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Supported sol–gel-derived membranes for neutral carrier-type ion-selective electrodes

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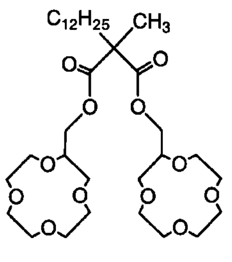
For neutral carrier-type ion-selective electrodes, several sol–gel-derived membranes were designed which encapsulate valinomycin and bis(crown ether) derivatives as the neutral carriers. Relatively large size ion-sensing membranes based on sol–gel-derived glass were successfully fabricated by using membrane filters and sintered glass filters as the membrane supports. Potassium and sodium ion-selective electrodes, at the tip of which sol–gel-derived membranes containing a neutral carrier and a poly(tetrafluoroethylene) membrane filter were incorporated, exhibit high electrode performance in their sensitivity, selectivity, and response time. The supported sol–gel-derived membranes were also modified chemically by alkoxysilylated bis(crown ether) derivatives and were tested for their usefulness as sensing membranes of ion-selective electrodes.

Introduction

Potentiometric ion sensors are convenient tools for selective determination of metal ions in solution.[1,2] Specifically, neutral carrier-type ion sensors, in which ionophores such as crown ethers can be used as ion-sensing materials, are highly selective for alkali and alkaline-earth metal ions[3] and are therefore useful for metal ion assays in biological and environmental situations.

Plasticized poly(vinyl chloride) (PVC) membranes, which are generally employed for neutral carrier-type ion sensors, require special plasticizers, but they have some problems associated with membrane durability due to the easy extrusion of their plasticizers and neutral carriers. Sol–gel-derived glasses are gaining increasing interest as functional glasses, into which various organic materials can be incorporated due to their low-temperature fabrication.[4,5] We have already applied sol–gel-derived ion-sensing membranes containing neutral carriers for ion-sensitive field-effect transistors (ISFETs), taking into account their low toxicity, high processibility, and high adhesiveness to FET gate surfaces.[6] The neutral carrier-type Na^+- and K^+-ISFETs with sol–gel-derived membranes on their gate surfaces showed high performance in areas such as biocompatibility.

The biocompatible neutral carrier-type ion-sensing membranes obtained by the sol–gel method are also expected to be useful for ion-selective electrodes (ISEs) that are more popular ion sensors than ISFETs. However, ISEs require large ion-sensing membranes (discs with larger than 5 mm diameter) as compared with ISFETs. Since the ion-sensing membranes possess a medium thickness of more than 0.1 mm, such large sol–gel-derived membranes are quite hard to fabricate due to their brittleness. This prompted us to adopt a membrane support for reinforcing sol–gel-derived membranes containing neutral carriers. This paper is concerned with the design of supported sol–gel-derived membranes incorporating valinomycin and bis(crown ether) derivatives **1** through **4** by encapsulation or chemical modification for neutral carrier-type ISEs. Some applicability of the resulting K^+- and Na^+-ISEs in metal ion assay is also described.



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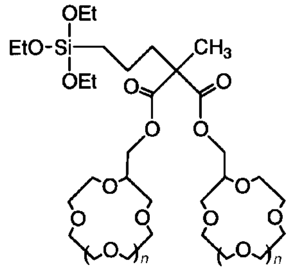

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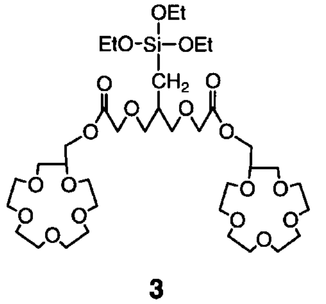

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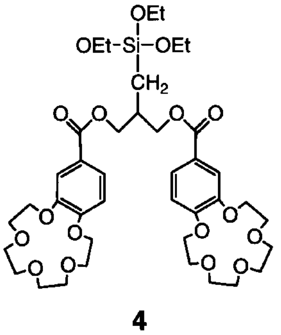

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Experimental

Syntheses

Bis(1,4,7,10,13-pentaoxacyclopentadecylmethyl)-2-allyl-2-methylmalonate or bis(15-crown-5-ylmethyl)-2-allyl-2-methylmalonate. 2-(Hydroxymethyl)-15-crown-5 was obtained with some modification of a published method.[7] To a dry benzene solution (600 mL) of 2-(hydroxymethyl)-15-crown-5 (90 mmol)

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and 2-allyl-2-methylmalonyl chloride (40 mmol) was added AgCN (20 g).[8] The mixture was refluxed for 7 days while stirring. After the reaction, the AgCN was filtered off and the filtrate was passed through a 2 cm Celite column. Solvent evaporation afforded a crude product. Purification by gel-permeation chromatography ($CHCl_3$) yielded the oily product, bis(15-crown-5-ylmethyl)-2-allyl-2-methylmalonate (viscous liquid, yield 30%): ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.17(3H, s, CH_3), 2.5–2.6(2H, m, CH_2C), 3.5–3.9(38H, m, CH_2OCH_2 and OCH), 4.1–4.3(4H, m, CO_2CH_2), 5.0–5.1(2H, m, $C\mathit{H}_2{=}CH$), 5.6–5.8(1H, m, $CH_2{=}C\mathit{H}$); IR(neat) 2910, 2870, 1730, 1645, and 1250 cm^{-1}; *m*/*z* (% rel intensity) 623(M^+,100). Anal. calcd for $C_{29}H_{50}O_{14}$: C, 55.93; H, 8.09; O, 35.98. Found: C, 56.16; H, 8.12%.

Bis(1,4,7,10,13-pentaoxacyclopentadecylmethyl)-2-triethoxysilylpropyl-2-methylmalonate or bis(15-crown-5-ylmethyl)-2-triethoxysilylpropyl-2-methylmalonate 2 (*n* = 2). To a dry benzene solution (30 mL) containing trichlorosilane (1.48 mmol) and bis(15-crown-5-ylmethyl)-2-allyl-2-methylmalonate (1.20 mmol) was added a tetrahydrofuran (THF) solution (2 mL) of $H_2PtCl_6{\cdot}H_2O$ (10 mg). The mixture was refluxed for 10 h while stirring in an argon atmosphere. After evaporating the benzene, dry ethanol (50 mL) was added to the residue and then the mixture was stirred in an ice bath. The catalyst was removed by centrifugation for half an hour. Evaporation of the excess ethanol and trichlorosilane gave the oily product, **2** (*n* = 2). The product was used for the subsequent sol–gel processing without further purification. **2** (*n* = 2) (viscous liquid, yield 86%): ^{1}H-NMR (270 MHz, $CDCl_3$) δ 0.07(2H, t, $J = 6.0$ Hz, $SiCH_2$), 1.21(9H, t, $J = 6.5$ Hz, $CH_2C\mathit{H}_3$), 1.42(3H, s, $CH_2CC\mathit{H}_3$), 1.6–1.9(4H, m, $SiCH_2(C\mathit{H}_2)_2C$), 3.4–3.9(44H, m, $SiOCH_2$, CH_2OCH_2, OCH), 4.1–4.3(4H, m, CO_2CH_2); IR(neat) 2960, 2870, 1730, 1260, 1100, and 805 cm^{-1}.

Bis(1,4,7,10,13-pentaoxacyclopentadecylmethyl)-5-methylene-3,7-dioxanonane-1,9-diate or bis(15-crown-5-ylmethyl)-5-methylene-3,7-dioxanonane-1,9-diate. To a dichloromethane solution (100 mL) of diethyl diazoacetate (2.25 mol) and 2-methylene-1,3-propanediol (1.12 mol) was added $BF_3{\cdot}OEt_2$ (1 mL) portionwise. The mixture was stirred at room temperature for an hour. Evaporation of the solvent afforded a pale-yellow viscous oil, diethyl(5-methyl-3,7-dioxanonanate). Hydrolysis of diethyl(5-methyl-3,7-dioxanonanate) by refluxing in EtOH/water (10/1) containing NaOH for 24 h yielded 5-methyl-3,7-dioxanonanoic acid, which was in turn chlorinated by refluxing with $SOCl_2$ in dry benzene (including several drops of *N*,*N*-dimethylformamide) for 12 h to give 5-methyl-3,7-dioxanonanoyl chloride.

The reaction of 2-(hydroxyethyl)-15-crown-5 and 5-methyl-3,7-dioxanonanonyl chloride in the presence of AgCN was carried out by a similar procedure to that for bis(15-crown-5-ylmethyl) 2-allyl-2-methylmalonate. Gel-permeation chromatography yielded an oily product, bis(15-crown-5-ylmethyl)-5-methylene-3,7-dioxanonane-1,9-diate (viscous liquid, yield 62%): ^{1}H-NMR (270 MHz, $CDCl_3$) δ 3.5–3.9(38H, m, $O(CH_2)_2O$ and $OCH_2C\mathit{H}O$), 4.00(4H, s, $C\mathit{H}_2C{=}CH_2$), 4.2–4.4(4H, m, CO_2CH_2), 4.70(4H, s, OCH_2CO), 5.2–5.3(2H, m, $CH_2{=}C$); IR(neat) 3510, 2870, 1750, 1450, 1130, and 940 cm^{-1}; *m*/*z* (% rel intensity) 669(M^+,19), 233(M^+,100). Anal. calcd for $C_{30}H_{52}O_{16}$: C, 53.88; H, 7.84; O, 38.28. Found: C, 54.17; H, 7.71%.

Bis(1,4,7,10,13-pentaoxacyclopentadecylmethyl)-5-triethoxysilyl-3,7-dioxanonane-1,9-diate or bis(15-crown-5-ylmethyl)-5-triethoxysilyl-3,7-dioxanonane-1,9-diate 3. A similar procedure for **2** (*n* = 2) afforded compound **3** (viscous liquid, yield 83%): ^{1}H-NMR (270 MHz, $CDCl_3$) δ 0.06(2H, s, $SiCH_2$), 1.2–1.3(9H, m, $CH_2C\mathit{H}_3$), 1.8–1.9(5H, m, $SiCH_2C\mathit{H}(C\mathit{H}_2)_2$), 3.4–3.9(44H, m, $SiOCH_2$, OCH_2, OCH), 4.1–4.3(4H, m, CO_2CH_2), 4.56(4H, s, OCH_2CO).

1,3-Bis[2,3-(1,4,7,10,13-pentaoxacyclopentadeca-2-ene)phenyl-4′-carboxyl]-2-methylene-propane or 1,3-bis[(benzo-15-crown-5-yl)phenyl-4′-carboxyl]-2-methylene-propane. The reaction of 2-methylene-1,3-propanediol and 4′-chlorocarbonylbenzo-15-crown-5 in the presence of AgCN was carried out by a similar procedure to the above-mentioned esterification. Gel-permeation chromatography ($CHCl_3$) yielded pure 1,3-bis[(benzo-15-crown-5-yl)phenylcarboxyl]-2-methylene-propane (colorless solid, yield 30%): mp 98 °C ^{1}H-NMR (270 MHz, $CDCl_3$) δ 3.7–4.2(32H, m, $O(CH_2)_2O$), 4.8–4.9(4H, m, CCH_2O), 6.8–7.7(8H, m, aromatic H); IR(neat) 3580, 2930, 1710, 1590, 1510, 1440, 1270, 1110, and 760 cm^{-1}; *m*/*z* (% rel intensity) 677(M^+,27), 163(100). Anal. calcd for $C_{34}H_{48}O_{14}$: C, 59.47; H, 6.46; O, 34.07. Found: C, 59.07; H, 6.35%.

1,3-Bis[2,3-(1,4,7,10,13-pentaoxacyclopentadeca-2-ene)phenyl-4′-carboxyl]-2-(triethoxysilylmethyl)propane or 1,3-bis[(benzo-15-crown-5-yl)phenyl-4′-carboxyl]-2-(triethoxysilylmethyl)propane 4. A similar procedure for **2** (*n* = 2) afforded compound **4** (glass, yield 91%): ^{1}H-NMR (270 MHz, $CDCl_3$) δ 0.05(2H, s, $SiCH_2$), 1.1–1.3(9H, m, $CH_2C\mathit{H}_3$), 1.8–1.9(5H, m, $SiCH_2C\mathit{H}(C\mathit{H}_2)_2$), 3.4–3.9(46H, m, $SiOCH_2$, OCH_2, OCH), 4.1–4.2(4H, m, $C\mathit{H}_2CHC\mathit{H}_2$), 4.3–4.4(1H, m, $CH_2C\mathit{H}CH_2$), 6.8–7.7(6H, m, aromatic H).

Other materials

The starting chemicals for the sol–gel processing, diethoxydimethylsilane (DEDMS) and tetraethoxysilane (TEOS), were purchased from Shin-Etsu Chemicals. Membrane filters made from polytetrafluoroethylene (PTFE) (Fluoropore FP-080, Sumitomo Electric, pore size of 0.8 μm and thickness of 0.1 mm) and cellulose nitrate (Advantec A045A047A, Toyo Roshi, pore size of 0.45 μm and thickness of 0.1 mm), and sintered glass filters with a No. 5 pore size were purchased for the supports of sol–gel-derived membranes. The thin glass substrate with an appropriate thickness was fabricated by extending a slide glass (Iwaki, Micro Slide Glass). The silicone-rubber precursor employed was of the alcohol-evolving-type (Shin-Etsu Chemicals, KE47T). PVC with an average polymerization degree of 1020 was purified by reprecipitation from THF in methanol. 2-Ethylhexyl sebacate (DOS) was purified by vacuum distillation.

Valinomycin and bis(12-crown-4-ylmethyl)-2-dodecyl-2-methylmalonate **1** were employed as received from Aldrich and Dojindo, respectively. Bis(12-crown-4-ylmethyl)-2-triethoxysilylpropyl-2-methylmalonate **2** (*n* = 1) was prepared in a previously reported procedure.[6] Potassium and sodium [4-(triethoxysilylpropyloxy)phenyl]triphenylborate (NaTESPB) and its corresponding potassium salt (KTESPB) were obtained with some modification of a method reported elsewhere.[6] Sodium tetrakis[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB) and potassium tetrakis(4-chlorophenyl)borate (KTpClPB) are commercially available from Dojindo. Alkali and alkaline-earth metal chlorides and ammonium chloride were of analytical reagent grade. Water was deionized.

The samples for control serum (Wako, control serum I) and urine (Bio-Rad, Lyphochek, urine control normal 1) are commercially available. The concentrations for Na^+ and K^+ are as follows: 143 (or 135) mM Na^+ and 4.1 (or 4.3) mM K^+ for the serum and 58 (or 60) mM Na^+ and 24 mM K^+ for the urine.

Fabrication of membranes and electrodes

The general procedure for casting ion-sensing membranes is as follows: TEOS (22 µL, 1.2×10^{-3} mol), DEDMS (62 µL, 3.5×10^{-3} mol), ethanol (69 µL), 0.1 N HCl aqueous solution (21 µL, 1.2×10^{-3} mol), a neutral carrier (1 mg), and a sodium tetraphenylborate derivative (0.2 mg) were mixed in a sample tube (2 mL) and the mixture was then allowed to stand for 15 h at ambient temperature (or 2 h at 50 °C) to afford a viscous sol–gel solution. In some cases, the solution also contained a small quantity (25 mol% to the neutral carrier employed) of an anion excluder. An aliquot (5 µL) of the sol–gel solution was placed on a membrane support fixed at the tip of an ion-selective electrode kit (DKK, Japan). The diameters for support membranes were 6 mm for the membrane filters and 9 mm for the sintered glass filter and thin glass substrate. The tip was allowed to stand at 50 °C for 2 days.

Silicone-rubber-based membranes were prepared by casting chloroform solution (0.6 mL) containing silicone rubber precursor (90 mg) and a neutral carrier (10 mg) on a PTFE-made Petri dish with an inner diameter of 17 mm. Gradual evaporation of the chloroform and hardening at ambient temperature for two days afforded an elastic, semitransparent membrane with a thickness of 0.1–0.2 mm. Similarly, PVC membranes were prepared by casting from a solution consisting of PVC (50 mg), DOS (100 mg), neutral carrier (10 mg), and THF (2 mL) on a flat Petri dish of 20 mm inner diameter. A 6 mm diameter disk was cut from the membrane with a cork borer and was then incorporated into the electrode body. The internal filling solution was 1×10^{-3} M NaCl aqueous solution. Conditioning of the electrodes was effected by soaking in the NaCl solution overnight.

Measurements

Potential measurements were made at 25 °C using a pH/mV meter (Toko, TP-1000). The external reference electrode was a double-junction-type Ag/AgCl electrode with a 3 M KCl internal solution and a 1 M CH_3CO_2Li external solution. The electrochemical cell was Ag | AgCl | 1×10^{-3} M KCl (or NaCl) | membrane | sample solution || 1 M CH_3CO_2Li || 3 M KCl | AgCl | Ag. The measuring metal ion activities were changed by injection of high-concentration solutions into the testing solutions, while stirring with a magnetic stir bar. The emf readings were made after the potential reached a constant value. The activity coefficients (γ) were calculated according to the Davies equation,[9] $\log \gamma = -0.511 z^2 I^{1/2}/(1 + 0.33\alpha I^{1/2}) - 0.1 z^2 I$, where z, α, and I stand for electric charge, ionic parameter (3 for K^+ and 4 for Na^+), and ionic strength, respectively. Selectivity coefficients for K^+ (or Na^+) with respect to other cations were determined by a mixed-solution method (FIM). The background cation concentrations for K^+ selectivity were 1 M for Na^+ and H^+, 5×10^{-1} M for Li^+, Ca^{2+}, and Mg^{2+}, and 1×10^{-1} M for H^+ and NH_4^+. Those for Na^+ selectivity were 1×10^{-1} M for K^+ and H^+, 5×10^{-1} M for Li^+, Ca^{2+}, and Mg^{2+}, and 1 M for NH_4^+. Response times (t_{90}) were determined on changing the Na^+ activity of the sample solution from 1×10^{-3} M to 3×10^{-3} M.

Membrane impedance was determined by an ac impedance method, using an impedance analyzer (Solartron, type 1253) equipped with a high-speed current amplifier. The membrane resistance was calculated from the Cole–Cole plot.

Potassium and sodium assays in serum and urine samples were carried out five times for each sample by Gran's plot method.[10] For the standard addition method, the volumes for the sample and adding solution (1×10^{-2} M KCl or 2 M NaCl aqueous solution) were 5 (for serum) or 10 (for urine) and 0.2 mL, respectively. The slopes were determined in advance by four-time addition of the standard solution.

Scanning electron microscopy (SEM) of the ion-sensing membranes was undertaken with 1000 magnifications at an acceleration voltage of 20 kV. Gold was evaporated on the sample surface.

Results and discussion

Sol–gel-derived membranes encapsulating neutral carriers

In general, functional sol–gel-derived glasses are fabricated on hard materials such as glasses and metals. Membrane supports, although not necessarily hard, are also required for the fabrication of sol–gel-derived membranes based on neutral carriers. Membrane filters made from PTFE and nitrocellulose, and sintered glass filters, thin borosilicate glasses were investigated for their usefulness as the membrane support. The alkoxysilane reagents for sol–gel processing were mixtures of TEOS and DEDMS. A TEOS/DEDMS ratio of 1/3 was adopted here, which was found to be the best for applications to ion-sensitive field-effect transistors (ISFETs).[6] In the ISFET applications, the TEOS/DEDMS ratios of 1/1 and 1/2 yielded ISFETs with sensing membranes which were too hard for high sensitivity, while the TEOS/DEDMS ratio of 1/4 afforded only sticky membranes.

Fig. 1 shows SEM photographs for the sol–gel-derived membranes containing valinomycin as the neutral carrier and PTFE and cellulose membrane filters and sintered glass filters

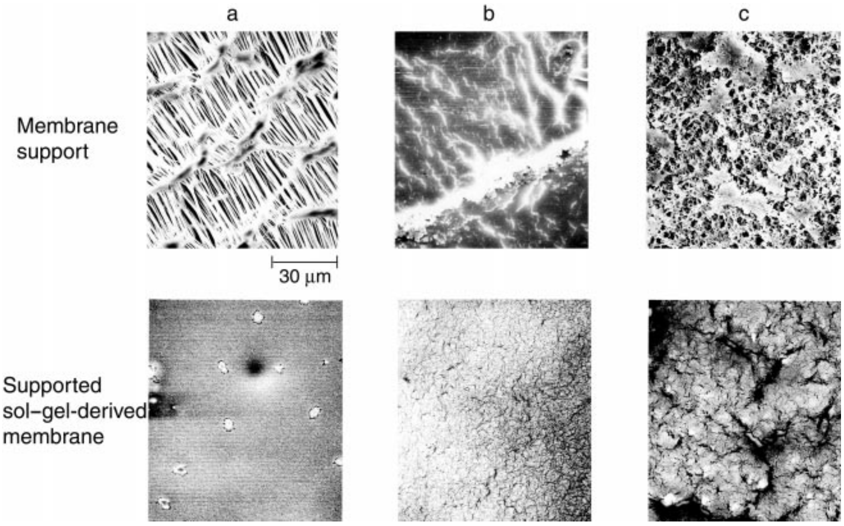

Fig. 1 Scanning electron micrographs for supported sol–gel-derived membranes and supports themselves. Membrane support: PTFE membrane filter (a); sintered glass filter (b); cellulose membrane filter (c).

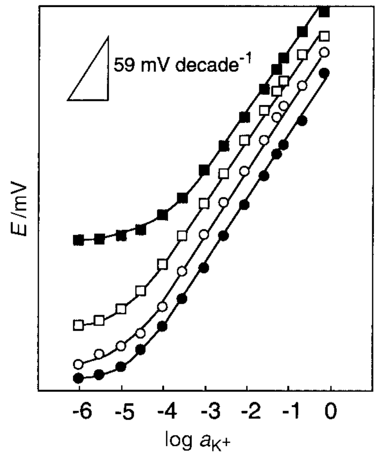

Fig. 2 Potential response of K^+-selective electrodes based on valinomycin-based sol–gel-derived membranes supported by PTFE membrane filter (○), sintered glass filter (●), cellulose membrane filter (□), and thin glass substrate (50 μm thickness) (■).

as the membrane support, together with the corresponding membrane supports themselves. The composite membranes based on a PTFE membrane filter possess a plain surface, which means that a sol–gel-derived membrane containing the neutral carrier was formed quite uniformly with high affinity to the membrane support. On the contrary, fine cracks were found on the surface of composite membranes based on a cellulose membrane filter and a sintered glass filter. The hydrophilic membrane supports do not seem to be very compatible with the sol–gel-derived component.

Typical potential responses to K^+ activity changes for the sol–gel-derived membrane K^+-selective electrodes based on valinomycin are shown in Fig. 2. The valinomycin-based ion-selective electrodes with sol–gel-derived membranes supported by a PTFE membrane filter and a sintered glass filter exhibited Nernstian responses (59 mV decade^{-1}) to K^+ activity changes over a wide activity range of 3×10^{-5}–3×10^{-1} M. The membrane supported by a cellulose membrane filter gave worse results in the electrode sensitivity, *i.e.*, a slope of 55 mV decade^{-1} over a K^+ activity range of 3×10^{-4}–3×10^{-1} M. A similar membrane supported by a thin borosilicate glass substrate was also tested as an ion-sensing membrane for K^+-selective electrodes. Interestingly, the membrane supported by a glass substrate with a thickness of 50 μm afforded a sub-Nernstian response, although the linear activity range is narrower than the other membranes supported by membrane and glass filters. The response times (t_{90}) for the above-mentioned K^+-selective electrodes of the valinomycin-based sol–gel-derived membranes ranged from several to several tens of seconds.

Membrane impedance (electrical resistance) for the supported sol–gel-derived membranes was measured to elucidate their effect on the sensitivity and response time of the resulting K^+-selective electrodes. Membranes based on plasticized PVC and silicone-rubber, and a sol–gel-derived membrane supported by a thick glass substrate were also employed for comparison. Fig. 3 summarizes the electrical resistance for various membranes, relating them to the electrode sensitivity (slope of K^+ calibration graph) and response time for the resulting K^+-selective electrodes. From the viewpoint of sensitivity, sol–gel-derived membranes with a medium membrane resistance, such as the membranes supported by PTFE and cellulose membrane filters and a sintered glass filter, allowed a high electrode sensitivity with Nernstian response for the K^+-selective electrodes, as is the case with the plasticized-PVC and silicone-rubber membranes. The much higher resistance, as seen in the sol–gel-derived membrane supported by a thick glass substrate, decreased the sensitivity in the K^+-selective electrode, probably due to the low ion-exchanging efficiency and the instability of the emf readings. Also, cellulose-supported sol–gel-derived membranes possessing too low a resistance afforded only poor sensitivity in the electrode. Probably, the efficient ionic conduction in the cellulose-supported membranes makes it difficult to establish a steady emf at the membrane interface. In the relationship between the membrane resistance and response time, low membrane resistance can generally realize fast electrode response in the ion-selective electrode, whereas high electrical resistance causes slow electrode response. Low membrane resistance can often be reflected in efficient ion exchange at the membrane interface, which in turn results in a fast electrode response. An anion excluder, KTpClPB, does not appear to improve the membrane resistance and therefore the performance of K^+ electrodes based on sol–gel-derived membranes supported by PTFE and cellulose membrane filters in this case.

The electrodes based on sol–gel-derived membranes containing valinomycin and a PTFE membrane filter or a sintered glass filter are highly K^+-selective, as shown in Fig. 4. The selectivity coefficient for K^+ with respect to Na^+ is 1.6×10^{-4}, reflecting the ion selectivity of the neutral carrier itself.[11]

The bis(12-crown-4) derivative **1** was also investigated as a Na^+ neutral carrier of sol–gel-derived membranes supported by a PTFE membrane filter. Since some anion interference was observed with Na^+-selective electrodes based on membranes of **1** in high Na^+ activity ranges, NaTFPB was also included in the membranes as the anion excluder. The resulting Na^+-selective electrodes responded to Na^+ activity changes in a Nernstian

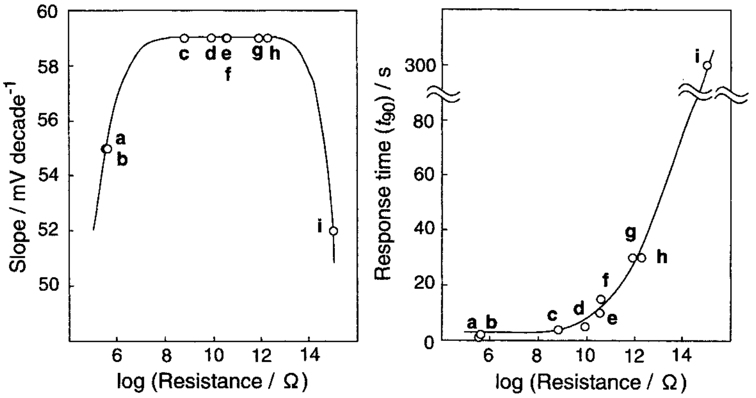

Fig. 3 Relationship between sensitivity (slope in calibration graph) or response time and membrane impedance for sol–gel-derived membranes of valinomycin supported by various supports. a: Cellulose membrane filter (also containing KTpClPB), b: cellulose membrane filter, c: DOS-plasticized PVC, d: silicone rubber, e: thin glass substrate (50 μm), f: sintered glass filter, g: PTFE membrane filter (also containing KTpClPB), h: PTFE membrane filter, i: thick glass substrate (150 μm).

Table 1 Metal-ion assay in control serum and urine samples by neutral carrier-type ion-selective electrodes based on PTFE-supported sol–gel-derived membranes

	K^+ concentrations/mM			Na^+ concentration/mM		
Sample	Actual	Found	Coefficient of variation (%)	Actual	Found	Coefficient of variation (%)
Serum	4.30	4.30	0.66	135.0	135.7	0.64
Urine	24.0	23.9	0.78	60.0	59.9	0.97

manner over the activity range 1×10^{-4}–3×10^{-1} M and with a short response time of 8 s.

Sol–gel-derived membranes modified chemically by neutral carriers

For neutral carrier-based sol–gel-derived membranes, incorporation of a neutral carrier by covalent bonding is preferable.[12] In the ion-sensing membranes simply encapsulating a neutral carrier, some exudation from the membrane phase to an aqueous phase occurs, which in turn brings about fast deterioration of the membrane and thereby of the electrode. Alkoxysilylated bis(crown ether) derivatives were employed as the neutral carriers for the chemical modification and were incorporated into sol–gel-derived membranes by their hydrolysis with TEOS and DEDMS, followed by condensation. Alkoxysilylated tetraphenylborate of K^+ and Na^+ (NaTESPB and KTESPB) were also used to incorporate a lipophilic salt through covalent bonding, together with one of the neutral carriers. In the application to ISFETs,[12] the covalent bonding of active materials on the sol–gel-derived membranes significantly improved the durability of the corresponding membranes simply encapsulating the active material. Therefore, covalent bonding was also employed here to obtain large-size, highly durable supported sol–gel-derived membranes for ISEs.

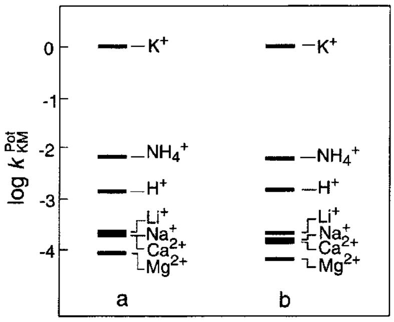

Fig. 4 Ion selectivities of K^+-selective electrodes based on valinomycin-based sol–gel-derived membranes supported by PTFE membrane filter (a) and sintered glass filter (b).

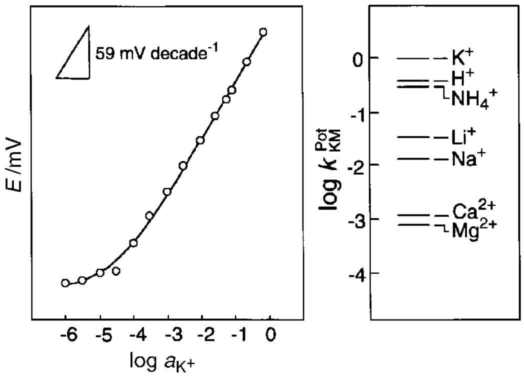

Fig. 5 Potential response and ion selectivities for K^+-selective electrodes based on PTFE-supported sol–gel-derived membrane modified chemically with bis(15-crown-5) **2** ($n=2$).

PTFE-supported sol–gel-derived membranes containing **2** ($n=2$) and KTESPB were fabricated, aiming at high-performance K^+-selective electrodes. The potential response for the K^+-selective electrodes showed a Nernstian response to K^+ activity changes over a wide activity range (Fig. 5). The ion selectivity for the K^+-selective electrode is fairly good, the selectivity coefficient for K^+ with respect to Na^+ being 1×10^{-2}. For improvement of the K^+ selectivity, two other bis(15-crown-5) derivatives **3** and **4** were also used as the K^+ neutral carrier instead of **2** ($n=2$), but higher K^+ selectivity against Na^+ could not be obtained. The potential response for the K^+-selective electrode based on a sol–gel-derived membrane modified chemically with **2** ($n=2$), **3**, and **4** was more sluggish than that for those based on sol–gel-derived membranes simply encapsulating a neutral carrier, the response times (t_{90}) being about several tens of seconds. This was the case with the Na^+-selective electrode based on PTFE-supported sol–gel-derived membranes modified chemically with **2** ($n=1$) and NaTESPB. The relatively slow potential response may be attributed to the low mobility of the neutral carriers, which is in turn derived from their immobilization by covalent bonding. Since ISFETs based on similar neutral-carrier-containing sol–gel-derived membranes without any membrane support exhibit fast potential response in ISFETs,[12] the adoption of membrane supports such as PTFE membrane filters in addition to the neutral carrier incorporation by chemical bonding probably decreases the neutral carrier mobility in the membrane even further.

Some applications for neutral carrier-type ion-selective electrodes based on sol–gel-derived membranes

Ion-selective electrodes based on PTFE-supported sol–gel-derived membranes containing a neutral carrier were tested for their applicability in the metal ion assay of human fluids. Assays of K^+ and Na^+ in control blood serum and urine samples were carried out with K^+- and Na^+-selective electrodes based on valinomycin and bis(12-crown-4) derivative **1**, respectively. The results are summarized in Table 1. The values of K^+ concentrations obtained with the neutral-carrier-based sol–gel-derived membrane K^+ electrode were in good agreement with the actual values, with variation coefficients of less than 1%. The table also shows that the Na^+ concentrations in the serum and urine samples were successfully determined by the Na^+-selective electrode.

Thus, the neutral carrier-type ion-selective electrodes based on supported sol–gel-derived membranes can afford reliable data for metal ion assay in human fluids and are quite promising for practical application in biological systems.

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