HIGH-PERFORMANCE SILICONE-RUBBER-BASED SENSING MEMBRANES FOR NEUTRAL-CARRIER-TYPE ION SENSORS

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SUMMARY

Polydimethylsiloxane (silicone rubber) is a candidate for membrane support for neutral-carrier-type ion sensors due to the high advantages over plasticized poly(vinyl chloride), such as biocompatibility and the lack of a need for special plasticizers. Neutral carriers can be dispersed in silicon-rubber membrane. The dispersibility of neutral carriers is enhanced by their molecular design, i.e., the incorporation of unsymmetrical structure and/ or oligo(siloxane) moiety. Chemical modification of silicone-rubber membranes is feasible with alkoxysilylated neutral carriers. The

silicone-rubber membranes encapsulating and chemically bonding a neutral carrier are applicable to practical use such as sodium assay in human body fluid.

1. INTRODUCTION

Neutral-carrier-type ion sensors are convenient tools for cation assay in solutions with high selectivity and sensitivity. Solvent-polymeric membranes are generally required as the ion-sensing membrane. A most popular ion-sensing membrane for neutral-carrier-type ion sensors is plasticized poly(vinyl chloride) (PVC). Plasticized-PVC ion-sensing membranes, however, still have some disadvantages. The ion-sensing membranes need high volume percents of a special plasticizer, which is easy to exude from the membranes to aqueous (sample) phases and therefore to deteriorate. The plasticizers as well as the neutral carriers are often toxic, and PVC is becoming a problem in environmental science. Plasticized-PVC membranes possess only poor biocompatibility since organic compounds such as proteins are adsorbed on them in serum metal-ion assay. It is sometimes claimed that neutral-carrier-type PVC membrane ion-selective electrodes may give some negative errors on metal-ion assay in biological systems, as described later. It is therefore almost impossible that ion sensors based on neutral-carrier-type PVC membranes can be applied to *in vivo* metal ion assays such as *in situ* ion monitoring at a patient's bedside. In addition, plasticized-PVC membranes are not very suitable for ion-sensitive field-effective transistors (ISFETs) mainly due to their poor adhesion to the FET gate surface.

An alternative membrane material for ion-selective electrodes is cross-linked polydimethylsiloxane, termed room-temperature vulcanizing-type (RTV) silicone rubber, which was first applied to heterogeneous precipitate-based membranes for silver ion-selective electrodes /1/. In general, no special plasticizer is required in silicone-rubber-based ion sensing membranes. Silicone-rubber-membrane ion-sensing electrodes can also be expected to show some biocompatibility on practical use in biological systems /2-4/.

However, silicone-rubber-based ion-sensing membranes possess high electrical resistivity derived from the low dispersibility of their active materials, which often causes unstable e.m.f. readings. This problem is especially serious in ion-sensing membranes containing electrically neutral ion-exchanger, neutral carriers. Another advantage of silicone-rubber membranes over plasticized-PVC membranes is easy chemical modification of the membrane materials with neutral carriers.

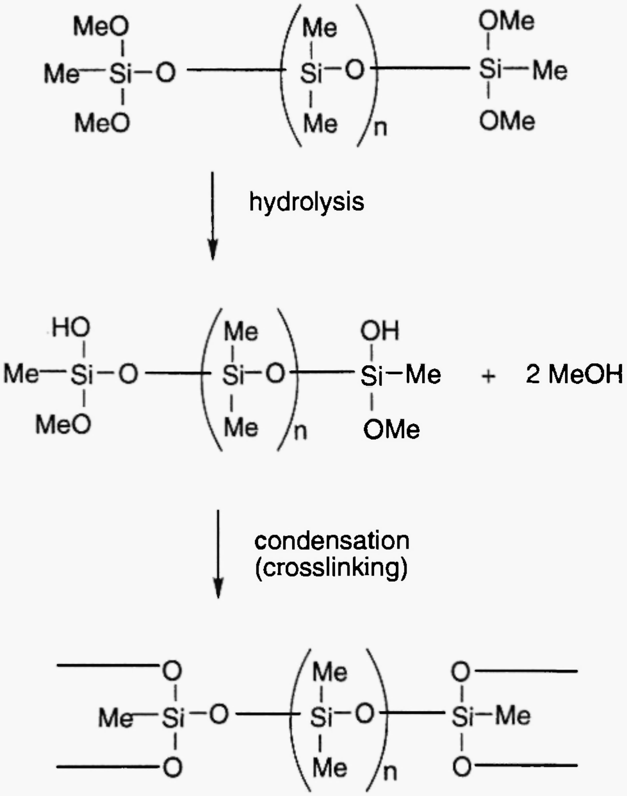
Here we describe the designing of useful neutral-carrier-based silicone-rubber membranes for potentiometrie ion sensors such as ion-sensing electrodes and ISFETs. We mention specifically how to overcome the low dispersibility of neutral carriers in silicone-rubber membranes and how to modify the membrane by neutral carriers, and the applicability of the resulting ion sensors based on neutral-carrier-based silicone-rubber membranes.

2. CROSSLINKED POLYDIMETHYLSILOXANE AS MEMBRANE SUPPORT FOR ION SENSOR

Formation of crosslinked polydimethylsiloxane (RTV silicone rubber) consists of two processes: i) the hydrolysis of active end groups of dimethylsiloxane oligomers, and ii) crosslinking by condensation of the active end groups with the resulting silanol groups. The active end groups are alkoxy, acetoxy, oxime, amino, amido groups, etc. In cases when the active end group is methoxy (alcohol-evolving type), the typical reaction mechanism for the silicone-rubber formation is as shown in Scheme 1.

Silicone rubber possesses several advantages: I) easy fabrication at ambient temperature; ii) high elasticity in wide temperature ranges (-60 ~ 30°C); iii) excellent adhesive properties, especially to inorganic materials; iv) excellent electrical properties; v) chemical inactivity, and vi) low toxicity. Therefore, silicone rubber can be generally applied to adhesion, sealing, coating, molding, and potting.

Silicone rubber may be an excellent candidate for membrane materials of ion sensors. Since the polymer material is highly elastic at ambient temperature, silicone-rubber-based ion-sensing membranes do not necessarily require special plasticizers to endow a neutral carrier with mobility enough for the ion exchange in the interface between the membrane and aqueous (sample) phases. This eliminates deterioration of the sensing membranes caused by the plasticizer exudation. Also, the excellent adhesive property of silicone rubber to inorganic materials is very advantageous in its use as ion-sensing membranes for ISFET. Furthermore, since silicone rubber is neither chemically active nor toxic, some biocompatibility can be expected in



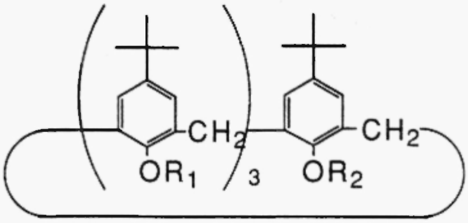
Scheme 1: Mechanism for formation of crosslinked poly(dimethylsiloxane) (silicone rubber).

the silicone-rubber-based ion-sensing membranes. This turns out to be a great advantage in the application of ion sensors in clinical analyses.

3. NEUTRAL-CARRIER ENCAPSULATION

One of the easiest ways to fabricate silicone rubber ion-sensing membranes based on neutral carriers is simply to encapsulate, that is, to disperse them in silicone rubber. However, there seems to be a flaw in the solubility or dispersibility of ion-sensing materials in silicone rubber. Silicone rubber is very different from plasticized PVC for ion-sensing membranes in that the latter contains a large quantity of plasticizer (or membrane solvent) in which neutral carriers can be dissolved easily. This problem is serious, especially in silicone-rubber membranes containing neutral carriers which show high crystallinity. Valinomycin, a typical K^+ ionophore, seems applicable to silicone-rubber-based K^+-selective electrodes without any significant problems /2,3,5-7/. Crown-ether-based neutral carriers are also quite soluble in silicone rubber.

Calix[4]arene derivatives carrying carbonyl linkages at the lower rim, which are generally Na^+ ionophores /8/, have proved to be excellent neutral carriers for PVC-membrane Na^+-selective electrodes /9-12/. Most calix[4]arene ionophores are quite soluble in plasticized-PVC membranes, but they are not necessarily soluble and applicable in silicone rubber. Highly soluble (or dispersible) calix[4]arene ionophores must have been designed for their application to silicone-rubber membranes.



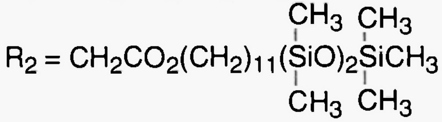
1: $R_1 = CH_2CO_2C_2H_5$
$R_2 = CH_2CON(C_{10}H_{21})_2$

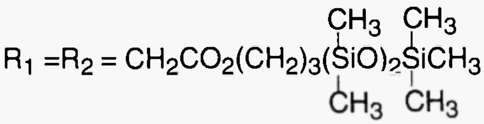
2: $R_1 = R_2 = CH_2CO_2C_2H_5$

3: $R_1 = CH_2CO_2C_2H_5$
$R_2 = CH_2CON(C_2H_5)_2$

4: $R_1 = CH_2CO_2C_2H_5$
$R_2 = CH_2CO_2C_{18}H_{37}$

5: $R_1 = CH_2CO_2CH_2CH_3$


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

6:

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

Design of Unsymmetrical Calix[4] Ionophores

Most calix[4]arene ionophores incorporating four ester or amide linkages, which possess *C4v* symmetry, show high crystallinity and high melting points. Probably due to the symmetrical structure, which in turn brings about comparably high molecular cohesion, the calix[4]arene ionophores are generally not very easy to dissolve in polymeric matrices, sometimes even in plasticized-PVC. Silicone rubber which has good adhesion to FET gates is suitable for the fabrication of stable ion-sensing membranes for ISFETs. Solubility of the calix[4]arene ionophores is generally worse in silicone-rubber membranes than in plasticized-PVC membranes. In order to improve the solubility of calix[4]arene ionophores in silicone rubber, an unsymmetrical calix[4]arene ionophore was designed which possesses three ethyl-ester linkages and an amide linkage with longer alkyl chains at the phenoxy positions, **1** /13/. As compared with a symmetrical calix[4]arene ionophore such as **2**, the unsymmetrical one possesses only low symmetry, i.e., *Cs* symmetry, and is therefore amorphous. Two other calix[4]arene ionophores with different unsymmetrical moieties were also synthesized for comparison with **1**. An amide carbonyl linkage without long aliphatic chains was incorporated into **3**. Calixarene ionophore **3** turns out to be an amorphous glass by melting, followed by cooling to room temperature. The other one, 4, has a dodecyl group as the long aliphatic chain and an ester carbonyl linkage instead of the amide one, still being unsymmetrical only with the aliphatic chain. All of the unsymmetrical calixarene ionophores possess a "cone" conformation, which is preferable to the three other conformations for Na^+ complexation.

A typical potential response of a Na^+-ISFET based on a silicone-rubber membrane containing 10 wt% unsymmetrical calix[4]arene ionophore **1** is shown in Figure 1. A one-component RTV silicone rubber of the oxime-evolving type was attempted as the membrane matrix. A Nernstian response to Na^+ activity changes was attained in the range of $1 \times 10^{-4} - 1$ M with the silicone rubber / **1** membrane. The potential response is very fast, the response time (t_{90}) being within 2 s. It should, however, be noted that a similar silicone-rubber membrane of symmetrical calix[4]arene ionophore **2**, when applied to Na^+-ISFETs, exhibits a poor response to Na^+ activity changes. The silicone-rubber membrane of **2** is very different in the ISFET response from the corresponding PVC membrane. This poor response of the silicone-rubber membrane of **2** is derived mainly from its low solubility in

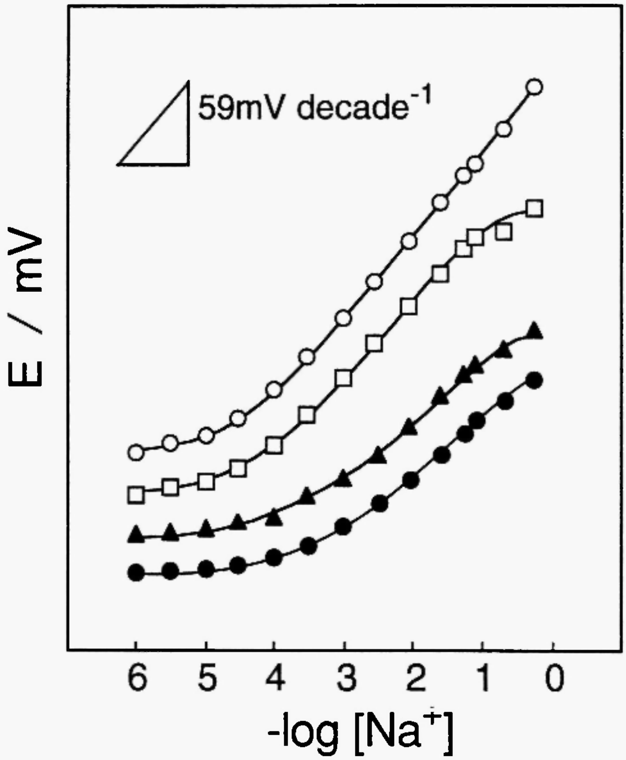


Fig. 1: Potential response to Na^+ activity changes of ISFETs based on silicone-rubber membranes of calixarene ionophores /13/. Calixarene ionophore: **1** (○), **2** (●), **3** (□), **4** (▲).

the polymer matrix. Scanning electron microscopy (SEM) gave some information about the solubility of **2** in silicone rubber. Microcrystals of **2**, which were not very compatible with silicone rubber, were observed on the silicone-rubber membrane of **2**, while the corresponding **1** membrane possesses a smooth surface, implying good compatibility of the unsymmetrical calix[4]arene ionophore with silicone rubber.

Calixarene ionophore **3** is also quite soluble in silicone rubber. In Na^+-ISFETS with **3** / silicone-rubber membranes, a Nernstian response was found in the range of $1 \times 10^{-4} - 1 \times 10^{-1}$ M Na^+, but the sensitivity was decreased more or less at the higher Na^+ activities. On the contrary, calixarene ionophore 4, although also unsymmetrical, bears only poor solubility in the polymer matrix. Serious phase separation was observed in the 4 / silicone-rubber membranes, thus resulting in poor ISFET sensitivity. The high solubility of 1 in silicone rubber and thereby high ISFET sensitivity are, therefore, attributable mainly to the unsymmetrization of calixarene ionophores by the incorporation of an amide carbonyl group. There also seems to be some contribution of the unsymmetrization by incorporating a long aliphatic chain to the solubility enhancement.

The highly dispersible calix[4]arene ionophores can also improve the durability for neutral-carrier-type ion sensors. Time-course changes in both sensitivity (slope for Na^+ calibration graph) and selectivity (selectivity coefficient for Na^+ with respect to K^+) were followed in the Na^+-ISFETs based on ion-sensing membranes of silicone rubber / 1, plasticized PVC / 1, and plasticized PVC / 2 (Figure 2). Serious deterioration proceeded quite quickly in the Na^+-ISFETs of plasticized PVC / 2; in about 30 days, both the Na^+ sensitivity and selectivity were drastically decreased. This occurred because the plasticizer and neutral carrier 2 dissolved out from the membrane into the aqueous phases. In the Na^+-ISFETs of plasticized PVC / 1 membranes, marked deterioration started at about 50 days, that is, much more gradually than the plasticized PVC / 2 system. This implies that the unsymmetrical calix[4]arene 1 is very superior to the symmetrical one, 2, in solubility even in PVC. Nevertheless, leaching of the plasticizer out from the plasticized PVC / 1 membrane led to such ISFET deterioration. On the other hand, attention should be drawn to the excellent durability of the Na^+-ISFETs containing silicone rubber / 1 membranes. In these ISFETs, their high Na^+ sensitivity and selectivity remained unchanged even after 120 days.

Incorporation of Oligo(siloxane) Group into Calix[4]arene Ionophores

As mentioned above, silicone rubber is a polymer consisting of siloxane units. It is therefore expected that incorporation of oligosiloxane moiety to calix[4]arene ionophores increases their affinity to silicone rubber and thereby enhances their solubility or dispersibility in the polymer matrix. For that purpose, oligosiloxane-modified calix[4]arene ionophores have been designed which are both unsymmetrical (5) and symmetrical (6) ones /14/. In the unsymmetrical oligosiloxane-modified calixarene ionophore, 5, one may also expect such dispersibility enhancement derived from the structural

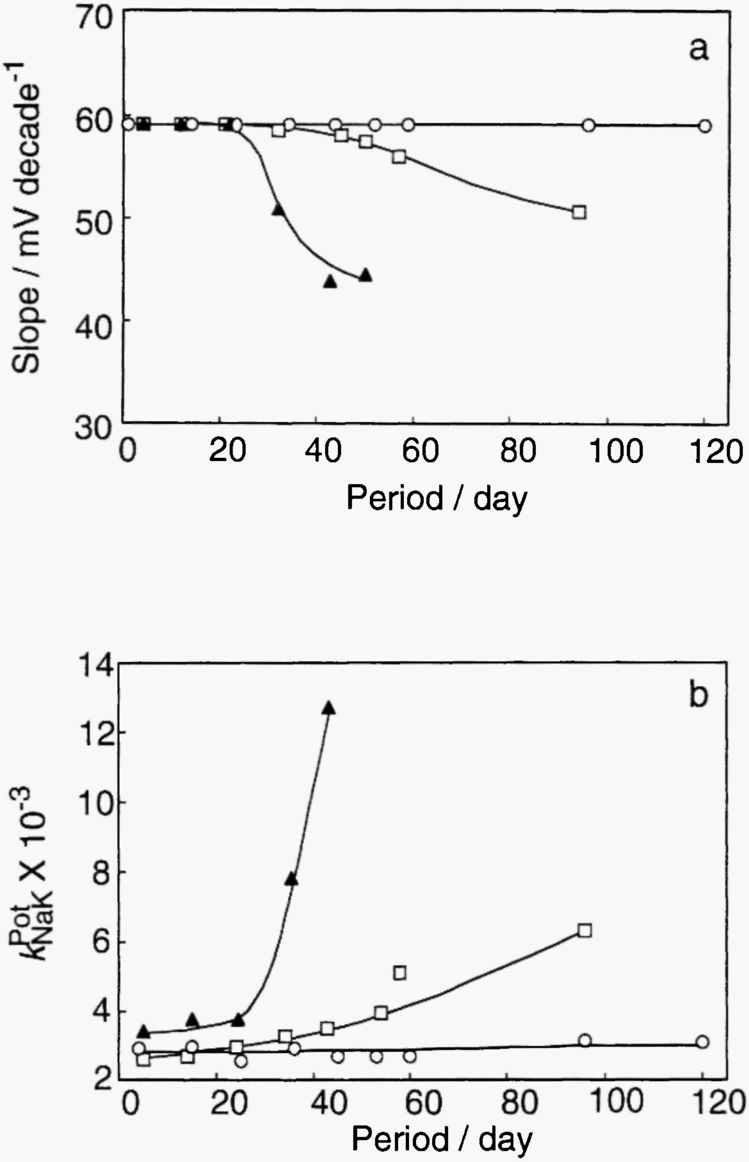


Fig. 2: Time-course changes of sensitivity (a) and selectivity (b) for calixarene-based Na$^+$-ISFETs /13/.
Silicone rubber / 1 (90/10 wt%) membrane (○), PVC/DOS / 1 (30/60/10 wt%) membrane (□), PVC/DOS / 2 (30/60 / 10 wt%) membrane (▲) (DOS is 2-ethylhexyl sebacate).

asymmetry of **1** as described above. Both **5** and **6** are viscous liquids, as anticipated from the properties of the oligosiloxane moiety.

Dispersibility of oligosiloxane-modified calixarene ionophores **5** and **6** in silicone rubber was first checked by microscopic observation. In the SEM photographs, the silicone-rubber membranes of **5** exhibit a smooth surface, which suggests high dispersibility of the oligosiloxane-modified calixarene ionophores in silicone rubber. This is also the case with the **6**-containing silicone-rubber membranes. Membrane impedance, obtained by ac impedance measurements in Na^+ aqueous solutions, may also give important information concerning the membrane dispersibility of the calixarene ionophores. Figure 3 depicts bulk membrane resistivities for the calixarene ionophore/silicone-rubber membranes under various conditions of Na^+ concentration in solution. The silicone-rubber membranes containing the

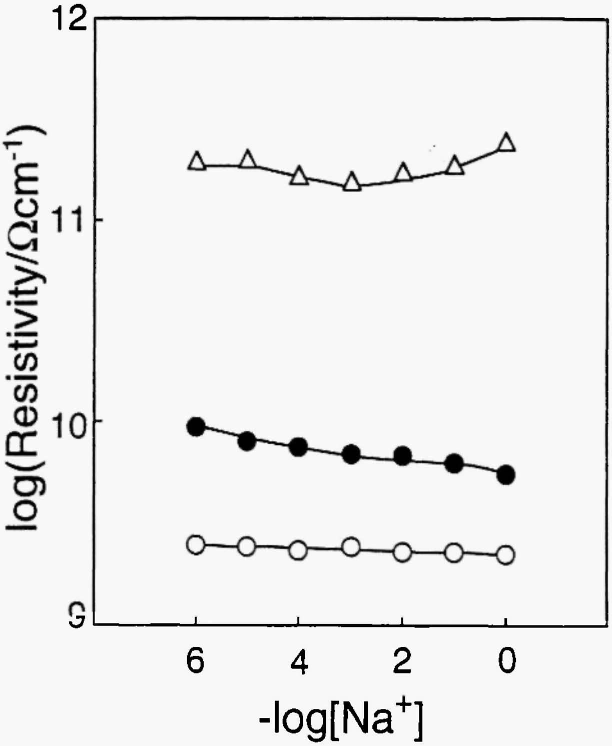


Fig. 3: Comparison in membrane impedance between silicone-rubber membranes systems of **5** (○), **6** (●), and **2** (△) /14/.

oligosiloxane-modified calix[4]arene ionophores, especially **5**, possess extremely low resistivity as silicone-rubber-type ion-sensing membranes. On the contrary, high resistivity was found with the membrane of **2**, which is equivalent to or even higher than the resistivity of silicone-rubber membranes without any ionophore. This fact strongly supports the supposition that both **5** and **6** are highly dispersible in silicone rubber, thus undergoing active cation diffusion in the membrane.

The Na^+-ISFETs of **5** and **6** exhibit high sensitivity with a Nernstian slope in a wide Na^+ activity range, which is very similar to that for **1** as shown in Figure 1. Since the response time of the Na^+ sensors is about 1 s, the potential response is fast enough for their practical use. Figure 4 summarizes the selectivity coefficients for Na^+ with respect to other ions in the Na^+-ISFETs

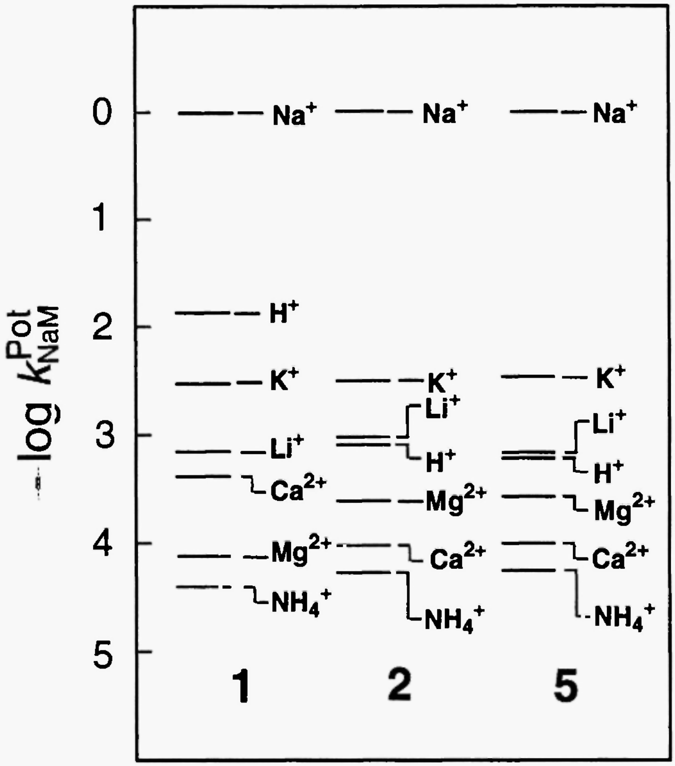


Fig. 4: Selectivity comparison between silicone-rubber membranes Na^+-ISFETs based on calixarene ionophores **1**, **2**, and **5** /13,14/.

based on calixarene ionophores **1**, **2**, and **5**. The Na^+ sensors based on calixarene ionophores **1** and **5** resemble that based on **2** in the Na^+ selectivity. For instance, the selectivity coefficient for Na^+ with respect to K^+ for the ISFET of **1** and **5** is about 3×10^{-3}, which is sufficient for Na^+ assay in blood sera.

Silicone rubber is also applicable to membrane support for neutral-carrier-type ion-selective electrodes. The problem of membrane impedance is, however, more serious in the ion-selective electrodes than in the ISFETs. The high membrane impedance often leads significantly to lowered sensitivity and slow potential response in the ion-selective electrodes. The silicone-rubber membrane based on oligosiloxane-modified calix[4]arene ionophore **5** still exhibits high performance when used for Na^+-selective electrodes /15/. Figure 5 shows a typical potential response for a

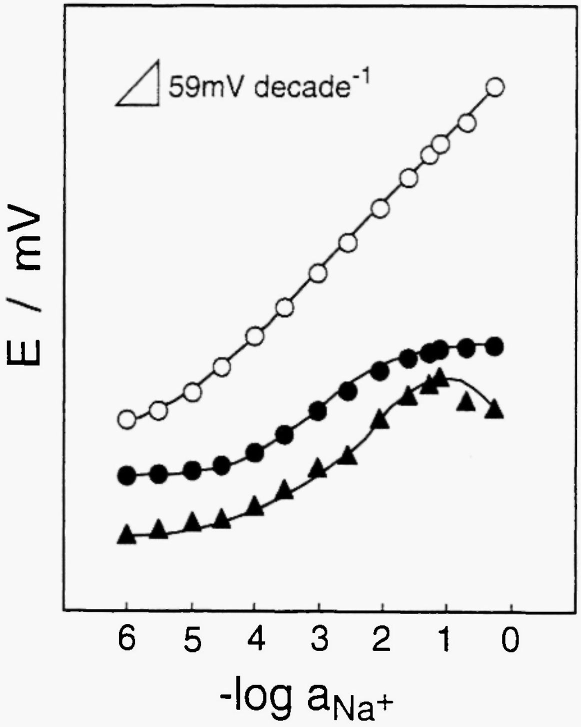


Fig. 5: Potential response to Na^+ activity changes of Na^+-selective electrodes with silicone-rubber membranes of neutral carriers **5** (○), **2** (●), and bis(12-crown-4) (▲) /15/.

silicone-rubber membranes Na^+-selective electrode based on calixarene ionophore **5**, together with those for **2** and a well-known Na^+ neutral carrier, bis[(12-crown-4)methyl] dodecylmethylmalonate or bis(12-crown-4) /16/. The silicone-rubber membrane of **5** afforded high electrode sensitivity with a Nernstian slope in the wide Na^+ activity range of $3 \times 10^{-5} - 1$ M. On the other hand, both the silicone-rubber membrane electrodes based on **2** and bis(12-crown-4) exhibited only poor sensitivity to Na^+ activity changes and even anion response in the high NaCl concentration range. Obviously, the poor dispersibility of neutral carriers **2** and bis(12-crown-4) in silicone rubber and therefore the high electrical resistivity of their silicone-rubber membranes lead to the low sensitivity for their Na^+-selective electrodes. A marked difference in response time was also found among Na^+-selective electrodes with silicone-rubber membranes of the three neutral carriers. Time-response profiles for the three silicone-rubber membrane electrodes are summarized in Figure 6. A fast response was attained in the electrodes with silicone-rubber membranes of **5**, the response time being about 2 s. The electrode response was, however, sluggish in the silicone-rubber membrane system of **2** and bis(12-crown-4). The response times in both the silicone-rubber membranes systems of **2** and bis(12-crown-4) were of the order of 1 minute. Thus, the high dispersibility of oligosiloxane-modified calixarene ionophore **5** in silicone rubber is reflected more remarkably in the performance of silicone-rubber membranes Na^+-selective electrodes than the corresponding ISFETs.

4. CHEMICAL MODIFICATION WITH NEUTRAL CARRIER

Exudation of neutral carriers and plasticizers from ion-sensing membranes results in the deterioration and contamination of the ion sensor in environmental and biological systems. In order to prevent the outflow of the neutral carriers from the ion-sensing membranes, several attempts have been made to immobilize neutral carriers to PVC membranes by chemical bonding /17-19/. However, if the neutral-carrier-type PVC membranes do not contain any special plasticizer, they do not seem to show very good electrode properties, i.e., non-Nernstian response and slow potential response. This is probably because PVC and the chemically-modified PVC themselves do not

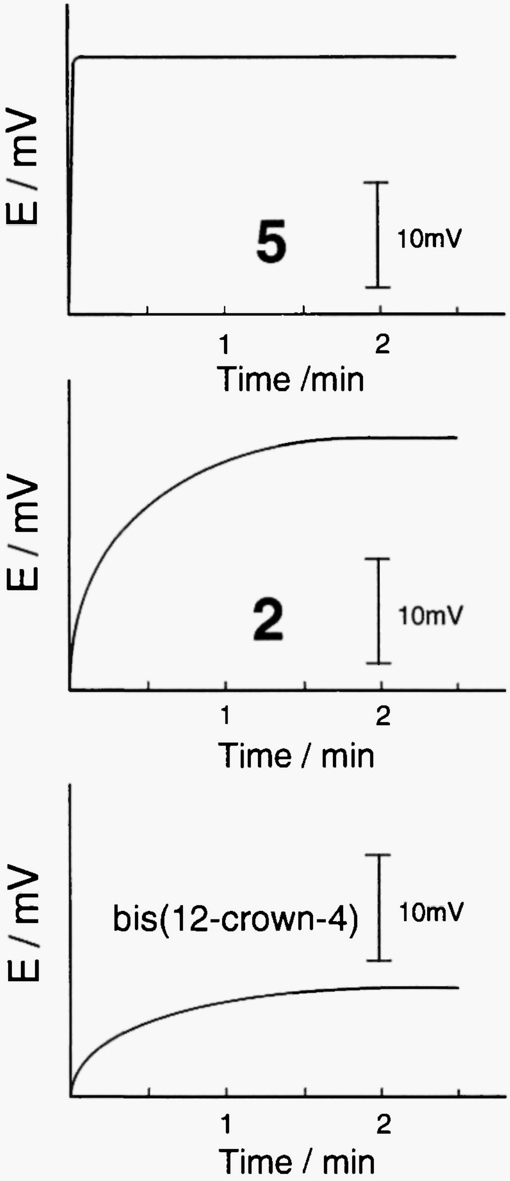


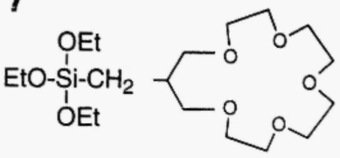
Fig. 6: Time-course changes of potential response for silicone-rubber membrane Na^+-selective electrodes based on neutral carriers **5**, **2**, and bis(12-crown-4) on changing Na^+ concentration from 1×10^{-3} to 3×10^{-3} M /15/.

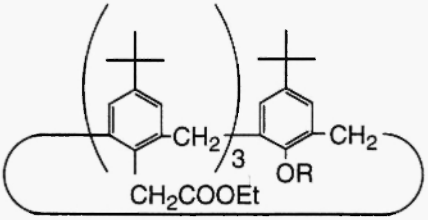
allow sufficiently high mobility for the chemically-bonded neutral carriers to exchange cations due to their high glass-transition temperatures (at least higher than room temperature).

Since silicone rubber is highly elastic even at room temperature, it does not require any special plasticizers in the ion-sensing membranes. Good biocompatibility is also expected in silicone rubber. On the formation of silicone rubber, in general, the hydrolysis of alkoxysilyl terminal groups of silicone-rubber precursors [poly(dimethylsiloxane) derivatives and crosslinking agents], followed by condensation, proceeds as already illustrated in Scheme 1. If an alkoxysilyl group can be incorporated into neutral carriers, the chemical bonding of neutral carriers to silicone rubber is feasible by a simple reaction of the silicone rubber precursor with alkoxysilylated neutral carriers on silicone-rubber fabrication, as schematically shown in Scheme 2 /20/.

As Na^+ neutral carriers, 16-crown-5 and calix[4]arene derivatives possessing a triethoxysilyl group **7** and **8** were designed for the chemical modification. Triethyoxysilylethyl-16-crown-5, 7, was then mixed with a

7





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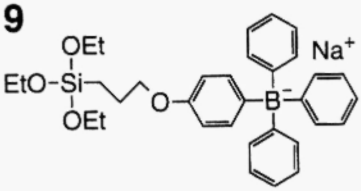
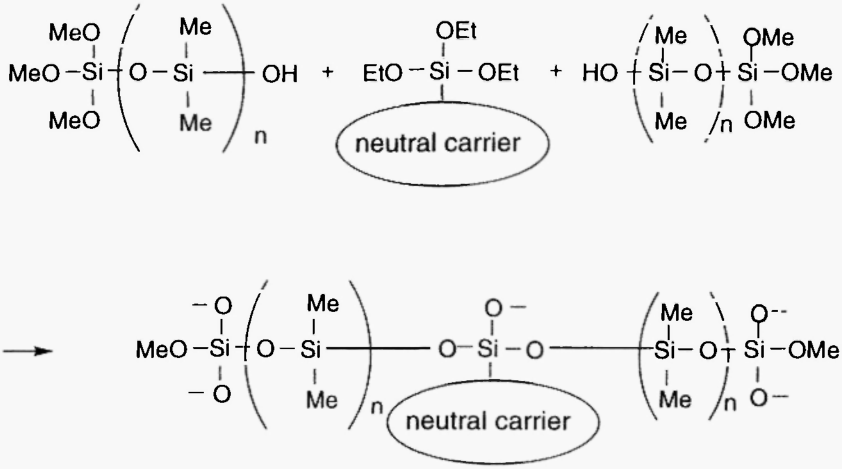


8

$$R=CH_2CO_2(CH_2)_{11}\underset{\textstyle OEt}{\overset{\textstyle OEt}{Si}}OEt$$

silicone-rubber precursor for the membrane fabrication accompanying covalent bonding of the neutral carrier. In order to check the covalent bonding of 16-crown-5 derivative to silicone-rubber membranes, IR spectra were measured before and after extraction of the non-bonded neutral carrier with benzene. In the IR spectrum for the silicone-rubber membranes fabricated using the ethoxysilylated 16-crown-5 which was Soxhlet-extracted with benzene for a whole day, a significant peak assigned to C–H deformation vibration for the 16-crown-5 ring was observed around 1350 cm^{-1}. Definitely, a considerable amount of 16-crown-5 moiety was bonded covalently to the silicone-rubber membranes fabricated using 7, as shown schematically in

Scheme 2. IR spectra between the silicone-rubber membranes fabricated using the alkoxysilylated calixarene 7 also confirmed the covalent bonding of the calix[4]arene ionophore to the silicone-rubber membranes.



Scheme 2: Mechanism for chemical modification of silicone-rubber membranes with a neutral carrier

Figure 7 gives a potential response for Na^+-selective electrodes based on silicone-rubber membranes modified chemically by alkoxysilylated calix[4]arene ionophore 8. The electrodes of the silicone-rubber membranes containing 8 without any other additive showed a very curious potential response, especially in the Na^+ activity changes between $1 \times 10^{-5} - 1 \times 10^{-2}$ M. This is probably due to the chemical modification of silicone-rubber membranes by a calixarene ionophore, which in turn brings about high membrane impedance (5.5×10^{10} S cm^{-1}). Therefore, sodium tetrakis [3,5-bis(trifluoromethyl)phenyl]borate (TFPB) was tested to improve the electrode response. The addition of TFPB to the silicone-rubber membranes of 8 alleviated the membrane impedance (2.1×10^{9} S cm^{-1}), thus affording the membranes high sensitivity to Na^+ activity changes with a Nernstian slope in the activity range of 1×10^{-5} and 1 M. TFPB, which is not bonded to silicone rubber, comes off easily from the membrane. For durability enhancement and

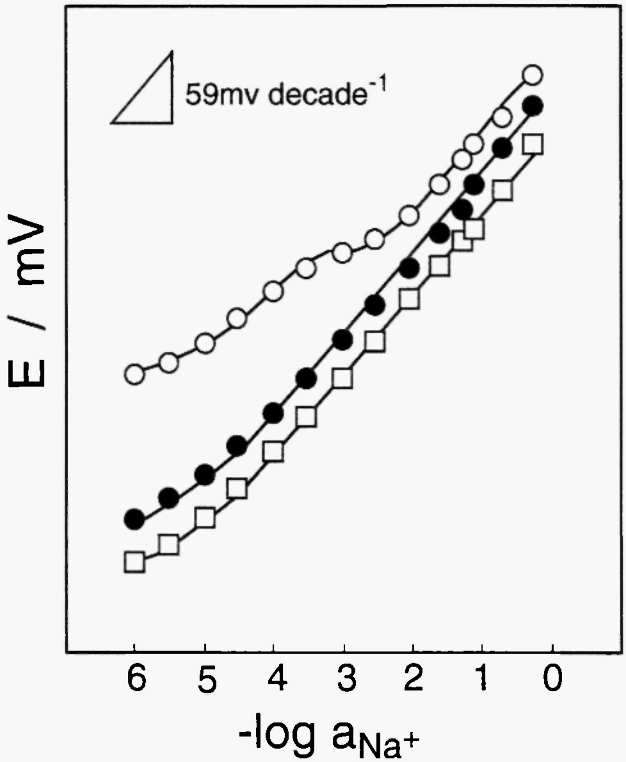


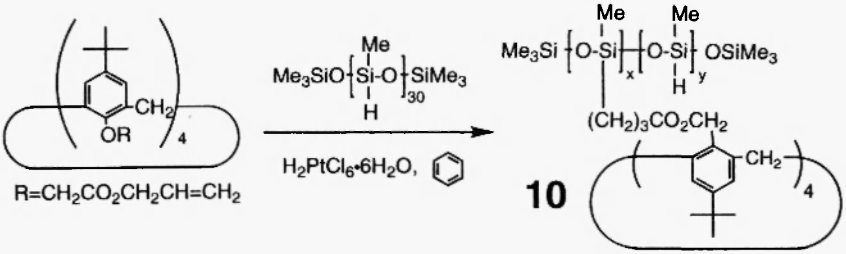
Fig. 7: Potential response to Na^+ activity changes for Na^+-selective electrodes based on silicone-rubber membranes modified chemically by triethoxysilylated calix[4]arene **8** /20/.
(○): without any anion excluder; (●): with TFPB; (□): also modified chemically by triethoxysilylated tetraphenylborate **9**.

toxicity alleviation of the ion-sensing membranes, sodium triphenyl-4-[3-(triethyoxysilyl)propyloxy]phenylborate **9** was also employed to immobilize an anion excluder covalently on silicone-rubber membranes together with the calixarene ionophore. A decreased membrane impedance (5.0 x 10^9 S cm^{-1}) was again attained in the silicone-rubber membranes incorporating both calixarene ionophore **8** and anion excluder **9**, although it is not as low as that for the membrane simply dispersing TFPB. The potential response profile for the membrane system containing **3** or **5** alone is quite similar to that for the membrane system of **3** and TFPB, showing a Nernstian response to Na^+

activity changes in the wide activity range. The response time in the electrodes was also improved by the addition of an anion excluder (TFPB or alkoxysilylated sodium tetraphenylborate **9**) to the silicone-rubber membranes modified chemically by calixarene ionophore **8**. Another way to immobilize neutral carriers to silicone rubber by covalent bonding may be copolymerization of vinyl-modified polysiloxane and neutral carrier vinyl monomers induced by UV-light irradiation and the resulting neutral-carrier-based membranes have been applied to K^+- and Na^+-ISFET /21,22/.

The neutral-carrier-modified silicone-rubber membranes often exhibit anomalous electrode response, unless a lipophilic salt was included in them to overcome their high membrane impedance. The design of an oligomeric neutral carrier with a medium molecular weight and its blending with a silicone rubber might alleviate the demerits of the silicone-rubber membranes modified chemically by a neutral carrier. The medium-molecular- weight weight neutral carrier is expected to improve the poor mobility of the covalently-immobilized neutral carriers and be less likely to exude from the membrane owing to its relatively high molecular weight.

For obtaining a medium-molecular-weight calixarene ionophore, calix[4]arene tetrallyl ester was incorporated into oligo(methylhydrosiloxane) by the hydrosilylation reaction (Scheme 3). The oligo(dimethylsiloxane) backbone is expected to enhance the solubility or dispersibility of the resulting calixarene ionophore in silicone-rubber membranes which also contain dimethylsiloxane repeating units. Each molecule of the resulting calixarene oligomer, **10**, was found to contain two or three calixarene units and to possess a molecular weight ranging from 4000 to 5000. The oligomeric calixarene ionophore, **10**, was blended with a silicone-rubber precursor



Scheme 3: Synthesis of oligomeric calixarene ionophore

in 10 weight %. The Na^+-selective electrodes based on silicone-rubber membranes containing the oligomeric calixarene ionophore **10** exhibited a Nernstian response to ten-time Na^+ activity changes in the wide activity range. That is to say, the curious potential response seen in the calixarene-bound silicone-rubber membranes modified chemically by **8** (Figure 7) was not observed in the silicone-rubber membranes containing the oligomeric calixarene ionophore **10**. This means that the calixarene moiety itself is more mobile, and it is easier to exchange Na^+ in the silicone-rubber membranes of oligomeric calixarene ionophore than in those modified chemically with a calixarene moiety. Another significant difference between the membranes containing oligomeric calixarene ionophore **10** and those incorporating a calixarene moiety by chemical bonding was also found in the response time. The response time is no shorter than 3 min in the system of the calixarene-chemically-bound membranes. In the present membrane system containing oligomeric calixarene ionophore **10**, on the other hand, the response time is already below 1 min. Thus, the employment of the silicone-rubber membranes containing oligomeric calixarene ionophore **10** instead of the calixarene-chemically-bound membranes has improved the electrode potential response and the response time thanks to the enhanced mobility of the calixarene moiety in the **10** system. Furthermore, the oligomeric calixarene ionophore **10** is much more insoluble in water than monomeric calix[4]arene ionophores such as calix[4]arene tetraethyl ester and is therefore less likely to exude from the membrane to aqueous phases.

5. APPLICATIONS

Attempts were made at first to determine Na^+ concentration in human blood sera with silicone-rubber membranes Na^+-ISFETs based on **5**, using the Na^+ calibration plots (Figure 8a) /23/. Two types of undiluted, 10-fold diluted, and 100-fold diluted control sera were employed here as the real serum sample. The results show that the values found for Na^+ concentration in both of the real samples are in good agreement with the Na^+ calibration plots. Even in the undiluted serum samples, only a slight potential shift was observed from the calibration. This indicates that the calixarene-based silicone-rubber membrane Na^+-ISFETs are reliable on serum Na^+-assay. For comparison of the silicone-rubber membrane Na^+-ISFETs based on calixarene ionophores, the corresponding plasticized-PVC membrane Na^+-

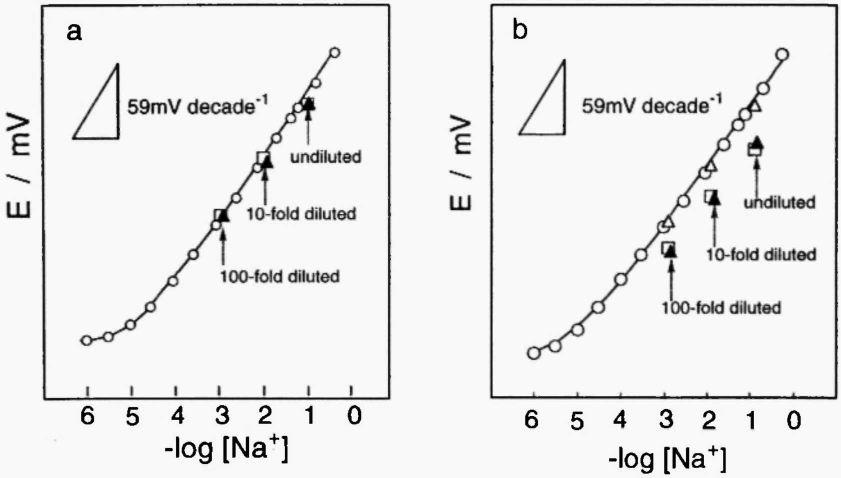


Fig. 8: Na$^+$ calibration plots and Na$^+$ concentrations in serum samples obtained with silicone-rubber membrane Na$^+$-ISFETs based on calixarene **5** (a) and with PVC/DOS membrane Na$^+$-ISFET based on calixarene **2** (b) by calibration method /23/.
(○) Na$^+$ calibration plots; (▲) found values for Serum I; (□) found values for Serum II; (△) dummy serum sample.

ISFETs were also tested for the Na$^+$ assay. The results for Na$^+$ assay in sera by the plasticized-PVC membranes ISFETs are depicted in Figure 8b. It is worth noting that the values of Na$^+$ concentration in the serum samples, obtained by the PVC-membrane Na$^+$-ISFET, deviate from the Na$^+$ calibration to a great extent. The negative potential shift from the Na$^+$ calibration was observed in the two different types of serum samples. Dummy serum samples containing the same concentrations of Na$^+$ and K$^+$ as the real serum samples (without any organic components such as proteins) were also employed for comparison. Hardly any significant deviation of Na$^+$ concentration value was found in the dummy samples. Obviously, the negative potential shift of the real serum samples cannot be ascribed to interference by K$^+$ but by organic components in sera, probably proteins. The PVC membranes containing calixarene ionophore **5** instead of **2** also brought about such a negative potential shift in serum Na$^+$ assay. For the Na$^+$ assay in human urine sample, the calixarene-based silicone-rubber membranes are superior to the corresponding plasticized-PVC ones as ion-sensing membranes for Na^+-ISFETs. Thus, the silicone-rubber membrane Na^+-ISFETs based on calixarene ionophore **5** are quite useful for Na^+ assay in human sera and urine, whereas the corresponding plasticized-PVC membranes ISFETs are likely to cause significant errors on the assay.

Adsorption of blood protein on the silicone-rubber membranes containing a neutral carrier was tested to obtain some information about biocompatibility of the ion-sensing membranes and therefore the resulting ISFETs on the Na^+ assay in blood. Comparison in the SEM photographs of a silicone-rubber membrane and its corresponding plasticized-PVC membrane indicates a striking difference in the protein adsorption. The silicone-rubber membrane hardly adsorbs blood protein, whereas a large amount of protein can be seen on the plasticized-PVC membrane. This indicates that the silicone- rubber ion-sensing membranes are superior to the corresponding plasticized-PVC membranes from the point of view of blood compatibility. The adsorption of organic components in blood on plasticized-PVC ion-sensing membranes often causes some negative errors on Na^+ assay in blood by the resulting membrane ion sensors. Also, due to the serious protein adhesion, it is difficult to use plasticized-PVC membrane ion sensors for cation monitoring *in vivo*. On the contrary, the markedly alleviated protein adhesion on the silicone-rubber membrane suggests the possibility of intra-arterial use of the neutral-carrier-type ISFETs designed here.

The Na^+-selective electrodes based on silicone-rubber membranes modified chemically by **8** and **9** were also attempted for Na^+ assay in control serum and urine (Table 1) /15/. The Na^+ concentration values found in both the serum and urine samples are in good agreement with their corresponding

Table 1

Sodium assay in control serum and urine by Na^+-selective electrodes based on silicone-rubber membranes modified chemically by calix[4]arene ionophore **8** and anion excluder **9**

Sample	Na^+ concentration (mM)		Coefficient of variation
	Actual	Found	(%)
Serum	143.0	143.1	0.69
Urine	54.0	54.8	1.37

actual values, with a relative standard deviation of about 1%. These results suggest that the Na^+-selective electrodes based on silicone-rubber membranes modified chemically by calix[4]arene ionophore 8 are reliable on Na^+ assay in human body fluid.

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