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Silicone-rubber membrane sodium-ion sensors based on calix[4]arene neutral carriers

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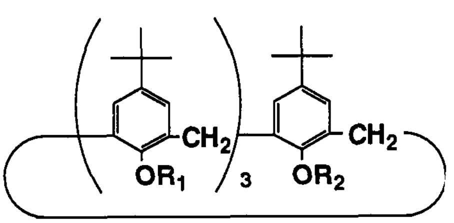
Abstract: Excellent calix[4]arene neutral carriers have been designed for sodium ion sensors, especially for sodium ion-sensitive field-effect transistors (Na^+ISFETs) based on silicone rubber membranes. Calix[4]arene neutral carriers incorporating unsymmetrical structures and/or oligosiloxane moieties, which are highly dispersible in silicone rubber, have realized high performance of silicone-rubber-based sodium-ion sensors, i.e., high sensitivity, selectivity, and durability. The Na^+ISFETs based on silicone rubber membranes of the modified calixarene neutral carriers have proved to be reliable for sodium assay in human body fluids.

Calixarene derivatives, cyclic oligomers of phenol-formaldehyde condensates, have been a new type of host compounds(1) since their tetramer, hexamer, octamer became easily available from *t*-butylphenol and formaldehyde. The calixarene derivatives show ionophoric properties by incorporation of carbonyl linkages into their phenoxy oxygen atoms. Specifically, the tetrameric (calix[4]arene) derivatives such as **1**, possess high Na^+ selectivity(2,3). Calix[4]arene derivatives bearing carbonyl linkages are, therefore, candidates for neutral carriers of Na^+-selective electrodes(4,5), as is the case with bis(crown ether)s(6) and acyclic polyether-amide(7) derivatives. We have been so far engaged in designing ion-selective electrodes and ISFETs based on various calix[4]arene neutral carriers, aiming at high performance Na^+ sensors. Firstly, we have designed calix[4]aryl tetradecyl ester **2** with high lipophilicity, which is an important requirement for neutral carriers of the ion-sensing membranes(8). Thus, plasticized poly(vinyl chloride) (PVC) membrane Na^+-selective electrodes based on calixarene neutral carrier **2** exhibited Nernstian response to Na^+ activity changes (Fig. 1). Optimization for the PVC/**2** membranes allowed high Na^+ selectivity against K^+, the selectivity coefficient for Na^+ with respect to K^+ being 3.8×10^{-3}.

Calix[4]arene neutral carriers can also be applied to Na^+ISFETs, but plasticized PVC membranes are not very suitable for ISFETs mainly due to the poor adhesion to the FET gate surface. Also, since their active materials and plasticizers are liable to exude from the PVC membranes into measuring sample solutions, the mandatory use of plasticizer may be another problem on clinical applications of the PVC membrane ion sensors. Room-temperature vulcanizing-type (RTV) silicone rubber may be an excellent alternative to plasticized PVC for organic ion-sensing membranes of ISFETs(9), because it is very adhesive to FET gates(10) and also does not need any special plasticizer. However, silicone rubber seems to have some drawback in solubility or dispersibility of ion-sensing material, unlike plasticized PVC for ion-sensing membranes. This is often the case with silicone rubber membranes containing calixarene neutral carriers. Most calix[4]arene neutral carriers incorporating four ester or amide linkages, which possess C_{4v} symmetry, show high crystallinity and high melting points. In general, calix[4]arenes are not very easy to dissolve in polymer matrices like silicone rubber, sometimes even in plasticized PVC, due to their symmetrical structure which in turn brings about comparably high molecular cohesion.

An approach to enhance dispersibility of calixarene neutral carriers in silicone rubber is, therefore, their structure unsymmetrization(11). An unsymmetrical calix[4]arene neutral carrier carrying three ethyl-ester linkages and an amide linkage with longer alkyl chains at the phenoxy positions, **3**, possesses only low symmetry of C_s symmetry, thus being quite amorphous and highly dispersible in silicone rubber. The unsymmetrical calixarene neutral carrier assumes a "cone" conformation, which is preferable to the three other conformations for Na^+ complexation, as evidenced by two pairs of doublets for the aromatic-ring-bridging methylenes in the ^{1}H-NMR spectra. Figure 2 shows a typical potential response of Na^+ISFETs based on silicone rubber membranes containing 10 wt% unsymmetrical calix[4]arene neutral carrier **3**. A Nernstian response to Na^+ activity changes was attained in the range of 1 X 10^{-4} — 1 M with the Na^+ISFETs of silicone rubber / **3** membrane. The potential response was very fast, the response time being within 2 seconds for a ten-time Na^+-activity increase. Similar silicone-rubber membrane Na^+ISFETs of symmetrical calix[4]arene neutral carrier **1**, on the other hand, exhibited a poor response to Na^+ activity changes. This poor response of the silicone rubber membrane of **1** is attributed mainly to its low solubility in silicone rubber. By scanning electron microscopy, microcrystals of **1**, which were not very compatible with silicone rubber, were observed on the membrane of **1**, while the corresponding **3** membrane possesses a smooth surface, implying good compatibility of the unsymmetrical calix[4]arene neutral carrier with silicone rubber. For durability check, time-course changes in sensor sensitivity (slope for Na^+ calibration graph) were followed in the Na^+ISFETs based on ion-sensing membranes of silicone rubber / **3** as well



1: $R_1 = R_2 = CH_2CO_2CH_2CH_3$

2: $R_1 = R_2 = CH_2CO_2C_{10}H_{21}$

3: $R_1 = CH_2CO_2CH_2CH_3$
$R_2 = CH_2CON(C_{10}H_{21})_2$

4: $R_1 = CH_2CO_2CH_2CH_3$
$R_2 = CH_2CO_2(CH_2)_{11}(\overset{CH_3}{\underset{CH_3}{Si}}O)_2\overset{CH_3}{\underset{CH_3}{Si}}CH_3$

5: $R_1 = R_2 = CH_2CO_2(CH_2)_3(\overset{CH_3}{\underset{CH_3}{Si}}O)_2\overset{CH_3}{\underset{CH_3}{Si}}CH_3$

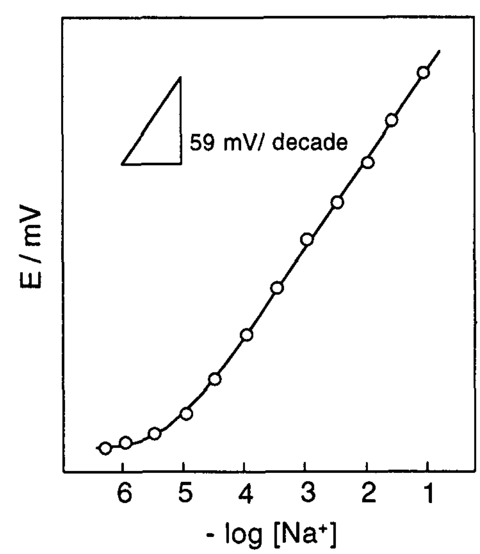


Fig. 1 Potential response for Na^+-selective electrodes based on plasticitized PVC membrane of calixarene neutral carrier **2**. Plasticizer: *o*-fluorophenyl *o*-nitrophenyl ether.

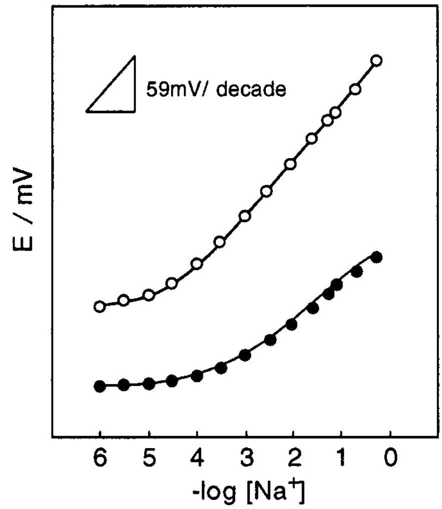


Fig. 2 Potential response for Na^+ISFETs based on silicone rubber membranes of calixarene neutral carriers **3** (○) and **1** (●).

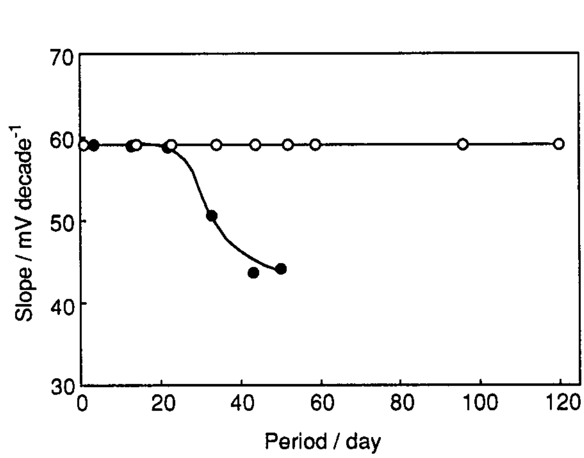


Fig. 3 Durability comparison between Na^+ISFETs based on silicone rubber membrane of **3** (○)and PVC membrane of **1** plasticized by bis(2-ethylhexyl) sebacate (●).

as of plasticized PVC / **1** (Fig. 3). The Na^+ sensitivity was drastically decreased in about 30 days in the Na^+ISFETs of plasticized PVC / **1** membranes. This means that deterioration proceeded quite quickly in the plasticized membrane system. In the Na^+ISFETs containing silicone rubber / **1** membranes, on the other hand, their high Na^+ sensitivity remained unchanged even after 120 days. Thus, the composite membranes of unsymmetrical calix[4]arene neutral carrier **3** and silicone rubber have realized long-lived Na^+ISFETs.

Since silicone rubber, the membrane matrix, is made up of siloxane units, another way to promote the dispersibility of calixarene neutral carriers in silicone rubber is incorporation of siloxane units to the neutral carriers(12). We have, therefore, designed unsymmetrical and symmetrical calix[4]arene tetraester derivatives bearing oligosiloxane moiety, **4** and **5**, and have applied them to Na^+-sensing silicone-rubber membranes for ISFETs. The oligosiloxane-modified calixarene neutral carriers **4** and **5** have unsymmetrical and symmetrical structures, respectively. In the unsymmetrical oligosiloxane-modified calixarene neutral carrier, **4**, one may also expect such dispersibility enhancement derived from its structural asymmetry as already described. Both **4** and **5** are viscous liquid as anticipated from the property of the oligosiloxane moiety. Scanning electron micrography confirmed the high dispersibility of the oligosiloxane-modified calixarene neutral carriers in silicone rubber. Membrane impedance in Na^+ aqueous solutions is also indicative of membrane dispersibility of the calixarene neutral carriers (Fig. 4). The silicone rubber membranes containing the oligosiloxane-modified calix[4]arene neutral carriers, especially **4**, exhibited extremely low electrical resistivity. On the contrary, high resistivity was found with the membrane of **1**, which is equivalent to or even higher in the resistivity than silicone-rubber membranes not containing any neutral carrier. This again suggests that both **4** and **5** are highly dispersible in silicone rubber, thus undergoing active cation diffusion in the membrane. The calixarene neutral carrier, **1**, on the other hand, can not work as the active neutral carrier in the membrane due to the poor dispersibility in the matrix. The Na^+ISFETs of **4** and **5** showed high sensitivity with a Nernstian slope in a wide Na^+ activity range, as is the case with those of **3**. Figure 5 summarizes the selectivity coefficients for Na^+ with respect to other ions in the Na^+ISFETs based on silicone rubber membranes of **3** and **4**, and a plasticized PVC membrane of **2** for

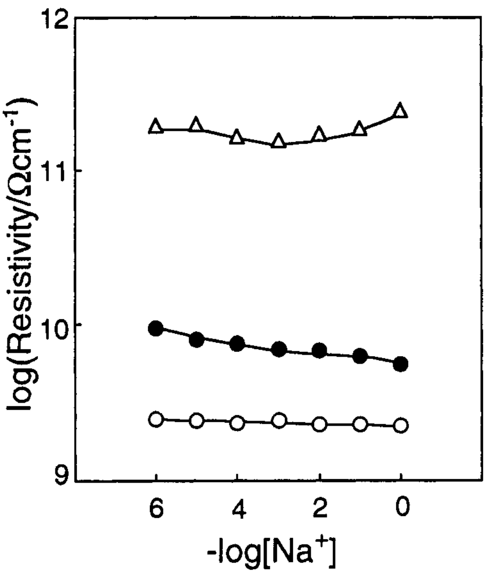


Fig. 4 Impedance comparison among silicone rubber membranes of **1** (△), **4** (○), and **5** (●).

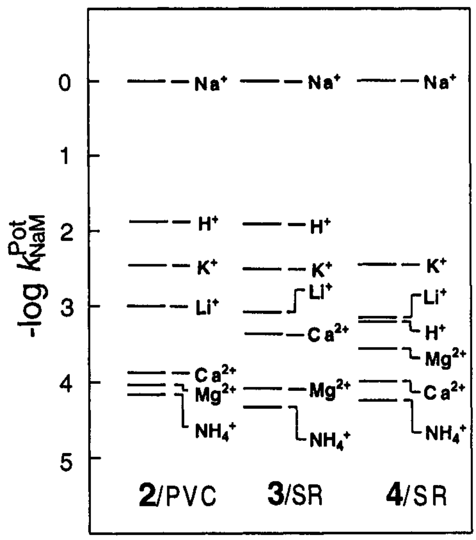


Fig. 5 Selectivity comparison among Na^+ISFETs based on silicone rubber (SR) membranes of **3** and **4** and PVC membrane of **2** plasticized by *o*-fluorophenyl *o*-nitrophenyl ether.

comparison. The Na^+ sensors with silicone rubber membranes of **3** and **4** are quite similar in the Na^+ selectivity to that with plasticized PVC membrane of **2**. The selectivity coefficients for Na^+ with respect to K^+ for the ISFETs of **3** and **4** are about 3 X 10^{-3}, which are sufficient for practical applications such as Na^+ assay in blood sera.

In order to check the applicability of the silicone-rubber membrane Na^+ISFETs thus obtained to clinical sodium assay, attempts were made to determine Na^+ concentration in human blood sera with the Na^+ISFETs of **4**, by using a Na^+ calibration graph (Fig. 6). Two types of undiluted, 10-fold diluted, and 100-fold diluted control sera were employed as the serum sample. The results show that the found values for Na^+ concentration in both of the samples are in good agreement with the Na^+ calibration graph. Even in the undiluted serum samples, little potential shift was observed from the calibration. This indicates that the calixarene-based silicone-rubber membrane Na^+ISFETs are quite reliable for serum sodium assay. Plasticized PVC, which is a popular, excellent membrane material for neutral-carrier-type ion-selective electrodes, has also been applied to Na^+ISFETs of calixarene neutral carrier **1** for comparison with silicone rubber. The results in Fig. 7 demonstrates that the found values of Na^+ concentration in the serum samples, obtained by the PVC membrane Na^+ISFET, deviate from the Na^+ calibration remarkably. The negative potential shift from the Na^+ calibration was observed in the two different type of serum samples. In dummy serum samples containing the same concentrations of Na^+ and K^+, there was hardly any significant deviation of the found Na^+-concentration values. Obviously, the negative potential shift of the serum samples is not originated from interference by K^+ but by organic components in sera, such as proteins. On sodium assay in human urine sample, too, the calixarene-based silicone-rubber membranes gave good results, although a slight deviation of the found value from the Na^+ calibration might be anticipated due to some K^+ interference in the undiluted urine sample.

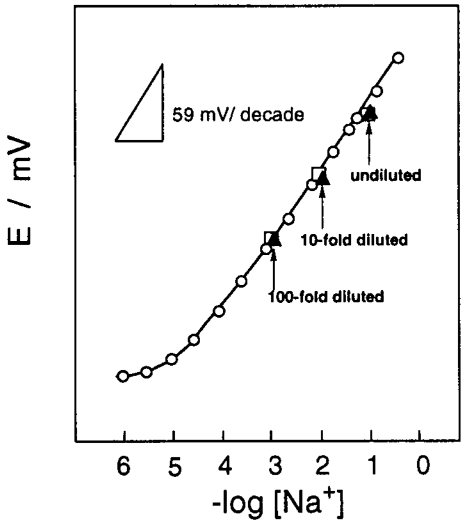


Fig. 6 Na^+ calibration graph and found values obtained in serum sodium assay with Na^+ISFET of silicone rubber membrane containing **4**. Na^+ calibration (○); found values for serum I (▲); found values for serum II (□).

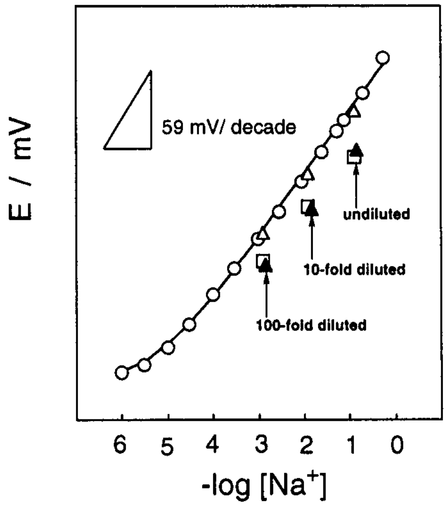


Fig. 7 Na^+ calibration graph and found values obtained in serum sodium assay with Na^+ISFET of silicone rubber membrane containing **4**. Na^+ calibration (○); found values for serum I (▲); found values for serum II (□); dummy sample containing Na^+ (145 mM) and K^+ (4.1 mM) (△).

In conclusion, the incorporation of unsymmetrical structures and/or siloxane units to calix[4]arene neutral carriers has made them highly-dispersible in silicone rubber which is an excellent membrane matrix for ISFETs. Thus, silicone rubber membranes containing unsymmetrical calix[4]arene neutral carrier **3** and oligosiloxane-modified calixarene neutral carriers **4** and **5** afforded high-performance Na^+ISFETs. Reliable sodium assay in blood sera and urine is attainable with the silicone-rubber membrane Na^+ISFETs based on the modified calixarene neutral carriers, which are also expected to have a great advantage of hemocompatibility of silicone rubber itself.

REFERENCES

1. C. D. Gutsche, *Acc. Chem. Res.*, **16**, 161 (1983).
2. S.-K. Chang and I. Cho, *J. Chem. Soc., Perkin Trans. 1*, **1986**, 211.
3. A. Arduini, A. Pochini, S. Reverberi, R. Ungaro, G. D. Andreetti, and F. Ugozzoli, *Tetrahedron*, **42**, 2089 (1986).
4. D. Diamond, G. Svehla, E. M. Seward, and M. A. McKervey, *Anal. Chim. Acta*, **204**, 223 (1988).
5. K. Kimura, M. Matsuo, and T. Shono, *Chem. Lett.*, **1988**, 615.
6. T. Shono, M. Okahara, I. Ikeda, K. Kimura, and H. Tamura, *J. Electroanal. Chem. Interfacial Electrochem.*, **132**, 99 (1982).
7. M. Güggi, M. Oehme, E. Pretsch, and W. Simon, *Helv. Chim. Acta*, **59**, 2417 (1976).
8. K. Kimura, T. Miura, M. Matsuo, and T. Shono, *Anal. Chem.*, **62**, 1510 (1990).
9. P. D. van der Wal, M. Skowronska-Ptasinska, A. van den Berg, P. Bergveld, E. J. R. Sudholter, and D. N. Reinhoudt, *Anal. Chim. Acta*, **231**, 41 (1990).
10. G. S. Cha, D. Liu, M. E. Meyerhoff, H. C. Cantor, A. R. Midgley, H. D. Goldberg, and R. B. Brown, *Anal. Chem.*, **63**, 1666 (1991).
11. K. Kimura, T. Matsuba, Y. Tsujimura, and M. Yokoyama, *Anal. Chem.*, **64**, 2508 (1992).
12. Y. Tsujimura, M. Yokoyama, and K. Kimura, *Electroanalysis*, **5**, 803 (1993).