evidence of intramolecular ionophore-ion complexation of the BME44 in the unplasticised membranes is demonstrated by the better potassium ion-selectivity of the electrodes, particularly against sodium, lithium and magnesium ions when compared to electrodes based on B143 (Figure 4). The enhancing effect of bis-crown ether complexation on potentiometric ion-selectivity is well documented [1,18]. Selectivity enhancement may also be attributed to the intramolecular interaction between the NH and NO<sub>2</sub> groups at the different ends of the bis-crown ether molecules. Such effect was not observed in NO<sub>2</sub>-substituted mono-crown ethers [2].

The study of potentiometric behaviour of NO<sub>2</sub>-substituted bis-crown or mono-crown ethers is not new and has been thoroughly investigated in plasticised PVC membranes [2]. But what is interesting here is that the potentiometric behaviour of these ionophores in the non-plasticised methacrylic-acrylic polymer is similar to those of plasticised PVC (Table 3). Despite the absence of plasticiser and a much higher glass transition temperature of the methacrylic-acrylic polymer when compared to plasticised PVC (-31°C cf. -68°C), various mechanisms which lead to complexation in the membrane probably can still occur. This also shows that for an ionophore to exhibit its complexation character, a high mobility environment such as a plasticised membrane may not be necessary. It is not sure whether

the methacrylic-acrylic polymer itself plays the role of the plasticiser. Further investigation is required for greater understanding of this behaviour.

#### ***BME44 based solid-state ISE for potassium assay in blood or urine***

The potassium ion-selectivity of the solid-state ISE against other physiological important cations is better than that required for clinical analysis of both blood and urine except for the selectivity against sodium ion where it is close to the required value (Table 4). The presence of artificial serum electrolyte as a background matrix has little effect on the performance of the solid-state potassium ISE. Only a slight decrease in both the response slope and linear response range and deterioration of the detection limit of the electrode was observed. The reproducibility of the electrode in the blood serum electrolyte matrix is satisfactory and better than that required for clinical assay of blood potassium (Table 4). These results showed that BME44 based solid-state potassium ISE fabricated from non-plasticised methacrylic-acrylic polymer membrane may be used for the clinical analysis of blood or urine potassium. Furthermore, the absence of plasticiser and the good adhesion properties of this polymer may be an advantage in the fabrication of solid-state devices for implantable ion sensors.

**Table 4 :** The performance of a BME ionophore based solid-state ISE in pure KCl and artificial serum electrolyte

	Measured Characteristics		Required characteristics	
	KCl Only	KCl in artificial serum electrolyte	Blood <sup>(a)</sup>	Urine <sup>(b)</sup>
Selectivity coefficient, (Log $K_{K,j}^{Pot}$ ):				
j =				
Li <sup>+</sup>	-3.5		<-1.7	
Na <sup>+</sup>	-3.1	-3.12	<-3.6	<-3.1
NH <sub>4</sub> <sup>+</sup>	-2.0			
Mg <sup>2+</sup>	-4.8		<-2.8	<-0.9
Ca <sup>2+</sup>	-4.5		<-2.9	<-0.9
Slope(mV/decade)	57.2	56.1		
Range (mM)	0.01-100	0.1-100		
Detection limit, (-logC)	5.0	4.0		
Reproducibility(mV) (n=5)			1.3	
1.0 mM KCl		0.4		
10.0 mM KCl		0.4		

(a)Reference 19 (b) Reference 20

**Conclusion**

The results of this work have shown that in a non-plasticised membrane based on the polymer of methacrylic-acrylic, NO<sub>2</sub>-substituted bis- or mono-crown ethers exhibited potentiometric behaviour similar to those based on conventional plasticised PVC membranes. The satisfactory selectivity and reproducibility of the solid-state ISEs based on BME44/non-plasticised methacrylic-acrylic membrane implies that such electrodes may be suitable for clinical analysis of potassium ion in body fluids.

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