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Polymeric Membrane Sodium-Selective Electrodes Based on Lipophilic Calix[4]arene Derivatives

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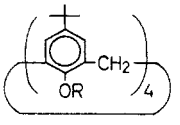
Three kinds of lipophilic calix[4]aryl ester and amide derivatives (1-3) have been designed for Na^+-selective polymeric membrane electrodes. The calixarene ionophores have thus proved to be excellent Na^+ neutral carriers. Furthermore, selection of the electrode membrane conditions such as membrane solvents and lipophilic salts allowed high Na^+ selectivities against K^+, the selectivity coefficient for Na^+ with respect to K^+ being 3.8×10^{-3} in the electrode based on calix[4]aryl decyl ester 1. The calixarene-based Na^+-selective electrode was successfully applied to Na^+ assay in human blood sera.

Considerable efforts have been directed toward the development of neutral carrier-type Na^+-selective electrodes as alternatives of Na^+-selective glass electrodes, because glass electrodes have some difficulties in clinical uses; i.e. special treatment is required at regular intervals to prevent electrode deterioration. Simon et al. have designed acyclic polyether-amide derivatives as neutral carriers for Na^+-selective electrodes (*1*) and applied them to Na^+ microelectrodes for intracellular studies (*2*). We have synthesized bis(12-crown-4) derivatives as Na^+ ionophores, taking advantage of high Na^+ selectivity based on the bis(crown ether) effect (*3*), and fabricated Na^+-selective electrodes of practical use (*4*).

Cyclic oligomers of *tert*-butylphenol-formaldehyde condensates, what we call calixarenes, when in the *cone* conformation, are apt to form inclusion compounds with small organic molecules being bound in the cavities. Incorporation of carbonyl groups such as ester, amide, and ketone linkages into the phenolic oxygen atoms of calixarenes provides them with ionophoric properties. In that case, a metal cation is in the cavity of the calixarene, interacting with the carbonyl and phenolic oxygen atoms. The ionophoric calixarene derivative are, therefore, candidates for neutral carriers of ion-selective electrodes. Diamond et al. and we independently applied calix[4]arenes carrying carbonyl groups to Na^+-selective electrodes (*5*, *6*). We have chosen lipophilic calix[4]arene derivatives incorporating alkoxycarbonyl and alkylcarbamoyl substituents, **1-3**, as the Na^+ neutral carriers. In this publication, we describe the electrode properties and membrane optimization of the Na^+-selective polymeric membrane electrodes based on the lipophilic calix[4]arene derivatives. As an application of the calixarene-based electrodes, Na^+ assay in human blood sera is also mentioned.



(1) R = $CH_2COOC_{10}H_{21}$

(2) R = $CH_2CON(n\text{-}C_4H_9)_2$

(3) R = CH_2COO⬡

EXPERIMENTAL SECTION

Synthesis of Calixarene Neutral Carriers. The parent calixarene, *tert*-butylcalix[4]arene, was prepared according to the Gutsche method (*7*). Decyl α-bromoacetate and cyclohexyl α-bromoacetate were obtained by esterification of bromoacetic acid with decyl and cyclohexyl alcohols in benzene in the presence of H_2SO_4. Treating chloroacetyl chloride with di-*n*-butylamine and triethylamine (HCl accepter) in benzene afforded *N,N*-di-*n*-butyl-α-chloroacetamide. Calix[4]aryl decyl ester **1** was synthesized by the following reaction of *tert*-butylcalix[4]arene with decyl bromoacetate. Under a nitrogen atmosphere, *tert*-butylcalix[4]arene (2.03 mmol) was dissolved in 150 mL of a dry mixed solvent (1:1) of *N,N*-dimethylformamide (DMF) and tetrahydrofuran (THF) by heating. To the solution, which had been cooled to room temperature, was added NaH (37.5 mmol) and decyl bromoacetate (23.1 mmol). The mixture was then refluxed for 3 h under a nitrogen atmosphere. After the reaction, the solvent was evaporated off and then water was added to the residue. The mixture was extracted with chloroform and the organic layer was dried over $MgSO_4$. The chloroform and the excess of decyl bromoacetate were removed under reduced pressure. The residue was subjected to silica gel column chromatography (benzene), which afforded pure product **1**. Calix[4]aryl dibutylamide, **2**, and calix[4]aryl cyclohexyl ester, **3**, were obtained by similar reactions using *N,N*-dibutylchloroacetamide and cyclohexyl bromoacetate, respectively. Silica gel chromatography using hexane/ethyl acetate gave the pure products. **1** (yield, 30%): colorless oil; ^1H NMR ($CDCl_3$) δ 0.87 (12 H, t, CH_3CH_2), 1.05 (36 H, s, $(CH_3)_4C$), 1.26 (64 H, s, $CH_3(CH_2)_8$), 3.18 and 4.84 (8 H, d, $PhCH_2Ph$), 4.10 (8 H, t, OCH_2CH_2), 4.77 (8 H, s, OCH_2CO), 6.77 (8 H, s, phenyl H). Anal. Calcd for $C_{92}H_{144}O_{12}$: C, 76.62; H, 10.06. Found: C, 76.54; H, 10.37. **2** (yield, 41%): white crystal; mp 208.5-209.5 °C; ^1H NMR ($CDCl_3$) δ 0.90 (24 H, t, CH_3CH_2), 1.08 (36 H, s, $(CH_3)_4C$), 1.2-1.6 (32 H, m, $CH_3(CH_2)_2$), 3.16 and 5.24 (8 H, d, $PhCH_2Ph$), 3.27 (16 H, t, CH_2N), 5.02 (8 H, s, OCH_2CO), 6.81 (8 H, s, phenyl, H). Anal. Calcd for $C_{84}H_{132}O_8N_4$: C, 76.09; H, 10.03; N, 4.23. Found: C, 75.77; H, 10.14; N, 4.13. **3** (yield, 31%): white crystal (from ethanol); mp 182-183 °C; ^1H NMR ($CDCl_3$) δ 1.08 (36 H, s, $(CH_3)_4C$), 1.2-2.0 (40 H, m, $CH_2(CH_2CH_2)_2CH_2CO$), 3.13 and 4.86 (8 H, d, $PhCH_2Ph$), 4.80 (12 H, s, OCH_2CO_2CH), 6.77 (8 H, s, phenyl H). Anal. Calcd for $C_{76}H_{104}O_{12}$: C, 75.46; H, 8.67. Found: C, 75.22; H, 8.70.

Other Chemicals. Poly(vinyl chloride) (PVC) with an average polymerization degree of 1100 was purified by reprecipitation from THF in methanol. The plasticizers or membrane solvents, *o*-nitrophenyl octyl ether (*8*), *o*-nitrophenyl phenyl ether (NPPE)

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(9), o-fluorophenyl o-nitrophenyl ether (FPNPE) (10), and dipentyl phthalate (DPP) (11) were prepared according to the procedures in the literature. Bis(2-ethylhexyl) sebacate (DOS) and tris(2-ethylhexyl) phosphate (TEHP), which were purchased from Wako, were purified by vacuum distillation. Potassium tetrakis(p-chlorophenyl)borate (KTpClPB) was prepared according to the reported procedure (12). Sodium dipicrylamide (NaDPA) and sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]-borate (NaTFPB) were used as received from Tokyo Kasei and Dotite, respectively. Alkali and alkaline-earth metal and ammonium salts employed were of analytical grade. Water was deionized and distilled.

Electrode Fabrication. The general procedure for casting the electrode membranes was as follows: PVC (50 mg), a plasticizer or membrane solvent (135 mg), a calixarene neutral carrier (10–30 mg), and a lipophilic salt (1 mg) were dissolved in THF (3 mL). The solution was poured into a flat Petri dish (21 mm i.d.). The THF was air-evaporated slowly to yield an elastic, translucent membrane of 0.1–0.2 mm thickness. For the experiments of the membrane solvent effect, the membrane composition was 25.5 wt % for PVC, 68.9 wt % for membrane solvent, 5.1 wt % for neutral carrier, and 0.5 wt % for NaDPA. The optimized membrane consisted of 24.3 wt % PVC, 65.5 wt % membrane solvent, 9.7 wt % neutral carrier, and 0.5 wt % NaDPA. A disk of 5 mm diameter was cut from the membranes and then incorporated into an electrode body of a Philips IS-561. An internal filling solution of 1×10^{-3} M NaCl was injected into the electrode, which was then conditioned by soaking in the NaCl solution overnight.

Emf Measurements. The measurements were performed at 25 °C by using a pH/mV meter. A double-junction type Ag·AgCl electrode was used as the external reference electrode. The representative electrochemical cell for the emf measurements was Ag·AgCl/1×10^{-3} M NaCl/PVC membrane/measured solution/1×10^{-1} M NH_4NO_3/4 M KCl/AgCl·Ag. The selectivity coefficients, k^{Pot}_{NaM}, were determined by a mixed solution method (Fixed Interference Method). The constant background concentrations of interfering ions were 5×10^{-2} M for alkali-metal ions and H^+, and 5×10^{-1} M for alkaline-earth metal ions and NH_4^+. The calculation details for the ion activities and selectivity coefficients are as described previously (13).

Serum Sodium Assay. The Na^+ assay in blood sera was carried out with the Na^+-selective electrodes by applying Gran's plot method (14). A control serum (Nescol-X, Kaketsuken, normal human blood) was diluted 10- or 20-fold and spiked with Na^+ in concentrations ranging from 100 to 200 mM. Real blood samples (10 mL each) of normal human were collected and allowed to stand for 40 min. The whole blood was then subjected to centrifugation, which produced about 4 mL of blood serum. The real blood sera were diluted 10-fold on the potentiometry. On the flame photometry for comparison, both the control and real sera were diluted 200-fold.

RESULTS AND DISCUSSION

Synthesis of Calixarene-Type Na^+ Neutral Carriers. We decided to synthesize *tert*-butylcalix[4]arenes incorporating ester- or amide-carbonyl groups at the phenolic oxygen atoms. We also envisaged enhancement in the compatibility of the calixarene neutral carriers with the membrane polymer (PVC) and therefore in the membrane stability by introducing lipophilic moieties to the parent calixarene. Thus, calix[4]aryl decyl ester (1), *N,N*-di-*n*-butylamide (2), and cyclohexyl ester (3) were selected as the calixarene neutral carriers and synthesized by the Williamson reaction of *tert*-butylcalix[4]arene with corresponding α-halogenated acetate and acetamide derivatives in the presence of sodium hydride as the base. This reaction was thought to afford mono-, di-, tri-, and tetrasubstituted products. A large excess of the α-halogenated acetate or acetamide derivatives was, therefore, employed to promote the formation of the tetrasubstituted products, 1 through 3. The products were purified by chromatography and recrystallization. ^1H NMR spectroscopy in any of the calixaryl ester and amide derivatives showed one pair of doublets for their $PhCH_2Ph$ methylene protons (see the NMR data in the Experimental Section), verifying their *cone* conformation (7) which is of crucial importance for the cation-complexing ability of the calixarene derivatives. Calixarene neutral carrier 1 holds quite high solubilities in popular organic solvents and high compatibilities with the membrane polymer and solvents (plasticizers) employed here. The other neutral carriers, 2 and 3, possess slightly lower solubilities in the plasticizers compared with 1, but they can still afford quite homogeneous membranes with the solvents except for TEHP.

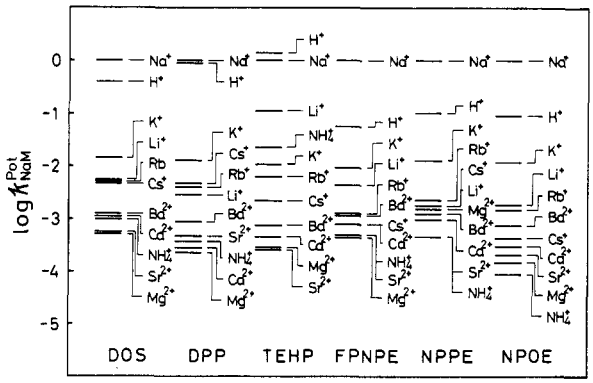

Figure 1. Selectivity coefficients for Na^+ in electrodes of calixaryl decyl ester 1 with different membrane solvents. For details see Experimental Section.

Optimization of Membrane Conditions. Effects of membrane solvents on the sensitivity and selectivity of the Na^+-selective electrodes based on three of the calix[4]arene neutral carriers, 1–3, were elucidated at first. Selected as the membrane polymer was PVC, which is the most popular membrane polymer for neutral-carrier-type ion-selective electrodes. Also, a trace amount of NaDPA was included in the electrode membranes to alleviate their membrane impedance. The membrane solvents tested were NPOE, NPPE, and FPNPE as the phenyl ether type solvents, DOS and DPP as the diester types, and TEHP as the phosphate type. Selectivity coefficients for Na^+ with respect to other alkali and alkaline-earth metal ions, NH_4^+, and H^+ in the electrodes of calixaryl decyl ester 1 are summarized in Figure 1. Any of the 1-based Na^+ electrodes with the membrane solvents showed a Nernstian or near-Nernstian response to Na^+ activity changes in the activity ranges of 3×10^{-5} or 1×10^{-4} to 1 M, the slopes for Na^+ calibration graphs ranging from 55 to 59 mV decade^{-1}. Although there is hardly any drastic effect of membrane solvent on the ion selectivity of the 1-based electrodes, the phenyl ether type solvents, especially FPNPE, are superior to the diester-type and phosphate-type ones in Na^+ selectivity against K^+, which is an important factor on practical uses of Na^+-selective electrodes. In the Na^+ electrodes of calixaryl amide 2, the diester-type solvents, DOS, and DPP, gave good results, allowing a Nernstian response to Na^+ activity changes in the activity range of 1×10^{-4} to 1 M and high Na^+ selectivities as seen in Figure 2. Without the lipophilic salt in the 2/DOS membrane, the membrane selectivities were changed slightly, the Na^+ selectivity against K^+ being somewhat diminished. On the contrary, the phenyl ether type solvents allowed a poor response of 45 mV decade^{-1} to the Na^+ activity changes in the 2-based electrodes. It might be due to the specific interaction of the phenyl ether type solvents with the calixaryl amide neutral carrier, possibly with the amide linkages, in the membranes. The 2-based electrode membranes with TEHP could not be fabricated due to the poor solubility of the neutral carrier in the solvent. In the Na^+ electrodes based on 3, a Nernstian or near-Nerstian response to Na^+ activity changes (slopes of 54–59 mV decade^{-1}) was also attained in the range of 1×10^{-4} to 1 M with the diester-type and phenyl ether type membrane solvents. The diester-type solvents, DOS and DPP, gave better results than

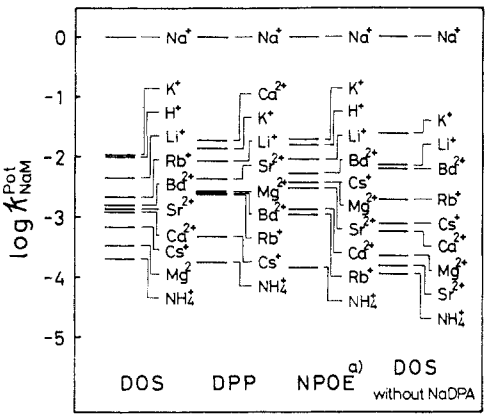

Figure 2. Selectivity coefficients for Na^+ in electrodes of calixaryl dibutylamide **2** with different membrane solvents, (a) apparent values.

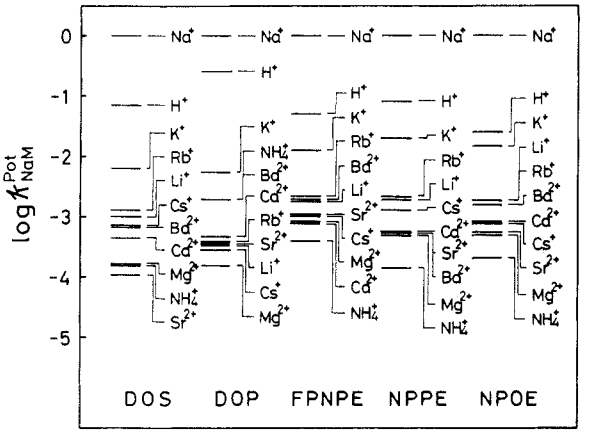

Figure 3. Selectivity coefficients for Na^+ in electrodes of calixaryl cyclohexyl ester **3** with different membrane solvents.

Table I. Selectivity Dependence on Neutral Carrier Concentration in Na^+-Selective Electrode with 1/FPNPE/NaDPA Membrane

neutral carrier concn, wt %	5.1	9.7	14
log k^{Pot}_{NaK}	−2.18	−2.42	−2.42

the phenyl ether type solvents in the Na^+ selectivity against K^+ in the **3**-based electrodes (Figure 3). This membrane solvent tendency in the **3**-based electrodes is different from that in the electrode based on **1**, which is also a calixaryl ester derivative. It is thus considered from the above examination regarding the membrane solvent effect that combinations of 1/FPNPE, **2**/DOS, and **3**/DOS, especially the first and last combinations, are prominent in the membrane systems of the Na^+-selective electrodes.

Effects of the neutral carrier concentration in the electrode membrane and the kind of lipophilic salt on the Na^+ selectivities were examined for the 1/FPNPE membrane system. Increasing neutral carrier concentration generally enhanced the Na^+ selectivity against K^+ in the 1/FPNPE membrane system, but even the concentration of 14 wt % allowed a similar Na^+ selectivity to that of 9.7 wt % (Table I). The neutral carrier concentration of 9.7 wt % seems reasonable to adopt in the 1/FPNPE membrane system. Since a trace amount of lipophilic salt may improve ion selectivities of neutral-carrier-based membranes more or less (*15*), two other lipophilic salts besides NaDPA were tested for their usefulness. A comparison of selectivity coefficients on the Na^+-selective 1/FPNPE membranes containing three different lipophilic salts is depicted in Figure 4, showing that NaTFPB can be an alternative of the lipophilic salt. NaDPA, however, gives still better results than NaTFPB in the Na^+ selectivities except against H^+. KTpClPB does not seem suitable for the 1/

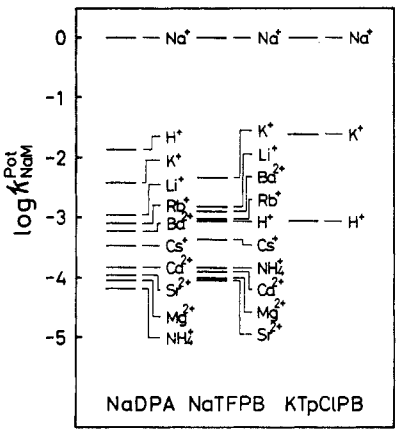

Figure 4. Selectivity coefficients for Na^+ in electrodes of 1/FPNPE membrane containing different lipophilic salts. The membrane composition is identical with that for the optimized membrane.

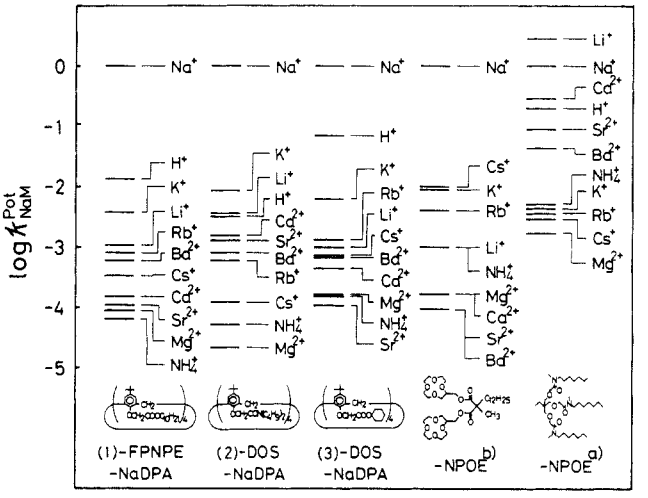

Figure 5. Selectivity comparison among Na^+-selective electrodes of lipophilic calixarene neutral carriers **1**–**3** and previous neutral-carrier-type PVC membrane Na^+ electrodes: (a) ref 1; (b) ref 4.

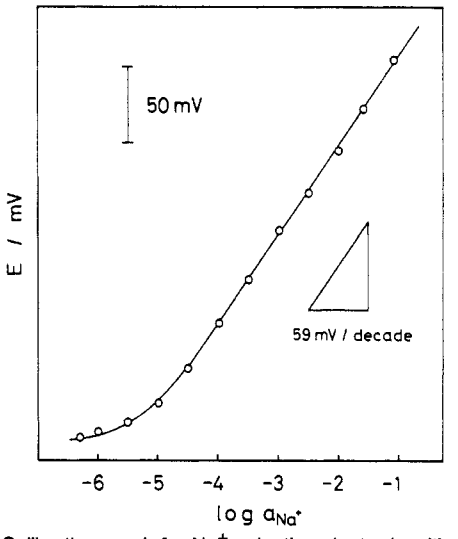

Figure 6. Calibration graph for Na^+-selective electrode with optimized 1/FPNPE membrane.

FPNPE membrane of the Na^+ electrode.

Figure 5 displays a selectivity comparison among the neutral-carrier-type Na^+-selective electrodes based on the three calixarene-containing membranes optimized here, and the PVC membranes of two previous Na^+ neutral carriers of Simon's (*1*) and ours (*4*), which possess one of the most excellent Na^+ selectivities against K^+ in the neutral-carrier-type

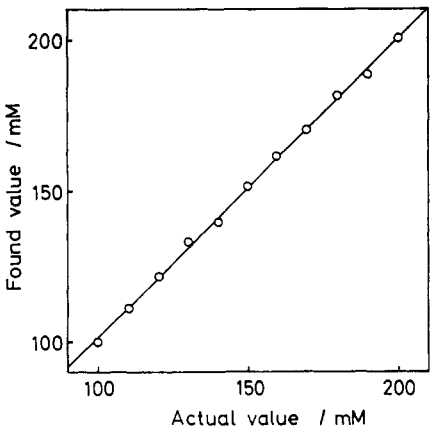

Figure 7. Correlation of actual values of Na^+ concentrations in control sera and those found with 1-based Na^+ electrode. For details see Experimental Section.

Table II. Sodium Assay in Human Blood Sera with Calixarene-Based Na^+-Selective Electrode with 1/FPNPE/NaDPA Membrane[a]

	Na^+ concn, mM		
sample	ISE method[b]	flame photometry	CV,[c] %
1	140.0	138.0	1.3
2	140.2	137.4	4.6
3	134.3	132.6	4.6
4	132.0	132.0	3.6
5	141.3	138.0	4.3

[a] For details see Experimental Section. [b] Mean of four repeat measurements. [c] Coefficient of variation.

membranes for Na^+-selective electrodes. Three of the calixarene-based membranes are nearly equal or even superior to the previous neutral-carrier-containing PVC membranes in the Na^+ selectivities with respect to K^+. In the Na^+ selectivity with respect to Ca^{2+}, Mg^{2+}, and NH_4^+, the calixarene-based membranes are comparable to the membrane of our bis(crown ether) derivative and are superior to that of Simon's polyether amide derivative. The 1/FPNPE membrane system presents the best Na^+ selectivity with respect to K^+ of the three calixarene-based membrane systems, the selectivity coefficient (k_{NaK}^{Pot}) being 3.8×10^{-3}. The selectivity coefficient of Na^+ with respect to K^+ is still not better than that for NAS_{11-18} glass electrode at high pH conditions (pH 11), 3.6×10^{-4}, which is the best known so far but is comparable to that at practical pH conditions (pH 7), 3.3×10^{-3} (*16*). Employment of pure liquid membrane might further promote the Na^+ selectivity of 1, as observed with a calixaryl methyl ester system by Diamond et al. (*5*). One of the advantages for the calixarene neutral carriers over the previous ones may be high lipophilicity that is required for high durability of the resulting membrane electrode.

Serum Na^+ Assay. Attempts were made to assay Na^+ in blood sera potentiometrically by using the Na^+-selective electrode with the optimized 1/FPNPE membrane, which exhibited a Nernstian response to Na^+ activity change in the activity range of 3×10^{-5} to 1 M (Figure 6). Employed as the samples were control sera spiked with Na^+ at the concentrations of 100–200 mM and real blood sera of normal humans. Grans' plot method was adopted here for the potentiometric Na^+ assay. The Na^+ concentrations in the serum samples obtained potentiometrically were compared with those obtained by flame photometry. The results for the Na^+-spiked control serum samples are demonstrated in Figure 7, showing that the Na^+ concentrations found with the calixarene-based Na^+-selective electrode are in good agreement with the flame-photometric data. This is also the case even in a Na^+ assay in the real blood sera, as shown in Table II. The coefficients of variation were within several percent. The results for Na^+ assay in both the control and real human sera indicated that the potentiometric method using the Na^+-selective electrode with the 1/FPNPE membrane is quite reliable. Accordingly, the Na^+-selective electrodes based on the lipophilic calix[4]arene derivative **1–3** are very promising for Na^+ assay in human blood sera.

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