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A Selective Optical Sensor Based on [9]Mercuracarborand-3, a New Type of Ionophore with a Chloride Complexing Cavity

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A highly selective optical sensor for chloride, based on the multidentate Lewis acid ionophore [9]mercuracarborand-3, is described herein. This sensor is constructed by embedding the mercuracarborand ionophore, a suitable pH-sensitive lipophilic dye, and lipophilic cationic sites in a plasticized polymeric membrane. The multiple complementary interactions offered by the preorganized complexing cavity of [9]mercuracarborand-3 is shown to control the anion selectivity pattern of the optical film. The film exhibits a significantly enhanced selectivity for chloride over a variety of lipophilic anions such as perchlorate, nitrate, salicylate, and thiocyanate. Furthermore, the optical selectivity coefficients obtained for chloride over other biologically relevant anions are shown to meet the selectivity requirements for the determination of chloride in physiological fluids, unlike previously reported chloride optical sensors. In addition, the optical film responds to chloride reversibly over a wide dynamic range (16 μM−136 mM) with fast response and recovery times.

Chloride plays a crucial role in a variety of biological/physiological processes. For instance, osmotic regularity and fluid secretion are controlled by the concentration level of serum electrolytes (Cl^-, Na^+, K^+, etc.). Further, abnormal regulation of the chloride ion in blood is thought to be involved in a number of diseases and pathological conditions, such as stroke, edema, cystic fibrosis, and atherosclerosis.[1,2] These facts make chloride an important target analyte. Conventional spectroscopic[3] and electrochemical[4] methods for chloride have been developed, but these methods are cumbersome, laborious, and, most importantly, not amenable for real-time monitoring or on-site applications (e.g., field applications or bedside monitoring). To overcome these limitations, ionophore-based chemical sensors, especially optical and potentiometric, have been proposed for the determination of chloride in clinical samples. The major challenge for all these sensors is their ability to discriminate against more hydrophobic anions, especially thiocyanate, an anion that is elevated in the blood of smokers, and salicylate.

Classical anion exchangers, such as quaternary ammonium salts and metal complexes, have been evaluated as ionophores in optical sensors for chloride, using a variety of polymer matrixes. In all cases, however, the developed sensors demonstrated a Hofmeister selectivity pattern: ClO_4^- > SCN^- > I^- > salicylate > NO_3^- > Br^- > Cl^- > SO_4^{2-}.[5−8] Additionally, these sensors did not meet the whole blood selectivity requirements (as calculated by Simon and co-workers)[8,9] for anions such as thiocyanate,

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(1) Tietz, N. W.; Pruden, E. L.; Siggaard-Anderson, O. In *Tietz Textbook of Clinical Chemistry*; Burtis, C. A., Ashwqood, E. R., Eds.; Saunders: Philadelphia, PA, 1994.
(2) Ross, P. E.; Garber, S. S.; Cahalan, M. D. *Biophys. J.* **1994**, *66*, 169−178.
(3) Marczenko, Z. *Separation and Specrophotometric Determination of Elements*; Wiley: New York, 1986.
(4) Hicks, J. M.; Aldrich, F. T.; Iosephson, M. *Clin. Chem.* **1976**, *22*, 1868−1871.
(5) Mohr, G. J.; Wolfbeis, O. S. *Anal. Chim. Acta* **1995**, *316*, 239−246.
(6) Huber, C.; Werner, T.; Krause, C.; Klimant, I.; Wolfbeis, O. S. *Anal. Chim. Acta* **1998**, *364*, 143−151.
(7) Ambrose, T. M.; Meyerhoff, M. E. *Anal. Chim. Acta* **1999**, *378*, 119−126.
(8) Tan, S. S. S.; Hauser, P. C.; Wang, K.; Fluri, K.; Seiler, K.; Rusterholz, B.; Suter, G.; Krüttli, M.; Spichiger, U. E.; Simon, W. *Anal. Chim. Acta* **1991**, *255*, 35−44.
(9) Oesch, U.; Ammann, D.; Simon, W. *Clin. Chem.* **1986**, *32*, 1448−1459.

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salicylate, nitrate, and, in some cases, sulfate. An indium(III) metalloporphyrin, which had been used in chloride-selective electrodes,[10] was also evaluated as an ionophore in optical sensors.[11−14] These optodes, similar to their electrochemical counterparts, still suffered from either inadequate ionophore stability and lipophilicity,[6] nontheoretical responses,[13] or insufficient selectivity against salicylate and thiocyanate to perform physiological analyses.[14] Optical sensors based on organotin compounds also have not met the selectivity requirements for chloride detection in blood because of potential interference from thiocyanate.[8,15−17] In fact, to date there have been no ionophore-based optical sensors that meet all of the selectivity requirements for chloride detection in human blood.

Organomercury compounds based on either one or two mercury centers have been used as ionophores in ion-selective electrodes and optodes.[18,19] Electrochemical studies with such ionophores, particularly dimercury compounds, have produced good selectivity data for chloride over many biologically relevant ions. These compounds, however, suffer from poor stability due to the trifluoroacetoxy groups bound to the mercury centers.[18] In the presence of strong interfering ions such as iodide, bromide, or thiocyanate, these compounds undergo a replacement of the substituents bound to mercury leading to the creation of a new ionophoric species and yielding a modified selectivity pattern.[18] In addition, recent attempts to incorporate one of these dimercury compounds into a hydrogel-based ion-selective optode yielded a sensor that demonstrated inadequate selectivity over bromide for physiological chloride analysis, possibly owing to the effect described earlier.[19]

The trioctyltin and metalloporphyrin compounds mentioned provide coordination between the chloride and a single metal center, while the organomercury compounds coordinate with ions via a two-metal acyclic system. We hypothesized that macrocyclic ionophores with electron-deficient, metal-containing cavities that can complex chloride might be more effective in the development of optical sensors that have the required chloride selectivity to perform physiological analyses. Herein, we report on the use of a macrocyclic, multidentate Lewis acid ionophore with a preorganized cavity for the development of chloride-sensitive and selective optical sensors. The ionophore, [9]mercuracarborand-3 (see Figure 1), is a charge-reverse analogue of [9]crown-3 ether and incorporates three electron-deficient mercury centers within a macrocyclic cavity. This new family of ionophores (i.e., mercuracarborands) offers many advantages as anion carriers.[20,21] First,

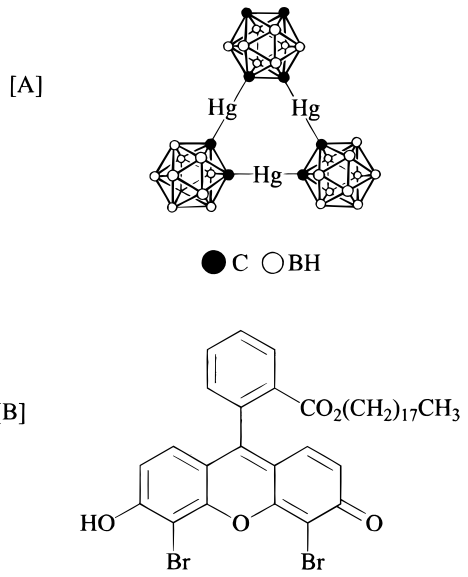

Figure 1. Structure of [9]mercuracarborand-3 ionophore [A] and the proton chromoionophore VI [B] used for the construction of the chloride optode membrane.

mercuracarborand ionophores can be prepared with different cavity sizes that can induce a preference for a given anion or a group of anions. Second, these compounds are chemically stable and lipophilic, which makes them easily soluble in plasticizers thereby enhancing the stability of the corresponding chemical sensors over time. Third, mercuracarborands are amenable to facile functionalization of the carborane ligands. Fourth, the multidentate nature of mercuracarborands and their preorganized cavity provide a means for improved analyte recognition (for a review on preorganization, see ref 22). Last, mercuracarborand ionophores neither fluoresce nor absorb in the visible region of the spectrum, making them well-suited for the development of optical sensors based on fluorescence or absorbance transduction when coupled with a suitable proton chromoionophore. The development of a highly selective [9]mercuracarborand-3-based optical chloride sensor that does not suffer interferences from thiocyanate and salicylate is described.

EXPERIMENTAL SECTION

Reagents. 9-(Diethylamino)-5-(octadecanoylimino)-5*H*-benzo[*a*]phenoxazine (proton chromoionophore I), 9-(dimethylamino)-5-[4-(16-butyl-2,14-dioxo-3,15-dioxaeicosyl)phenylimino]benzo[*a*]phenoxazine (proton chromoionophore II), 9-(diethylamino)-5-[(2-octyldecyl)imino]benzo[*a*]phenoxazine (proton chromoionophore III), 4′,5′-dibromofluorescein octadecyl ester (proton chromoionophore VI), high molecular weight poly(vinyl chloride) (PVC), *o*-nitrophenyloctyl ether (*o*-NPOE), tridodecylmethylammonium chloride (TDMAC), and Selectophore grade tetrahydrofuran (THF) were obtained from Fluka (Ronkonkoma, NY). 2-Morpholinoethanesulfonic acid (MES) was obtained from Research Organics (Cleveland, OH). The sodium salts of thiocyanate and sulfate

(10) Park, S. B.; Matuszewski, W.; Meyerhoff, M. E.; Liu, Y. H.; Kadish, K. M.; *Electroanalysis* **1991**, *3*, 909−916.
(11) Wang, E.; Meyerhoff, M. E. *Anal. Lett.* **1993**, *26*, 1519−1533.
(12) Wang, E.; Meyerhoff, M. E. *Anal. Chim. Acta* **1993**, *283*, 673−682.
(13) Barker, S. L. R.; Shortreed, M. R.; Kopelman, R. *Anal. Chem.* **1997**, *69*, 990−995.
(14) Barker, S. L. R.; Thorsrud, B. A.; Kopelman, R. *Anal. Chem.* **1998**, *70*, 100−104.
(15) Tan, S. S. S.; Hauser, P. C.; Chaniotakis, N. A.; Suter, G.; Simon, W. *Chimia* **1989**, *43*, 257−261.
(16) Spichiger, U. E.; Freiner, D.; Bakker, E.; Rosatzin, T.; Simon, W. *Sens. Actuators B* **1993**, *11*, 263−271.
(17) Hisamoto, H.; Miyashita, N.; Watanbe, K.; Nakagawa, E.; Yamamoto, N.; Suzuki, K. *Sens. Actuators B* **1995**, *29*, 378−385.
(18) Rothmaier, M.; Schaller, U.; Morf, W. E.; Pretsch, E. *Anal. Chim. Acta* **1996**, *327*, 17−28.
(19) Huber, C.; Werner, T.; Krause, C.; Wolfbeis, O. S. *Analyst* **1999**, *124*, 1617−1622.
(20) Badr, I. H. A.; Diaz, M.; Hawthorne, M. F.; Bachas, L. G. *Anal. Chem.* **1999**, *71*, 1371−1377.
(21) Hawthorne, M. F.; Zheng, Z. *Acc. Chem. Res.* **1997**, *30*, 267−276.
(22) Toner, J. L. In *Crown Ethers and Analogues*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1989; pp 77−205.

were obtained from Matheson Coleman & Bell (Cincinnati, OH). The sodium salts of iodide, nitrate, and fluoride were obtained from J. T. Baker (Philipsburg, NJ); those of chloride and formate were obtained from Fisher Scientific (Cincinnati, OH). Sodium salts of perchlorate, salicylate, and monobasic phosphate were purchased from Sigma (St. Louis, MO). The [9]mercuracarborand-3 ionophore was prepared as described previously from *o*-carborane and was characterized by melting point, ^{1}H NMR, ^{199}Hg{H} NMR, and IR.[23] All aqueous solutions were prepared using 14-MΩ deionized distilled water produced by a Milli-Q water purification system (Millipore, Bedford, MA).

Preparation of Sensor Membranes and Measurement Setup. The sensor membranes were prepared using a spin-coating technique. A membrane cocktail was prepared by dissolving 53 mg of PVC, 106 mg of *o*-NPOE, 5.1 mg of [9]mercuracarborand-3, 1.9 mg of chromoionophore VI, and 1.4 mg of TDMAC in 1.5 mL of tetrahydrofuran. This composition corresponds to a 2:1:1 molar ratio of ionophore to chromoionophore to lipophilic additive. A dust-free square-shaped glass plate of ∼35 mm in length (Fisher Scientific; Pittsburgh, PA) was positioned in a SSEC spin coater (model 102; Fort Washington, PA) by applying vacuum. The glass plate was rotated at 600 rpm, and 200 μL of the membrane cocktail was injected onto the rotating glass plate. After spinning for ∼4 s, the glass plate coated with the optical film was removed from the spin coater and dried in air for ∼10 min. When not in use, the membrane was stored in the dark.

Two identical membranes were mounted in a custom-built spectrophotometer flow cell modeled after Seiler et al.[24] The flow cell was mounted into a Hewlett-Packard diode array spectrophotometer (model 8453; San Diego, CA). Using a Gilson Minipuls peristaltic pump (Villiers, France), a buffer was allowed to flow for 20 min with a flow rate of 2.0 mL/min to condition the membranes. The response of the optical sensing membranes toward different anions was evaluated by adding known aliquots of the test solution to a stirred reservoir containing 50 mL of buffer. The resulting buffered salt solution was pumped through the cell and recirculated into the buffer reservoir. After reaching equilibrium, the spectrum of the optical films was recorded in the range between 400 and 600 nm. To correct for the background absorbance, the spectrum of a flow cell assembled by mounting two glass plates coated with chromoionophore-free membrane cocktail was recorded. For calculation of α values, the absorbance values at 534 nm corresponding to the fully deprotonated and protonated forms of the chromoionophore were measured by conditioning the sensing membrane in 10 mM Na_2HPO_4 and 10 mM H_2SO_4, respectively. Theoretical response functions were fitted to experimental data for each anion by performing a nonlinear least-squares regression with the Microsoft Excel SOLVER function.

RESULTS AND DISCUSSION

The sensing mechanism of the [9]mercuracarborand-3-based chloride-sensitive optical film is based on a coextraction process into the membrane film, upon reaching equilibrium with an aqueous sample solution.[25−27] In this sensing mechanism, the selective binding of the [9]mercuracarborand-3 ionophore (L) with chloride is accompanied by protonation of the chromoionophore (Ind^-) to maintain the electroneutrality of the membrane, which is controlled by the lipophilic cationic sites (R^+) in the membrane phase. This coextraction process is summarized in eq 1. The

$$L + Ind^- + R^+ + H^+_{(a)} + Cl^-_{(a)} \rightleftarrows IndH + LCl^- + R^+ \qquad (1)$$

subscripts denote ions in the aqueous sample solution, while all other chemical entities are in the membrane phase.

The coextraction constant (K) corresponding to this equilibrium is expressed as

$$K = \frac{[IndH][LCl^-]}{[Ind^-][L]} \cdot \frac{1}{a_{H^+}a_{Cl^-}} \qquad (2)$$

To describe the response characteristics of this sensor system, it is quite useful to use the relative absorbance, α,[27] which is the fraction of the total indicator (Ind_T) that is present in the deprotonated form ($[Ind^-]$). The parameter α can be expressed in terms of measurable quantities as follows:

$$\alpha = (A - A_0)/(A_1 - A_0) = [Ind^-]/Ind_T \qquad (3)$$

where A is the measured absorbance at a given concentration and a particular wavelength and A_0 and A_1 are the absorbance values of the completely protonated and deprotonated forms of the proton chromoionophore at the same wavelength, respectively.

The response function of the [9]mercuracarborand-3-based optode can be obtained from eqs 2 and 3, the mass balance equations ($L_T = [L] + [LCl^-]$ and $Ind_T = [Ind^-] + [IndH]$), and the electroneutrality equation ($R_T^+ = [Ind^-] + [LCl^-]$) as follows,

$$K = \frac{1}{a_{H^+}a_{Cl^-}} \frac{(1-\alpha)}{\alpha} \frac{(R_T^+/Ind_T) - \alpha}{[(L_T/Ind_T) - (R_T^+/Ind_T - \alpha)]} \qquad (4)$$

This equation can be simplified for certain membrane compositions. In the case of equimolar concentrations of chromoionophore and cationic sites (i.e., when $Ind_T = R_T^+$), eq 4 becomes

$$Ka_{H^+}a_{Cl^-} = \frac{1}{\alpha} \frac{(1-\alpha)^2}{(L_T/Ind_T) - (1-\alpha)} \qquad (5)$$

All theoretical curves herein have been obtained using eq 5. In a manner analogous to that for ion-selective electrodes,[27] the relationship between the parameter α, the coextraction constant, the activity of protons, the total amount of the ionophore, and the

(23) Yang, X.; Zheng, Z.; Knobler, C. B.; Hawthorne, M. F. *J. Am. Chem. Soc.* **1993**, *115*, 193−195.

(24) Seiler, K.; Morf, W. E.; Rusterholz, B.; Simon, W. *Anal. Sci.* **1989**, *5*, 557−561.

(25) Morf, W. E.; Seiler, K.; Lehmann, B.; Behringer, C.; Hartman, K.; Simon, W. *Pure Appl. Chem.* **1989**, *61*, 1613−1618.

(26) Morf, W. E.; Seiler, K.; Lehmann, B.; Behringer, C.; Tan, S.; Hartman, K.; Sörensen, P.; Simon, W. In *Ion-Selective Electrodes*; Pungor, E., Ed.; Akadémiai Kiadó: Budapest, 1989; Vol. 5, pp 115−131.

(27) Morf, W. E.; Seiler, K.; Sörensen, P.; Simon, W. In *Ion-Selective Electrodes*; Pungor, E., Ed.; Akadémiai Kiadó: Budapest, 1989; Vol. 5, pp 141−152.

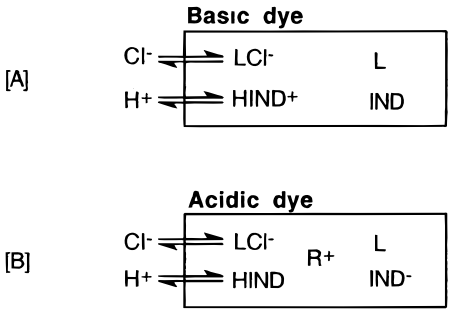

Figure 2. Sensing schemes for chloride using a neutral carrier (L) coupled with basic chromoionophore [A] and acidic chromoionophore [B].

optical selectivity coefficient, $K^{opt}_{Cl^-,Y^-}$ in the presence of interfering anion (Y^-), is given by eq 6:

$$Ka_{H^+}(a_{Cl^-} + \sum_{Y^-} K^{opt}_{Cl^-,Y^-} a_{Y^-}) = \frac{1}{\alpha} \frac{(1-\alpha)^2}{(L_T/Ind_T) - (1-\alpha)} \quad (6)$$

There are two possible schemes for the development of bulk optode membranes utilizing the recognition chemistry of the [9]-mercuracarborand-3 neutral carrier. As can be seen in Figure 2A, the first scheme is based on coextraction of protons and chloride by a basic dye (Ind) and anion carrier (L), respectively. Protonation of a basic dye upon anion coextraction is the key for the absorbance change of the optical film. Initial studies using several basic dyes with different basicities (e.g., chromoionophores I−III) were performed to evaluate this sensing scheme for the development of anion optodes based on the [9]mercuracarborand-3 ionophore. However, due to the protonation of the basic dye upon conditioning of the optical films in 10 mM buffer solution (formate buffer pH 3.5, phosphate buffer pH 4.2, or MES buffer pH 5.5), no or very little absorbance change was observed upon addition of chloride to the buffer. Increasing the pH of the measuring buffer to higher values (10 mM phosphate pH 7.5 or 9) almost led to full protonation of the chromoionophore, possibly due to coextraction of hydroxide ion by [9]mercuracarborand-3 and protons by the proton chromoionophore. Thus, this sensing scheme was found to be inappropriate for the development of a chloride optode.

Therefore, we utilized the second scheme for the development of a chloride optode membrane based on the mercuracarborand ionophore. In this scheme (see Figure 2B), anion binding by [9]-mercuracarborand-3 is followed by a concomitant protonation of the acid dye (chromoionophore VI) to ensure electroneutrality balance (as governed by a fixed concentration of cationic sites, R^+). Such protonation of the proton chromoionophore leads to a large change in the absorbance of the optical film. Indeed, as shown in Figure 3, the absorbance spectrum of a plasticized PVC optode membrane (impregnated with [9]mercuracarborand-3, chromoionophore VI, and TDMAC), measured in 10 mM MES, pH 5.5, is highly dependent on the chloride ion concentration. With increasing chloride concentrations, the extent of protonation of the acid dye by proton coextraction becomes greater, yielding a lower absorbance of the deprotonated form at 534 nm.

Plotting the absorbance values at 534 nm versus the concentration of chloride (Figure 4) demonstrates that the optode membrane responds with high sensitivity to chloride, and that it has a wide dynamic range of 16 μM to 136 mM. Changing the pH of the measuring solution to pH 3.5 (10 mM formate buffer) results in a loss of chloride sensitivity (i.e., decrease of the response slope) and a shift of the dynamic range to lower chloride concentrations. The loss of sensitivity is likely due to a partial protonation of the acid chromoionophore in the membrane phase at lower pH values. This notion was supported by the decrease of absorbance values at 534 nm (i.e., absorbance of the deprotonated form of the chromoionophore) measured at pH 3.5, as compared

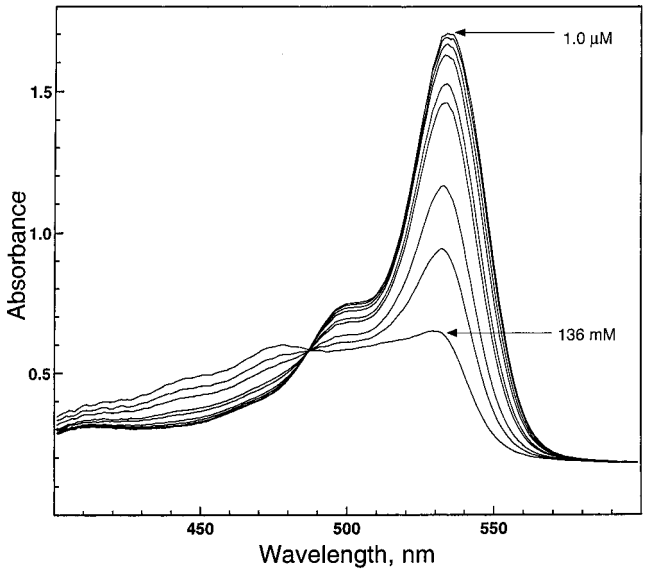

Figure 3. Absorption spectra of a plasticized PVC chloride optode membrane doped with [9]mercuracarborand-3 and chromoionophore VI measured in 10 mM MES buffer, pH 5.5, and at varying chloride concentrations (1, 16, and 66 μM and 0.16, 0.65, 1.2, 6.0, 11, 57, and 136 mM). The peak absorbance at 534 nm decreased monotonically with increasing chloride concentration.

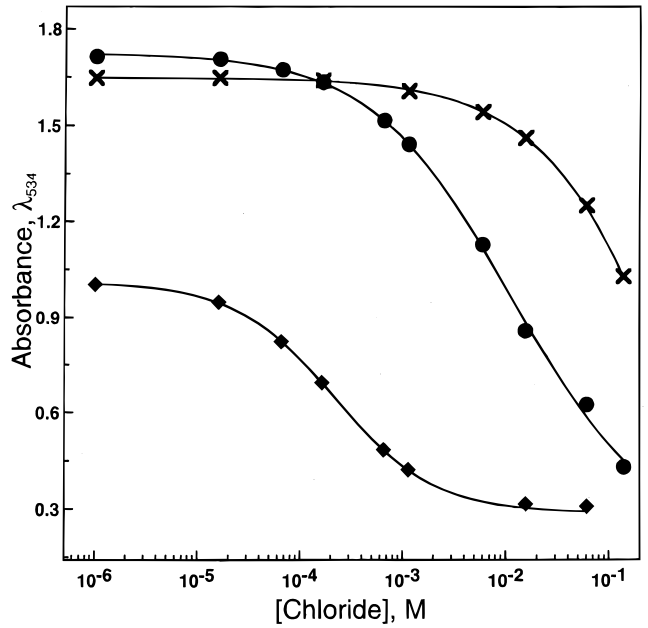

Figure 4. Absorbance of [9]mercuracarborand-3-based chloride-sensitive optode at 534 nm as a function of chloride concentration at different pH values: (●) 10 mM MES, pH 5.5, (◆) 10 mM formate, pH 3.5, and (×) 10 mM phosphate, pH 7.4.

Table 1. Selectivity Coefficients of MC-3 Based Optical Film, Measured in MES Buffer, pH 5.5, in Comparison with Ion-Exchanger-Based Optical Membrane

anion	95% normal concn range (mM)	log $K^{opt}_{Cl,anion}$ required[9]	anion-exchanger optode[8]	MC3-based optode
Cl^-	95−110	0.0	0	0
Br^-	0.041−0.11[a]	≤0.94[a]	0.4	0.85
SCN^-	0.007−0.017 (0.15)[b]	≤1.7 (0.8)[b]	2.8	−1.41
HPO_4^{2-}	0.26−0.89	≤−1.2		−5.21
I^-	0.0003−0.0005	3.3	1.8	2.10
SO_4^{2-}	0.3−1.0	≤−1.3	−1.0	−5.34
NO_3^-	0−0.1	1.0	1.1	−4.16
salicylate	0.5−1.5 [1]	≤−0.2		−1.85
ClO_4^-			3.2	−3.90

[a] As calculated from range given in ref 29. [b] For smokers.

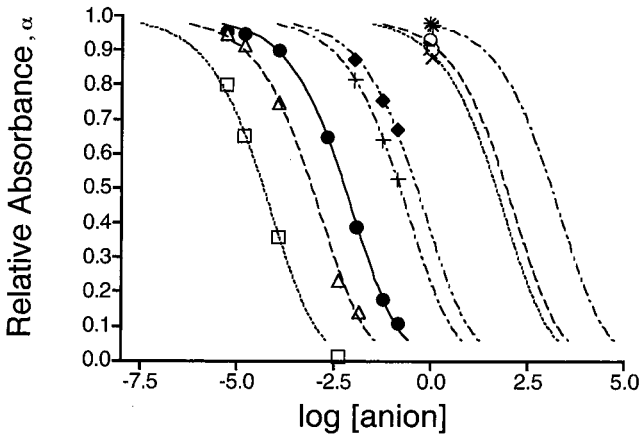

Figure 5. Optical response of the [9]mercuracarborand-3-based sensing film toward various anions as measured in 10 mM MES, pH 5.5: (●) chloride, (×) perchlorate, (+) thiocyanate, (□) iodide, (◆) salicylate, (○) nitrate, (△) bromide, (*) sulfate. Lines are the theoretical response functions of the optode membrane toward various anions calculated using eq 5.

to those measured at higher pH values (see data in Figure 4). On the other hand, increasing the pH of the measuring buffer to 7.4 (10 mM phosphate buffer) led to a shift in the dynamic range to much higher concentrations. The ability to tune the dynamic range of the sensor by changing the pH of the measuring buffer is one of the useful features of membrane-based optodes.[25−27] The dynamic range of the sensor at pH values of 5.5 and 7.4 is suitable for many applications, including chloride assay in physiological fluids where the 95% normal concentration range of chloride is 95−110 mM. The remainder of the experiments was performed in 10 mM MES buffer, pH 5.5.

The optical anion selectivity of the mercuracarborand-based chloride optodes is demonstrated in Figure 5. The anion optical selectivity coefficients summarized in Table 1 were determined at $\alpha = 0.5$, as previously reported in the literature.[28] As shown in Figure 5 and Table 1, the [9]mercuracarborand-3-based chloride-sensitive optode showed excellent selectivity over the common anions and lipophilic anions, such as thiocyanate, salicylate, perchlorate, and nitrate. The anion optical selectivity sequence obtained with the optode membrane is as follows: iodide > bromide > chloride > thiocyanate > salicylate > perchlorate ∼ nitrate > sulfate (see data in Table 1). This selectivity pattern deviates from the Hofmeister selectivity pattern that is based solely on lipophilicity. Furthermore, this pattern indicates the existence of a selective interaction between [9]mercuracarborand-3 and the chloride ion, which is consistent with the data reported previously using ^{199}Hg NMR titrations and the results obtained using [9]mercuracarborand-3 based membrane electrodes.[20]

Table 1 also gives the physiological normal concentration range of anions and the required selectivity coefficients calculated via the worst case scenario (i.e., by considering the lowest value of the normal physiological activity range for chloride and the highest value for interfering anions) for whole blood, plasma, and serum applications. All values listed were calculated and reported previously by Simon and co-workers, except for bromide.[8,9] Higher concentrations of bromide in physiological fluids arise from its use in sedation therapy. This therapy, however, is outdated and is being replaced with more targeted therapies.[1,29] Thus, the presence of high concentrations of bromide in physiological samples is very infrequent and is becoming less of a concern in chloride analysis as opposed to potential interferences from thiocyanate, iodide, and salicylate. Also, recent reports on the concentration of bromide in physiological fluids have prompted studies to establish a true reference level for the ion in human blood.[30] Consequently, the required selectivity coefficient has been recalculated based on the level of the ion in human blood reported by Olzowy and co-workers.[30] The recalculation of the required optical selectivity coefficient for chloride over bromide is necessary due to discrepancies between the values of the old reference interval and newer available data. Calculations are based on a 95% normal bromide concentration range of 0.041−0.11 mM determined from the logarithmic transformation (to achieve normality) of physiological bromide concentration data yielded by wavelength-dispersive X-ray fluorescence spectrometry.[30] As can be seen, the selectivity coefficients obtained with the [9]mercuracarborand-3 based-chloride optode system fulfill the selectivity requirements for chloride assay in physiological fluids. In comparison with the ion-exchanger-based optode, the [9]mercuracarborand-3-based

(28) Morf, W. E.; Seiler, K.; Rusterholz, B.; Simon, W. *Anal. Chem.* **1990**, *62*, 738−742.

(29) Panteghini, M.; Bonmora, R.; Malchiodi, A.; Calarco, M. *Clin. Biochem.* **1986**, *19*, 20−25.

(30) Olzowy, H. A.; Rossiter, J.; Hegarty, J.; Geoghegan, P.; Haswell-Elkins, M. *J. Anal. Toxicol.* **1998**, *22*, 225−230.

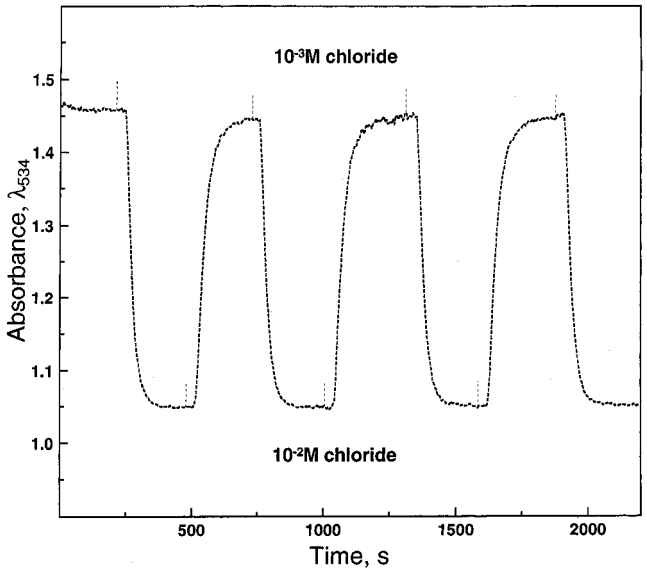

Figure 6. Response time and reproducibility of [9]mercuracarborand-3-based chloride optode membrane measured by alternating 1 and 10 mM chloride solutions prepared in 10 mM MES, pH 5.5.

optode membrane demonstrated an improved selectivity over more lipophilic anions, except in the case of bromide. The presently discussed optode, however, still demonstrates sufficient selectivity to analyze for chloride in the presence of bromide. In addition, the [9]mercuracarborand-3-based optode outperforms other previously reported chloride-selective optodes with respect to selectivity over ions such as bromide, thiocyanate, and salicylate.

It should also be noted that the analysis of chloride in urine samples is more challenging than in blood since urinary chloride is at levels that approach the limit of commercial analyzer, which use classical ion-exchanger-based electrodes.[31] At such levels of chloride, commercially available analyzers yield measurements with significant positive biases that have been attributed to uncontrollable interferences.[31] For example, selectivity coefficients for urine chloride analysis are more stringent than for measurements in blood for some analytes; typical urine chloride levels are 11−25 mM, while thiocyanate is present at 0.002−0.007 (nonsmokers) and 0.012−0.029 (smokers) mM levels,[1] yielding required selectivity coefficients of 1.2 and 0.58, respectively. The mercuracarborand-based optode described herein has sufficient detection limits for urinary chloride determination.

To evaluate the response time and the signal reproducibility of the chloride optode membrane, two concentration levels of chloride (1 and 10 mM) prepared in 10 mM MES, pH 5.5, were pumped alternately through the optode cell. The signal reproducibility is high as can be seen in Figure 6, with an average absorbance of 1.451 ± 0.003 and 1.050 ± 0.001 for 1 and 10 mM chloride concentrations ($n = 4$), respectively. The response time of the optode membrane, calculated as the t_{90} value, was found to be 58.2 ± 1.5 s ($n = 4$), and the recovery time at t_{90} was found to be 84.7 ± 2.0 s ($n = 3$).

CONCLUSIONS

In summary, we have utilized a novel host−guest recognition system based on a mercuracarborand macrocyclic ionophore for the development of a chloride-sensitive optical membrane. The multiple complementary interactions provided by the ionophore led to a selectivity pattern that was completely different from that of the classical anion-exchanger-based optical sensors. In fact, all previously reported optodes for chloride, including those based on anion exchangers, metalloporphyrins, organotin, and organomercury compounds, although demonstrating good selectivity toward chloride, do not meet full selectivity requirements for chloride analysis in physiological fluids. While, in most cases, the selectivity coefficients for these optodes toward all biologically relevant ions is not reported, each report describes insufficient selectivity toward at least one important ion. The optode described herein, however, meets all required selectivity coefficients for physiological analyses. This mercuracarborand ionophore effectively discriminates for chloride over ions such as salicylate, thiocyanate, bromide, and nitrate. In addition, the [9]mercuracarborand-3-based chloride optical sensor showed a chloride response over a wide dynamic range, and this response was fast and reversible.

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(31) Komaromy-Hiller, G.; Swallow, K. L.; Moulton, L. *Ann. Clin. Lab. Med.* **1998**, *28*, 347−353.