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Mercuracarborand "Anti-Crown Ether"-Based Chloride-Sensitive Liquid/Polymeric Membrane Electrodes

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Highly sensitive and selective chloride liquid/polymeric membrane electrodes are described that employ [9]-mercuracarborand-3 (MC3), a neutral preorganized macrocyclic Lewis acid, as the anion carrier. MC3-based chloride-sensitive membrane electrodes, doped with different mole percentages of cationic additives (5, 10, and 60 mol % tridodecylmethylammonium chloride) relative to the amount of the carrier, exhibit enhanced potentiometric selectivity for chloride over other anions, including more lipophilic anions such as perchlorate, nitrate, and thiocyanate. In addition, the selectivity coefficients obtained are shown to meet the requirement for clinical applications. The obtained selectivity pattern is shown to correlate very well with ^{199}Hg NMR titrations of MC3 with various anions, performed in organic solvents. Optimized membrane electrodes show a near-Nernstian response toward chloride over a wide concentration range and have micromolar detection limits. MC3-based chloride sensors show a fast response time (in the order of few seconds), as well as short recovery time. The developed mercuracarborand-based sensors do not practically respond to pH changes over the pH range of 2.5−7.0. Response characteristics (e.g., detection limit, linear range, response slope, and selectivity) of the [9]mercuracarborand-3 based chloride sensors remain essentially the same over a period of ∼2 months, reflecting remarkable stability and well-defined chemistry of the macrocyclic Lewis acid ionophore.

Great achievements in the development of highly selective liquid/polymeric membrane electrodes for cations have led to the replacement of conventional techniques for routine analysis of many cations with membrane electrodes. In contrast, only a few anion-selective liquid/polymeric membrane electrodes are used routinely in the assessment of anion concentrations in real samples.[1,2] In particular, the development of selective and sensitive liquid/polymeric membrane electrodes for hydrophilic anions, such as chloride, remains a formidable challenge and is of increasing interest for clinical, environmental, and industrial applications. Various approaches have been attempted to develop chloride liquid/polymeric membrane electrodes. For example, anion exchangers have been explored as the electroactive species for the development of chloride-sensitive liquid/polymeric membrane electrodes.[3−5] However, these anion-exchanger-based chloride sensors lack selectivity over more lipophilic anions, and the sensors respond significantly to more lipophilic anions according to the well-known Hofmeister series[6] (i.e., the response pattern is based on the lipophilicity of the anions). Indeed, chloride determination in physiological fluids (e.g., serum and plasma) using anion-exchanger-based membrane electrodes gave misleading results with high positive errors due to insufficient selectivity over the more lipophilic anions[2,7−9] and some of the hydrophilic anions.[10,11]

The inherent lack of selectivity of anion-exchanger-based chloride electrodes could be overcome by utilizing anion carriers that selectively bind the chloride anion. For instance, a host of organotin compounds have been used as chloride carriers in liquid/polymeric membranes.[12,13] Such chloride-sensitive membrane electrodes demonstrated a selectivity pattern that clearly deviated from the lipophilicity-based Hofmeister selectivity. However, these chloride electrodes were not amenable for practical applications due to long response time, non-Nernstian response, high memory effect, and potentiometric signal instability.[14] More recently, chloride-sensitive membrane electrodes based on Mn(III)−[15] and In(III)−porphyrins,[16] as well as Hg(II)−organic

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compounds,[17,18] have been reported. However, nonclassical behavior,[17,18] chemical instability,[18] insufficient selectivity, and/or protein fouling impeded the use of these electrodes in direct monitoring of chloride in undiluted physiological samples.[16] Very recently, a chloride-sensitive membrane electrode based on a hydrogen-bonding ionophore was developed.[19] This hydrogen-bonding-based chloride-sensing mode is very sensitive to membrane composition and sample solution measuring buffer.

The use of macrocyclic Lewis bases as cation carriers in liquid/polymeric membrane electrodes is well documented in the literature, and a host of highly selective cation membrane sensors based on macrocyclic Lewis bases (e.g., crown ethers, cyclodepsipeptide antibiotics, and calixarenes) have been developed and are routinely used for analyses of various cations in real samples.[20,21] In contrast, anion sensors have yet to reach the same level of achievement, due to the slower pace of anion carrier chemistry. Recently, a new class of carborane-supported macrocyclic Lewis acid hosts (mercuracarborands) was introduced.[22−26] In comparison with cation−macrocyclic systems (e.g., crown ethers), mercuracarborand compounds have a larger highly preorganized Lewis acid cavity that can accommodate guest anions. Moreover, mercuracarborands are air-stable at room temperature and have good lipophilicities, making them rather soluble in most organic solvents (e.g., plasticizers). More importantly, mercuracarborands can be prepared and functionalized with bulky, lipophilic, electron-withdrawing or electron-donating groups and with varying cavity sizes. Such unique features make mercuracarborand anion carriers promising candidates for the development of novel liquid/polymeric membrane electrodes and membrane optodes for anions. Development of chloride optical sensors based on mercuracarborand ionophores is within the scope of another paper.

We herein describe the utilization of a highly preorganized macrocyclic ionophore, [9]mercuracarborand-3 (see Figure 1 for structure), a charge-reverse analogue of the well-known 9-crown-3 ether, for the development of sensitive and selective liquid/polymeric membrane electrodes for chloride. This is the first example of utilizing this class of highly preorganized, macrocyclic, multidentate mercuracarborand Lewis acids as anion carriers in a solvent polymeric membrane.

EXPERIMENTAL SECTION

Reagents. High-molecular-weight poly(vinyl chloride) (PVC), *o*-NPOE, tridodecylmethylammonium chloride (TDMAC), and Selectophore tetrahydrofuran (THF) were obtained from Fluka (Ronkonkoma, NY). *N*-(2-Hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (HEPES) and 2-morpholinoethanesulfonic acid (MES) were obtained from Research Organics (Cleveland, OH). The sodium salts of thiocyanate, chloride, hydrogen sulfite, and sulfate were obtained from Matheson Coleman & Bell (Cincinnati, OH). The sodium salts of iodide, nitrate, and fluoride were obtained from J. T. Baker (Philipsburg, NJ). *n*-Bu_4NClO_4, and sodium salts of bromide, perchlorate, salicylate, acetate, and hydrogen carbonate were purchased from Sigma (St. Louis, MO). *n*-Bu_4NCl, *n*-Bu_4NSCN, and *n*-Bu_4NNO_3 were obtained from Aldrich (Milwaukee, WI). Tetrabutylammonium salts were used as received. Deuterated solvents were obtained form Cambridge Isotope Laboratories (Andover, MA). All other reagents were of analytical grade. Deionized distilled water (14 MΩ) was used for the preparation of all aqueous solutions.

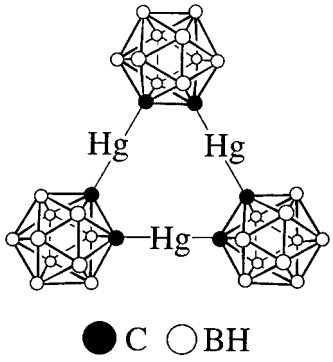

Figure 1. Chemical structure of [9]mercuracarborand-3 ionophore.

Synthesis of [9]Mercuracarborand-3. The [9]mercuracarborand-3 (MC3) ionophore was prepared as described previously[24] by adding a solution of *n*-butyllithium (58.3 mmol) in hexane to an ethereal solution of *o*-carborane (27.7 mmol) at 0 °C and stirring the resulting slurry at room temperature under argon. The mixture was treated with solid $Hg(OAc)_2$ (27.7 mmol) and stirred overnight at room temperature. The mixture was then treated with H_2O and dried over $MgSO_4$. The residue was recrystallized from ether to give MC3 in a 65% yield: mp >300 °C; ^{1}H NMR (200 MHz, $(CD_3)_2CO$, 25 °C) δ 1.0−3.6; ^{13}C{H} NMR (90 MHz, $(CD_3)_2CO$, 25 °C) δ 95; ^{11}B{H} NMR (160 MHz, $(CH_3)_2CO$, 25 °C, $BF_3{\cdot}Et_2O$ external) δ 1.6, −5.4, −8.5 (2:2:6); ^{199}Hg{H} NMR (89.6 MHz, $(CD_3)_2CO$, 25 °C) δ −1364; IR (KBr) ν (cm^{-1}) 2561 (BH).

^{199}Hg{H} NMR Titration. The ^{199}Hg{H} NMR spectra were obtained using a Bruker ARX 500 spectrometer (Billerica, CA) and were recorded at room temperature. The ^{199}Hg{H} NMR spectra were measured in 10-mm sample tubes at 89.4 MHz by using broad-band decoupling. External 1.0 M PhHgCl/DMSO-d_6 solution was used as the reference at −1187 ppm relative to neat Me_2Hg. The ^{199}Hg NMR data were collected at different guest/host ratios for each individual anion in acetone solution. Spectroscopic data: MC3−Cl(*n*-Bu_4N) δ −975, MC3−SCN(*n*-Bu_4N) δ −1139, MC3−NO_3(*n*-Bu_4N) δ −1359, MC3−ClO_4(*n*-Bu_4N) δ −1371.

Preparation of Membrane Electrodes and Potentiometric Measurements. The PVC-based ISE membranes were prepared as described elsewhere[14] using 2.0 wt % MC3, 65 wt % plasticizer (*o*-NPOE), 33 wt % PVC, and different mole percentages of ionic additives (TDMAC) (relative to the ionophore weight). Working electrodes were assembled by cutting 5.5-mm-diameter disks of

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the master membrane and mounting these disks onto Philips electrode bodies (Philips ISE-561; Gläsblaserei Möller, Zurich). A solution of 10.0 mM NaCl prepared in deionized water was used as the internal filling solution for the chloride-sensitive membrane electrodes. Before use, the membrane electrodes were conditioned overnight in a 10.0 mM NaCl or in 10.0 mM NaCl prepared in 10.0 mM HEPES buffer, pH 7.4. During evaluation of the lifetime of the MC3-based chloride sensors, the electrodes were stored in 10 mM NaCl or in 10 mM NaCl buffered at pH 7.4 with HEPES. All potentiometric measurements were made at ambient temperature (25 °C). The galvanic cell used was as follows: Ag/AgCl(s) | 4.0 M KCl || 1.0 M CH_3COOLi || sample solution | ISE membrane | internal reference filling solution | AgCl(s)/Ag. Cell potentials were measured using a Macintosh Quadra 700 computer equipped with a MacADIOS ABO analog/digital input/output board (GW Instruments, Somerville, MA) and a custom-built electrode interface module controlled by SuperScope II software (GW Instruments).[27]

Evaluation of Potentiometric Anion Selectivity and pH Response. Following the IUPAC recommendations, selectivity coefficients of the MC3-based membrane electrodes were calculated using the matched potential method (MPM).[28] According to the MPM method, the sensors are calibrated with potential interfering anions and chloride individually. The selectivity coefficient is given by the concentration ratio of chloride anion to that of the interference anion at a given potential. Selectivity coefficients were determined in the 1.7×10^{-2}–2×10^{-2} M chloride concentration range. However, for highly discriminated anions (e.g., perchlorate, nitrate, acetate, bicarbonate, sulfate, phosphate, and sulfite) the chloride concentration range from 2×10^{-6} to 8×10^{-6} M chloride was selected for accurate determination of selectivity coefficients. The solutions were buffered using 10.0 mM HEPES buffer, adjusted to pH 7.40 ± 0.01. No correction was made for the slight changes in the liquid junction potential of the cell as a function of increasing anion activities. Concentrations were used rather than activities due to difficulty in calculating the activity coefficients in zwitterionic buffers.

The response of the MC3-based chloride-sensitive membrane electrodes toward pH was obtained by titrating 1.0 mM and 10.0 mM NaCl, dissolved in 10.0 mM phosphoric acid, with very small aliquots of concentrated NaOH or H_2SO_4 to span the entire pH range of 2–13 pH units. A glass pH electrode in conjunction with a double-junction reference electrode filled with 1.0 M CH_3COOLi, as the outer bridge solution, was used to monitor the pH of the test solution. The effect of pH on the detection limit was evaluated by calibrating MC3-based membrane electrodes for chloride in 10.0 mM MES buffer, pH 5.5, and 10.0 mM HEPES buffer, pH 7.4.

RESULTS AND DISCUSSION

Various modes of anion–macrocycle interactions have been explored to develop anion-selective liquid/polymeric membrane electrodes that deviate significantly from the Hofmeister selectivity pattern. The majority of such anion sensors are based on a coordinate bond formation between a central metal ion in transition metal complexes or organometallics (e.g., vitamin B_{12} derivatives,[29,30] metalloporphyrins,[16,31,32] metallophthalocyanines,[35,36] and Sn–,[12,13,35] Hg–,[17,18] and Pd–organic compounds[36]) and the target analyte anion. Such an approach has been extensively used for the development of anion sensors for various analytes. Interactions that mimic protein binding with oxoanions (e.g., Coulombic and H-bonding interactions) were also utilized to design a series of guanidinium-based ionophores for hydrogen sulfite/sulfur dioxide and salicylate.[37–39] Other modes of useful interactions that have been used in the development of non-Hofmeister anion-sensitive membrane electrodes include adduct formation,[40] redox interactions,[41,42] and hydrogen bonding.[19]

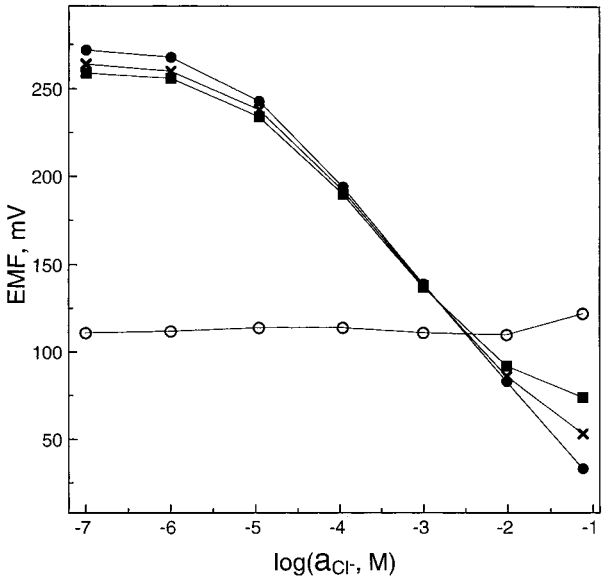

Figure 2. Chloride potentiometric response of [9]mercuracarborand-3 based membrane electrodes doped with different mole percentages of TDMAC: 0 (○), 5 (■), 10 (×), and 60 (●).

Multiple complementary interactions between a preorganized Lewis acid macrocyclic host (see Figure 1 for structure) and chloride ion are utilized herein. As can be seen in Figure 2 and Table 1, liquid/polymeric membrane electrodes based on [9]-mercuracarborand-3, prepared with *o*-NPOE/PVC (2:1) and dif-

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Table 1. Response Characteristics of [9]Mercuracarborand-3-Based Chloride-Sensitive Membrane Electrodes Doped with Various Amounts of TDMAC (Relative to the Ionophore Weight): 5 (Mem-I), 10 (Mem-II), and 60 mol % (Mem-III)

parameter	Mem-I	Mem-II	Mem-III
TDMAC, mol %	5	10	60
slope, mV/decade	−53.4	−53.4	−56.8
linear range, M	10^{-5}–10^{-2}	10^{-5}–10^{-2}	10^{-5}–10^{-1}
detection limit, μM	3.6	5.9	7.1
response time,[a] $t_{90\%}$ (10^{-4} M), s	13 ± 2	8 ± 2	5 ± 0
response time,[a] $t_{90\%}$ (10^{-3} M), s	12 ± 2	8 ± 2	6 ± 3
recovery time,[a] $t_{90\%}$, s	30 ± 9	24 ± 8	43 ± 22

[a] Data shown are averages ±SD (n = 3).

ferent mole percentages of TDMAC as a membrane additive, showed a near-Nernstian response toward chloride ions, over a wide concentration range, and with micromolar detection limits. It was found that the linear range and the slope of the response at high concentrations increase with increasing concentrations of cationic additives in the membrane phase. At lower TDMAC concentrations in the membrane, the calibration plots become nonlinear at higher chloride concentrations due to Donnan failure. In this case, it is plausible that strong binding between [9]mercuracarborand-3 and the chloride ion results in a coextraction of counterions at such high chloride salt concentrations. Counterion coextraction, as predicted theoretically and proved experimentally (see, for example, ref 43 and references therein), leads to a decrease in the response slope and the linear range, especially at high salt concentrations. The presence of high concentrations of cationic additives in the membrane phase minimizes counterions coextraction (i.e., increases anion permselectivity) and, consequently, increases both the linear range and slope of the response.

[9]Mercuracarborand-3 is expected to act as a neutral carrier for anions. Accordingly, membrane electrodes prepared without added lipophilic cationic additives should not induce any anionic response, while such an anionic response is expected only in the presence of cationic sites in the membrane.[36,44] These cationic additives induce the anion permselectivity of the membrane and work as the counterion for the chloride complex of mercuracarborand. Indeed, as shown in Figure 2, an anionic response was obtained only when MC3-based membranes were doped with cationic sites (TDMAC). Membrane electrodes prepared without TDMAC, however, showed a rather cationic response at higher concentrations, due to the presence of endogenous anionic impurities in both PVC and plasticizer.[45,46] This response is a typical behavior for liquid/polymeric membrane electrodes based on neutral anion carriers.[44] This is in contrast to the nonclassical response behavior of chloride sensors based on other Hg(II)–organic compounds,[17] where membrane electrodes prepared without cationic sites induced a super-Nernstian response toward chloride. In such cases, it is possible that the ionophore is involved in an equilibrium or engaged in a reaction that generates cationic sites in the membrane phase.[36] This behavior is anticipated based on the chemical instability and ease of hydrolysis of the Hg–OCO bond.[18,47]

The response and recovery times of chloride-sensitive liquid/polymeric membrane electrodes based on [9]mercuracarborand-3 and doped with cationic additives (5, 10, and 60 mol %), are shown in Figure 3. As demonstrated, MC3-based chloride sensors exhibit a fast response time (t_{90} is in the order of seconds), and an equilibrium potential was reached within few seconds after changing the sample chloride concentrations either from zero to 1×10^{-4} M or from 1×10^{-4} to 1×10^{-3} M. In addition, after exposure to 1×10^{-3} M chloride, MC3-based chloride sensors achieved recovery to baseline potential in less than 1 min (see also data in Table 1). The chloride response signals obtained for MC3-based membrane electrodes at 1×10^{-4} and 1×10^{-3} M were also highly reproducible. Such features (e.g., fast response, good recovery, and good signal reproducibility) are certainly important for the use of MC3-based chloride sensors in automated analyses or in a flow injection mode.

According to well-known host–guest principles, preorganized macrocyclic carriers offer many advantages in comparison with the corresponding acyclic analogues owing to the "macrocyclic effect".[48,49] The preorganized cavity of the host and its multiple, complementary interactions with the guest significantly increase the binding ability of the carrier toward the guest and provide a basis for the selectivity toward the guest species. Indeed, as can be seen in Table 2, liquid/polymeric membrane electrodes based on MC3 and doped with various mole percentages of TDMAC showed a remarkable enhancement in potentiometric selectivity for chloride over other anions in comparison with anion-exchanger-based membrane electrodes. For instance, MC3-based chloride sensors doped with 5 mol % TDMAC (relative to the ionophore weight) showed a significant enhancement of chloride selectivity over all the studied anions, including highly lipophilic anions such as perchlorate, salicylate, and thiocyanate. The optimum amount of TDMAC is reached at 10 mol % (relative to the ionophore weight), as shown in Table 2. At higher mole percentage of TDMAC (60 mol %; see Table 2), however, a partial loss of potentiometric selectivity was obtained, yet the sensors nearly maintained the same selectivity order with significant enhancement over the anion-exchanger-based membrane electrodes.

^{199}Hg NMR experiments were performed to gain a better understanding of the high chloride selectivity obtained for MC3-based membrane electrodes over the more lipophilic anions. A deuterated acetone solution of MC3 was titrated with deuterated acetone solutions of tetrabutylammonium salts of different anions. As can be seen in Figure 4, the chemical shift of the ^{199}Hg NMR peak correlates very well with the obtained potentiometric selectivity sequence. A large chemical shift of the ^{199}Hg NMR peak

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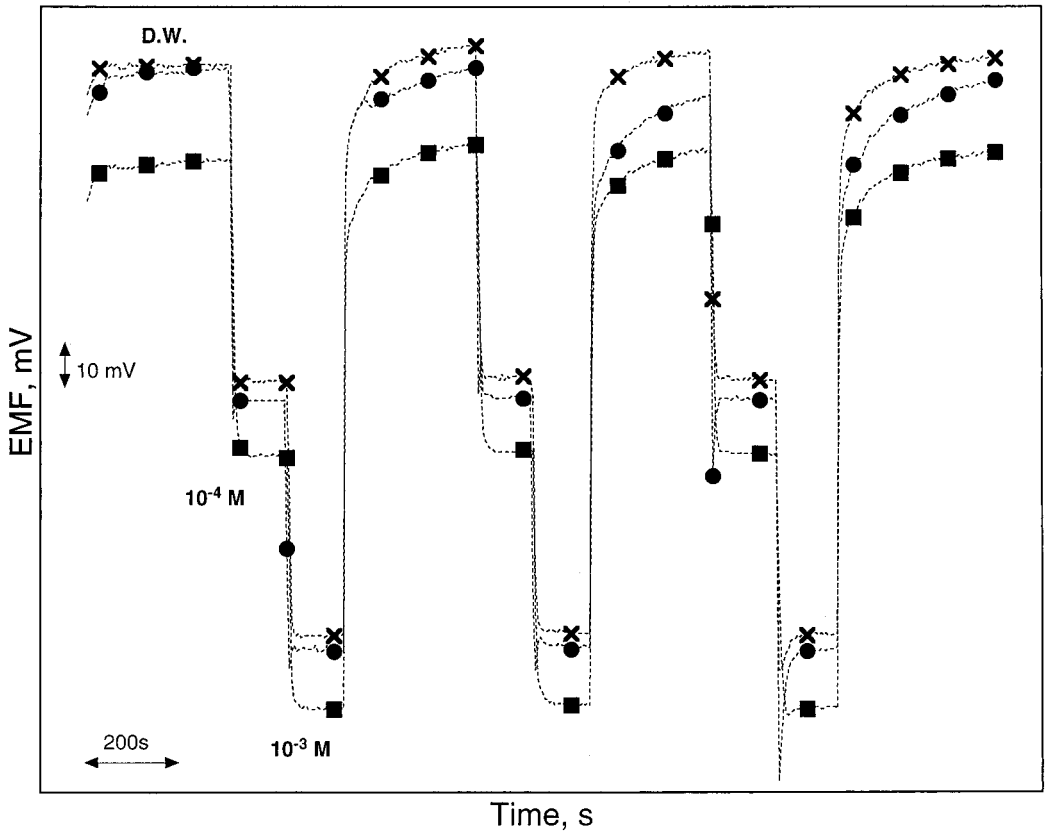

Figure 3. Response time and potentiometric signal reproducibility of [9]mercuracarborand-3-based chloride membrane electrodes doped with different mole percentages of TDMAC: 5 (■), 10 (×), and 60 (●). D. W., deionized distilled water.

Table 2. Selectivity Coefficients of [9]Mercuracarborand-3-Based Electrodes Doped with Various Amounts of TDMAC (Relative to the Ionophore Weight) in Comparison with Anion-Exchanger-Based Membrane (2 wt % TDMAC)[a]

	$\log K^{pot}_{Cl^-,anion}$, MPM				required
anion	2 wt % TDMAC	Mem-I	Mem-II	Mem-II	selectvty coeff[b]
Cl^-	0	0	0	0	0
SCN^-	3.25	−1.43	−1.85	−0.16	1.7 (0.8)[c]
salicylate	3.07	−2.4	−2.25	−0.62	−0.3
Br^-	1.58	1.32	1.25	1.04	0.7
ClO_4^-	3.72	*d*	*d*	−2.79	
I^-	3.14	1.43	1.25	2.34	
NO_3^-	2.36	−4.00	−4.33	−2.71	
SO_4^{2-}	−0.61	*d*	*d*	*d*	
HSO_3^-/SO_3^{2-}	−0.70	*d*	*d*	*d*	
$H_2PO_4^-/HPO_4^{2-}$	−1.29	*d*	*d*	*d*	
HCO_3^-	−1.95	*d*	*d*	*d*	
$CH_3CO_2^-$	−2.55	*d*	*d*	*d*	
F^-	−3.01	*d*	*d*	*d*	

[a] Required selectivity coefficients for physiologically relevant anions are included for comparison with the obtained potentiometric selectivity. [b] Calculated for a minimum error of 1% due to the presence of interfering anion. [c] For smokers. [d] No or minimal response was observed for these anions up to 0.1 M concentration. Accurate values for selectivity coefficients based on the MPM were not accessible in such cases.

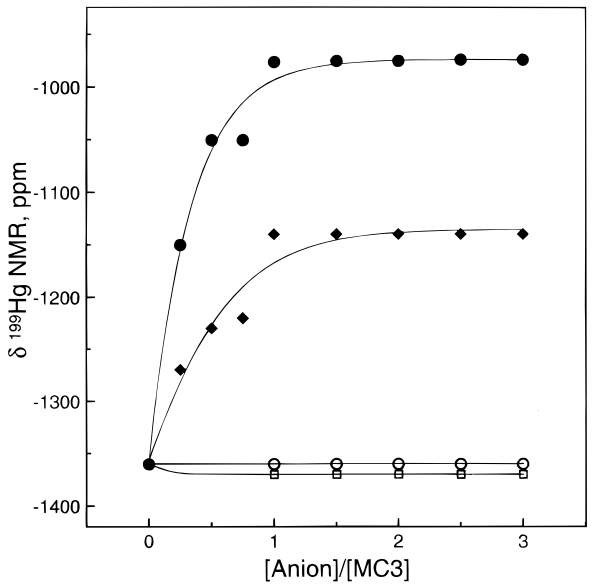

Figure 4. ^{199}Hg NMR titration of [9]mercuracarborand-3 with tetrabutylammonium salt of different anions: chloride (●), thiocyanate (◆), nitrate (○), and perchlorate (□).

is obtained when MC3 was titrated with tetrabutylammonium chloride. However, no change in the ^{199}Hg NMR peak was observed by adding either nitrate or perchlorate salts of tetrabutylammonium, which indicates that very little or no binding occurs between these anions and MC3. On the other hand, the chemical shift observed when MC3 was titrated with the thiocyanate salt is less than that obtained with the chloride salt, which again is highly consistent with the potentiometric selectivity of MC3-based membrane electrodes. The data in Figure 4 also demonstrate that MC-3 forms 1:1 complexes with chloride.

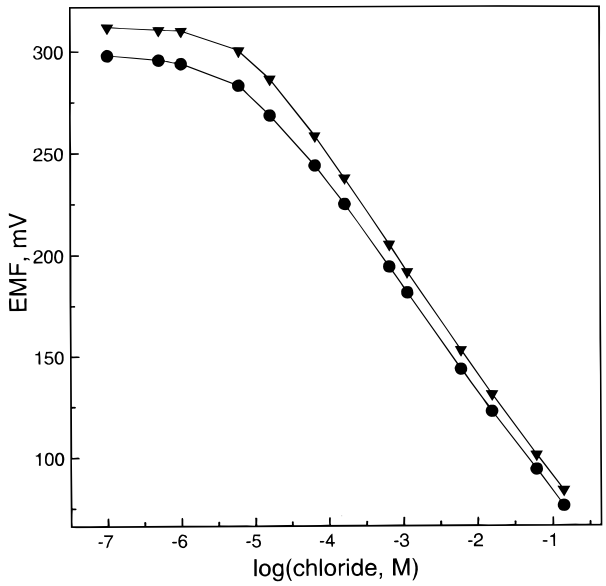

Figure 5. Potentiometric response of [9]mercuracarborand-3-based membrane electrode, doped with 60 mol % TDMAC, toward chloride and measured at different pH values: 10.0 mM MES, pH 5.5 (▼), and 10.0 mM HEPES, pH 7.4 (●).

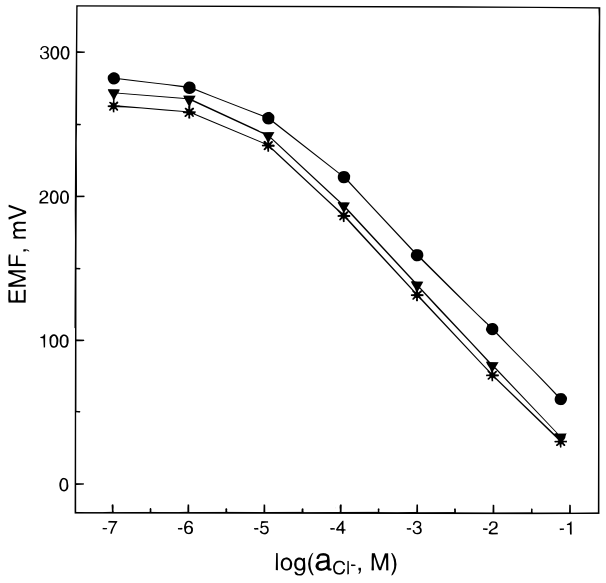

Figure 6. Chloride response curves of [9]mercuracarborand-3-based membrane electrodes (doped with 60 mol % TDMAC) measured over time: 1st (▼), 25th day (∗), and 53rd day (●).

In comparison to anion-exchanger-based electrodes, an improvement in chloride selectivity over iodide and bromide was obtained using MC3 (see data in Table 2). However, MC3-based electrodes respond slightly better to iodide and bromide than chloride, and the selectivity obtained is in the order, iodide > bromide > chloride. Such a selectivity order relative to bromide and iodide was expected based on previous ^{199}Hg NMR studies performed in organic solvents to probe halide interactions with compounds structurally similar to MC3 (e.g., octaethyl [9]-mercuracarborand-3).[26] In these studies, it was found that the presence of halide anions causes a chemical shift of the ^{199}Hg NMR peak in the order: iodide > bromide > chloride.

The use of potentiometric sensors in practical applications depends to a large extent on their selectivity. For comparison purposes, required selectivity coefficients for chloride assay in physiological fluids calculated using the separate solution method (SSM)[2,50] are reported in Table 2. Only anions that exhibit a Nernstian or near-Nernstian response (i.e., chloride, thiocyanate, salicylate, and bromide) are considered. This is because in this case K^{pot} values calculated with the SSM could be compared with those calculated based on the MPM.[28] In comparison with the anion-exchanger-based membrane electrodes that suffer severely from lipophilic anion interferences, the high chloride selectivity of MC3-based chloride-sensitive membrane electrodes over biologically important anions should allow the determination of chloride in physiological fluids. The chloride selectivity coefficients obtained for MC3-based membrane electrodes doped with various mole percentages of TDMAC are much smaller than those required for determination of chloride with <1% error due to interfering anions except in the case of bromide. In the case of bromide, the error in the worst case (i.e., by considering the lowest value of the normal physiological activity range for chloride and the highest value for bromide) is 3.7%, 3.2%, and 1.96% for Mem-I, Mem-II, and Mem-III, respectively. It is worth mentioning that the presence of bromide in physiological fluids (e.g., serum) is very infrequent, since sedation therapy (main source of bromide in addition to bromide poisoning) is obsolete.[9] Further, other physiologically important anions such as bicarbonate, phosphate, and sulfate induce minimal or no response. Therefore, for these anions, it is not possible to calculate accurate selectivity coefficient values based on MPM. This high discrimination against these anions indicates that they should not interfere with the chloride determination in physiological fluids.

Quite interestingly, we found that mercuracarborand-based membrane electrodes do not show any response toward cysteine in HEPES, pH 7.4 (cysteine was obtained as the free base, and the pH was adjusted with H_2SO_4). On the other hand, the response curve toward cysteine (chloride instead of sulfate as counterion) was found to be similar to the chloride calibration curves obtained with simple chloride salts such as sodium chloride, indicating that the response obtained with cysteine hydrochloride is due to chloride ions alone. This finding indicates that applications of the MC-3-based chloride membrane electrodes in physiological fluids may not be hindered by protein interactions through cysteine residues with mercury binding centers of the mercuracarborand ionophore. Unlike other mercury(II)-based ionophores that showed a strong interaction with sulfite/hydrogen sulfite,[41] the response of MC3-based membrane electrodes toward sulfite/hydrogen sulfite, measured in 0.01 M HEPES buffer, pH 7.4, was found to be minimal (see data in Table 2).

The effect of pH on the response of MC3-based membrane electrodes toward chloride was evaluated. It was found that chloride-sensitive membrane electrodes based on MC3 showed virtually no pH response over the range of 2.5−7 pH units, at 1 mM and 10.0 mM chloride ion concentrations (data not shown), which should enable the utilization of such sensors at physiological pH (pH ∼7.4) without a significant loss of either detection limit or slope of the chloride response. Indeed, as shown in Figure 5, the MC3-based electrode (doped with 60 mol % TDMAC) gave a

(50) Oesch, U.; Ammann, D.; Simon, W. *J. Chem. Soc., Faraday Trans.* **1986**, *82*, 1179−1186.

Table 3. Effect of Aging up to 53 Days on the Potentiometric Selectivity of the MC3-Based Chloride-Sensitive Membrane Electrodes, Measured in 10.0 mM HEPES Buffer, pH 7.4[a]

	$\log K^{pot}_{Cl^-,anion}$, MPM		
	perchlorate	nitrate	thiocyanate
Mem-I	*b*	−4.14 [−4.00]	−1.56 [−1.43]
Mem-II	*b*	−4.22 [−4.33]	−1.41 [−1.85]
Mem-III	−2.4 [−2.79]	−2.34 [−2.71]	−0.44 [−0.16]

[a] Values in brackets are for freshly prepared membranes. [b] No or minimal response was observed up to 0.1 M concentration. Accurate values for selectivity coefficients based on the MPM were not accessible in such cases.

similar detection limit and slope of the calibration plot toward chloride in 10.0 mM MES buffer, pH 5.5, and in 10.0 mM HEPES buffer, pH 7.4. Similar results were obtained for membrane electrodes doped with 5 and 10 mol % TDMAC (data not shown).

Membrane electrodes prepared with MC3 and doped with 60 mol % TDMAC exhibited a good lifetime, as demonstrated in Figure 6. The slope and detection limit of MC3-based membrane electrodes doped with 60 mol % TDMAC, measured in deionized distilled water, remained unchanged over a period of ∼2 months (53 days). Similar results were obtained for chloride calibration curves generated in buffered solutions. In addition, the selectivity of "aged" MC3-based chloride-sensitive membrane electrodes doped with 60 mol % TDMAC was practically very close to that of freshly prepared sensors (data in brackets), as depicted in Table 3. Membrane electrodes doped with 5 and 10 mol % TDMAC showed a slightly higher detection limit after 2 months of soaking (shift to higher concentration by ∼0.4 logarithmic concentration unit, data not shown). Such a slight loss of lower detection limit is possibly due to leaching of the ionophore at lower mole ratios of TDMAC.

CONCLUSIONS

A new class of anion carriers has been utilized for the development of chloride-sensitive liquid polymeric/membrane electrodes. It has been demonstrated that [9]mercuracarborand-3 binds chloride via the neutral carrier mechanism, and the membrane induces anion permselectivity only in the presence of added cationic sites. Unlike anion-exchanger-based membrane electrodes, MC3-based chloride sensors were shown to induce a selectivity pattern completely different from the Hofmeister pattern, with a high preference for chloride ions over the other anions, including most of the lipophilic anions. The obtained selectivity pattern was shown to correlate very well with ^{199}Hg NMR titration data obtained in organic solvents. The selectivity coefficients of MC3-based chloride sensors were shown to fulfill the required values for chloride analyses in physiological fluids. In addition, it was found that chloride-sensitive MC3-based membrane electrodes exhibit essentially no pH dependence at physiological pH. Furthermore, chloride-sensitive membrane electrodes based on the mercuracarborand ionophore displayed a very short response time and fast recovery. Finally, we found that aging for up to ∼2 months for membrane electrodes doped with high TDMAC concentrations has no practical effect on response characteristics including selectivity, slope of the response, detection limit, and linear range.

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