Technical Notes

Characterization of Epoxy Resin-Based Anion-Responsive Polymers: Applicability to Chloride Sensing in Physiological Samples

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The potentiometric properties of an epoxy resin-based anion-responsive polymer and its feasibility as a chloride sensing membrane are described. The response mechanism of the epoxy resin-based membrane is studied by varying the types and contents of epoxy components (e.g., resin, hardener, diluent, and plasticizer). It is found that epoxy resins cured with polyamine-type hardeners have excellent characteristics as chloride-sensing membranes, in terms of their selectivity, stability, sensor lifetime, and adhesion for constructing an all-solid-state sensing device. In addition, effects of the diluent or plasticizer added in an epoxy resin/hardener system on the potentiometric behaviors are investigated. To demonstrate the practical analytical utility of the epoxy resin-based electrodes, chloride levels in various controls and biological specimens (e.g., human sera and whole blood) are determined with a flow-cell system.

The accurate and rapid determination of chloride in biological fluids is of utmost relevance in the diagnosis and treatment of various health disorders related to hyper- or hypochloremia.[1] Direct potentiometric measurements of chloride using ion-selective electrodes (ISEs) have attracted considerable interest over the conventional coulometric and colorimetric titrimetry.[2] Such an ISE technique not only provides high throughput in measurements but also is inexpensive and simple to microfabricate and integrate in sensing cartridges. These intrinsic properties of ISEs have expedited the development of point-of-care testing devices capable of rapidly monitoring patient conditions at the bedside.[3,4]

Two types of ISEs have been used for the potentiometric determination of chloride in physiological samples: Ag/AgCl-based solid-state electrodes and liquid/polymeric membrane-based electrodes. The former suffers from a poor discrimination of bromide and uric acid, and also from protein adsorption to the

surface of the Ag/AgCl electrode. The latter, which is mostly based on classical anion exchangers (e.g., quaternary ammonium chloride), is used in several commercial clinical analyzers.[5,6] However, the applicability of those anion-exchanger-based electrodes has often been limited due to their higher selectivities toward lipophilic anions (e.g., salicylate, thiocyanate, etc.), which are known to be dictated by the Hofmeister sequence.[7,8]

Several approaches of reducing interference from lipophilic anions have been reported previously. For instance, the development of chloride-selective electrodes based on neutral carrier-type metal complexes such as metalloporphyrins,[9−12] mercury organic derivatives,[13−15] and organotin compounds[16−18] has been of prime interest to modify the selectivity pattern. However, the practical use of these electrodes for clinical analysis is still limited owing to severe salicylate interference or instability in aqueous solutions. Umezawa and co-workers reported that chloride ISEs based on neutral, hydrogen-bond-forming ionophores (e.g., bis-thiourea compounds) are highly selective for chloride over other anions including salicylate and thiocyanate.[19,20] In another approach, the

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(1) Burtis, C. A.; Ashwood, E. R. *Tietz Textbook of Clinical Chemistry,* 3rd ed.; W. B. Saunders Co.: Philadelphia, PA, 1999; Chapter 32.
(2) Oka, S.; Sibazaki, Y.; Tahara, S. *Anal. Chem.* **1981,** *53,* 588−593.
(3) Meyerhoff, M. E. *Clin. Chem.* **1990,** *36,* 1567−1572.
(4) Mock, T.; Morrison, D.; Yatscoff, R. *Clin. Biochem.* **1995,** *28,* 187−192.

(5) Oesch, U.; Ammann, D.; Pham, H. V.; Wuthier, U.; Zund, R.; Simon, W. *J. Chem. Soc., Faraday Trans. 1* **1986,** *82,* 1179−1186.
(6) Lee, J. S.; Lee, S. D.; Cui, G.; Lee, H. J.; Shin, J. H.; Cha, G. S.; Nam, H. *Electroanalysis* **1999,** *11,* 260−267.
(7) Wang, T.; Diamandis, E. P.; Lane, A.; Baines, A. D. *Clin. Biochem.* **1994,** *27,* 37−41.
(8) Ozawa, S.; Miyagi, H.; Shibata, Y.; Oki, N.; Kunitake, T.; Keller, W. E. *Anal. Chem.* **1996,** *68,* 4149−4152.
(9) Chaniotakis, N. A.; Chasser, A. M.; Meyerhoff, M. E. *Anal. Chem.* **1988,** *60,* 185−188.
(10) Bakker, E.; Malinowska, E.; Schiller, R. D.; Meyerhoff, M. E. *Talanta* **1994,** *41,* 881−890.
(11) Yoon, I. J.; Shin, J. H.; Paeng I. R.; Nam, H.; Cha, G. S.; Paeng, K.-J. *Anal. Chim. Acta* **1998,** *367,* 175−181.
(12) Buhlmann, P.; Pretsch, E.; Bakker, E. *Chem. Rev.* **1998,** *98,* 1598−1687.
(13) Rothmaier, M.; Simon, W. *Anal. Chim. Acta* **1993,** *271,* 135−141.
(14) Rothmaier, M.; Schaller, U.; Morf, W. E.; Pretsch, E. *Anal. Chim. Acta* **1996,** *327,* 17−28.
(15) Badr, I. H. A.; Diaz, M.; Hawthorne, M. F.; Bachas, L. G. *Anal. Chem.* **1999,** *71,* 1371−1377.
(16) Wuthier, U.; Pham, H. V.; Zund, R.; Welti, D.; Funck, R. J. J.; Bezegh, A.; Ammann, D.; Pretsch, E.; Simon, W. *Anal. Chem.* **1984,** *56,* 535−538.
(17) Fluri, K.; Koudelka, J.; Simon, W. *Helv. Chim. Acta* **1992,** *75,* 1012−1022.
(18) Hauser, P. C. *Anal. Chim. Acta* **1993,** *278,* 227−232.
(19) Nishizawa, S.; Buhlmann, P.; Iwao, M.; Umezawa, Y. *Tetrahedron Lett.* **1995,** *36,* 6483−6486.
(20) Xiao, K. P.; Buhlmann, P.; Nishizawa, S.; Amemiya, S.; Umezawa, Y. *Anal. Chem.* **1997,** *69,* 1038−1044.

use of asymmetric membrane electrodes with cellulose triacetate or hydrophilic polyurethane overcoat at the membrane/sample interface was proposed to minimize salicylate interference in serum chloride measurements.[21,22]

We[23] and other groups[24] recently reported that epoxy resin membranes exhibit unsuspected, challenging potentiometric response to various anions, particularly to chloride. Epoxy resin, a material that contains two or more glycidyl groups per molecule, is widely used as adhesives and structural polymers, especially in composites.[25,26] Most commercial epoxy resins are low molecular weight oligomers and form relatively tough infusible thermoset products when cross-linked with an appropriate hardener (curing agent). The hardeners may be classified as primary or secondary polyamines, acid anhydrides, polyamides, and other catalysts. The epoxy compositions employ other constituents such as diluents, plasticizers, fillers, and accelerators to facilitate processing and to modify properties of the cured resin. In previous studies,[23,24] preliminary results showing the potential utility of epoxy resin in preparing chloride-responsive membranes were presented. However, studies on the response mechanism and the practical application of the epoxy resin membrane have rarely been found in the literature.

The primary aim of this work is to investigate the response mechanism of such an epoxy resin-based membrane system. In this regard, effects of varying the epoxy membrane constituents, particularly the type of hardener, on the response behaviors of the resulting membranes are examined. The formulation of the membrane as a chloride sensor is optimized by varying the types and contents of epoxy components (i.e., resin, hardener, plasticizer, and diluent) in both conventional and planar all-solid-state electrode formats. The practical analytical utility of the epoxy resin-based electrode is demonstrated by determining chloride levels in various controls and biological specimens (e.g., human sera and whole blood).

EXPERIMENTAL SECTION

Reagents. The sources of reagents used were as follows: Alteco F-05 5 Minutes epoxy adhesive based on *N*-(3-aminopropyl)-*N*,*N*-dimethylpropane-1,3-diamine as a hardener from Alteco (Osaka, Japan); Devcon 14250 5 Minutes epoxy adhesive based on polyamine/polymercaptan mixture as a hardener from Devcon (Danvers, MA); bis(2-ethylhexyl) adipate (DOA), 2-nitrophenyl octyl ether (NPOE), tetrahydrofuran (THF), poly(vinyl chloride) (PVC), tridodecylmethylammonium chloride (TDMACl), and [4,5-dimethyl-3,6-dioctyl-1,2-phenylene]-bis(mercurytrifluoroacetate) (ETH 9009) from Fluka (Buchs, Switzerland); *N*-(2-hydroxyethyl)-piperazine-*N'*-(3-propanesulfonic acid) (HEPPS) from Sigma (St.

Louis, MO); bisphenol A proxylate diglycidyl ether (BP-A-PGE) and bis(4-glycidyloxyphenyl)methane (BGPM) from Aldrich (Milwaukee, WI); *m*-phenylene diamine (MPDA), benzyldimethylamine (BDMA), tetraethylenepentamine (TEPA), methyl nadic anhydride (MNA), and 1,2,4-benzene-tricarboxylic anhydride (BTCA) from Acros Organics (Morris Plains, NJ); two types of calibration solutions (S5185 and S5195) for ABL clinical analyzer systems from Radiometer (Copenhagen, Denmark); human sera (Model Suitrol N and A) from Nissui Pharmaceutical (Tokyo, Japan); and blood gas/electrolyte control samples (Model A700-001) from Alko Diagnostic (Holliston, MA). Whole blood samples were obtained from a local blood bank. All other chemicals used were of analytical-reagent grade. Standard solutions and buffers were prepared with deionized water.

Preparation and Evaluation of ISE Membranes. The planar type all-solid-state microelectrodes with multiple sensing sites were fabricated by screen-printing silver and dielectric pastes on an alumina plate.[6] The exposed silver electrodes were then immersed in 0.1 M $FeCl_3$ for 30 min to form a AgCl layer. Chloride sensors were prepared by covering the Ag/AgCl electrode with an epoxy or PVC film (ca. 20 μm in thickness; 1.0 mm in diameter). The polymeric epoxy membrane was deposited by dispensing 5 μL of a casting mixture consisting of 150 mg of a resin (i.e., the resin part of Alteco and Devcon adhesives, BP-A-PGE, and BGPM) and 150 mg of a hardener (i.e., the hardener part of Alteco and Devcon adhesives, BDMA, TEPA, MNA, and BTCA). A diluent (300 μL; e.g., THF) or a plasticizer (75 μL; e.g., DOA and NPOE), when used, was mixed with the casting paste. The epoxy membranes were then cured overnight at room temperature (for resin/polyamine composites) or 150 °C (for resin/anhydride composites). The PVC-based layer was formed by applying 3 μL of a stock casting solution containing 66 mg of PVC, 2 mg of ETH 9009, 1 mg of TDMACl, and 143 mg of DOA dissolved in 450 μL of THF.

Response and calibration curves for chloride and other anions were obtained through the addition of standard solutions (e.g., NaCl solution) to 200 mL of a background electrolyte (0.05 M HEPPS–NaOH, pH 7.4) with stirring. The potential differences between the working and the reference electrodes were measured using an IBM-compatible computer equipped with a custom-built high-impedance input 16-channel analog-to-digital converter. The response properties of the electrodes toward pH were examined by adding aliquots of NaOH to a solution of 11.4 mM boric acid/6.7 mM citric acid/10.0 mM NaH_2PO_4 at room temperature. Selectivity coefficients were estimated by using the matched potential method at an interfering ion concentration of 0.1 M.[27,28]

Evaluation of Membrane Resistance. The relative bulk membrane electrical resistance was evaluated according to a method described in detail elsewhere.[29,30] Briefly, impedance plots for ISE membranes were obtained using an EG&G potentiostat/galvanostat (Model 273A) equipped with a lock-in amplifier (Model 5210). Small disks of membranes were punched from epoxy films cast into a glass ring (i.d. 22 mm) placed on a Teflon plate, and they were tested in Philips electrode bodies (IS-561; Glasblaserei

(21) Cha, M. J.; Shin, J. H.; Oh, B. K.; Kim, C. Y.; Cha, G. S.; Shin, D. S.; Kim, B. *Anal. Chim. Acta* **1995**, *315*, 311−319.

(22) Sakong, D. S.; Cha, M. J.; Shin, J. H.; Cha, G. S.; Ryu, M. S.; Hower, R. W.; Brown, R. B. *Sens. Actuators, B* **1996**, *32*, 161−166.

(23) Lee, H. L.; Shin, J. H.; Lee, M. H.; Oh, S. H.; Nam, H.; Cha, G. S. 87th Annual Meeting of the Korean Chemical Society, Seoul, Korea, Apr. 20−21, 2001; p 306.

(24) Trojanowicz, M.; Opara, W. *Talanta* **2002**, *56*, 213−217.

(25) Lee, H.; Neville, K. *Handbook of Epoxy Resins*; McGraw-Hill: New York, 1967.

(26) McAdams. L. V.; Gannon, J. A. Emulsion Polymerization to Fibers, Manufacture. In *Encyclopedia of Polymer Science and Engineering*; Mark, H. F., Bikales, N. M., Overberger, C. G., Menges, G., Kroschwitz, J. I., Eds.; John Wiley & Sons: New York, 1986; Vol. 6, pp 322−382.

(27) IUPAC Recommendations. *Pure Appl. Chem.* **1994**, *66*, 2527−2536.

(28) IUPAC Recommendations. *Pure Appl. Chem.* **1995**, *67*, 507−518.

(29) Armstrong, R. D.; Proud, W. G. *J. Electroanal. Chem.* **1990**, *295*, 163−168.

(30) Tsujimura, Y.; Yokoyama, M.; Kimura, K. *Anal. Chem.* **1995**, *67*, 2401−2404.
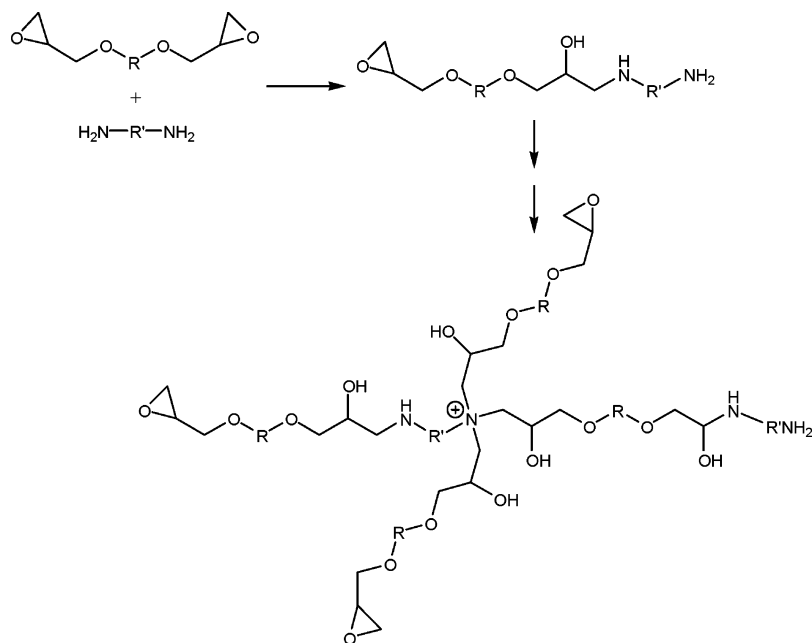
Figure 1. Reaction mechanism of glycidyl epoxy resin and polyamine curing agent.

Moller, Zurich, Switzerland) with an Ag/AgCl double junction electrode as an external reference (Model 90-02, Orion; Cambridge, MA). For all epoxy resin-based electrodes, 0.1 M NaCl was used as the internal filling solution, and measurements were made after soaking for 1 h in 0.1 M NaCl. The amplitude of the applied sinusoidal voltage and the frequency range were 10 mV and 100000−0.01 Hz, respectively. The bulk membrane resistance was calculated by Nyquist plots.

Determination of Chloride Levels in Samples. To evaluate the analytical usefulness of the epoxy resin-based chloride sensor for the determination of chloride levels in various control samples and biological specimens (e.g., human sera and whole blood), its potentiometric response was measured with the flow cell described in the previous work.[6] Blood chloride concentrations were determined with two commercial standard solutions (S5185 and S5195 for the ABL clinical analyzer systems). The performance of the microchip-based chloride sensor was compared with that of a commercial blood gas/electrolyte analyzer (Stat Profile Ultra M, Nova Biomedical; Waltham, MA) as the laboratory reference.

RESULTS AND DISCUSSION

Characterization of Epoxy Resin-Based Anion-Responsive Polymers. To investigate the response mechanism of the epoxy resin-based membrane system, two types of hardeners (i.e., polyamines and acid anhydrides) were employed in this study. For the purpose of excluding effects of other additives (e.g., accelerators and fillers), which are typically included in commercial epoxy adhesive products, on the potentiometric behaviors, epoxy films were formulated with pure resins (e.g., BP-A-PGE and BGPM) and hardeners (e.g., MPDA, BDMA, TEPA, MNA, and BTCA).

All membrane combinations of resins with anhydride-type hardeners (i.e., BP-A-PGE/MNA, BP-A-PGE/BTCA, BGPM/MNA, and BGPM/BTCA) exhibited negligible potentiometric response to both anions and cations. On the other hand, epoxy resin-based electrodes employing polyamine-type hardeners (i.e.,

MPDA, BDMA, and TEPA) showed a highly responsive nature toward anions. Furthermore, their potentiometric properties (e.g., slopes for chloride in the range of $10^{-3}-10^{-1}$ M and selectivities over salicylate, log K_{Cl^-,Sal^-}^{pot}) were largely governed by the epoxy compositions used: −52.5 mV/dec and −0.8 for BP-A-PGE/TEPA; −47.7 mV/dec and 2.4 for BGPM/MPDA; and −47.4 mV/dec and 1.2 for BGPM/BDMA, respectively.

To further consider the functions of polyamines in anion-responsive epoxy resins, only polyamine was incorporated into a PVC matrix with different contents: 10, 20, and 40 mg of a polyamine in 33 mg of PVC dissolved in 450 μL of THF. These polyamine-doped PVC membranes did not yield any significant anionic response. Through a series of experiments, it was confirmed that amino moieties of the hardener have a critical role in the potentiometric response properties of epoxy resin-based electrodes. It was also revealed that free polyamines without participating in the curing process of resins hardly contribute to the response mechanism. On the basis of these observations, it is proposed here that quaternary ammonium sites are eventually generated on the "cured" epoxy resins via consecutive transformations of amines (i.e., primary → secondary → tertiary amine → quaternary ammonium) and that these sites act as an anion exchanger in the membrane phase (see Figure 1).[31−33]

Two commercial epoxy adhesives (i.e., Alteco and Devcon products) were investigated for use as a chloride-sensing material (see Table 1). Dramatically improved selectivities over lipophilic anions (e.g., bromide, thiocyanate, and salicylate) of epoxy-based chloride sensors, when compared to the conventional TDMACl-based solvent/polymeric membrane, were observed. It may be attributed to the following several reasons. A reduced flexibility of ionophoric moieties covalently bound to epoxy networks and

(31) Yang, C.-P.; Ting, C.-Y. *J. Appl. Polym. Sci.* **1994**, *51*, 1597−1605.
(32) Li, M.-S.; Su, Y.-F.; Ma, C.-C. M.; Chen, J.-L.; Lu, M.-S.; Chang, F.-C. *Polymer* **1996**, *37*, 3899−3905.
(33) Ochi, M.; Takahashi, R. *J. Polym. Sci., Part B: Polym. Phys.* **2001**, *39*, 1071−1084.

Table 1. Potentiometric Response Characteristics of Epoxy Resin-Based Chloride-Selective Electrodes[a]

electrode type[b]	slope[c] mV/dec	detection limit log[Cl⁻]	selectivity[d] log $K^{pot}_{Cl^-,j}$					preconditioning time[e] t_{pre}, min	drift mV/h	impedance[f] MΩ
			Br⁻	Sal⁻ [g]	SCN⁻	ClO₄⁻	HCO₃⁻			
Alteco	−45.5 ± 0.3	−4.1 ± 0.3	0.45	0.49	1.44	1.08	−0.98			
Devcon	−57.1 ± 0.3	−4.5 ± 0.2	0.45	0.64	1.12	1.05	−0.88	>3 h	1.5 ± 0.32	1.17 ± 0.5
Devcon/THF	−56.5 ± 0.3	−4.4 ± 0.2	0.43	0.69	1.09	1.01	−0.84	4.8 ± 0.5	0.3 ± 0.07	0.08 ± 0.01
Devcon/DOA	−56.2 ± 0.3	−4.4 ± 0.2	0.41	0.59	1.11	1.01	−0.96	6.8 ± 0.5	0.3 ± 0.09	0.09 ± 0.02
Devcon/NPOE	−56.3 ± 0.4	−4.6 ± 0.3	0.47	0.88	1.35	1.16	−0.92			
TDMACl[h]			1.4	3.2	3.8					
required[i]			≤0.7		≤1.7					

[a] Number of samples: $n = 3$ or 5. [b] Alteco, F-05 5 Minutes epoxy adhesive from Alteco (Osaka, Japan); Devcon, 14250 5 Minutes epoxy adhesive from Devcon (Danvers, MA); THF, tetrahydrofuran; DOA, bis(2-ethylhexyl) adipate; NPOE, 2-nitrophenyl octyl ether. [c] Range, $10^{-4}-10^{-1}$ M. [d] Evaluated in 0.05 M HEPPS−NaOH, pH 7.4. Values were determined by using the matched potential method at an interfering ion concentration of 0.1 M. [e] Time required by virgin electrodes to reach 0.1 mV/min drift, which is a limiting stability recommended by IUPAC for clinical applications. [f] Values were obtained with ac-impedance method. [g] Salicylate ion. [h] Data from refs 34 and 35. [i] Required selectivity coefficients for physiologically important anions in the blood chloride measurements.[5] Calculated for a maximal interference of 1% by interfering anions.
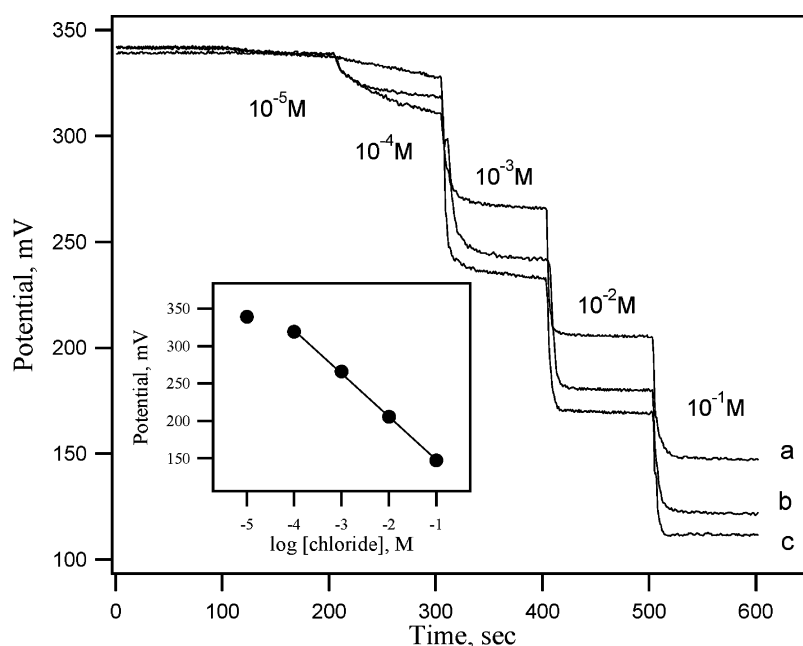

Figure 2. Dynamic response toward (a) chloride, (b) bromide, and (c) salicylate, and a calibration curve for chloride observed with the Devcon/THF-based chloride-selective electrode.

an increased steric hindrance toward large, lipophilic anions might contribute to the modification of the Hofmeister series. Scholefield et al. demonstrated that quaternary ammonium salts attached to polymer backbones could show considerably improved selectivity.[36,37] In addition, it has been reported that the use of hydrophobic quaternary ammonium molecules with long carbon chains[8,37] or diquaternary ammonium salts with two quaternary ammonium sites closely spaced by a short carbon chain[38] yielded the deviation in Hofmeister selectivity pattern. Quaternary ammonium moieties covalently networked and closely packed in an epoxy matrix could possess such unique properties (i.e., hydrophobic multi-quaternary ammonium groups).

As listed in Table 1, the response properties, especially in chloride sensitivity (i.e., slope) of the Alteco-based epoxy electrode, were slightly inferior to those of the Devcon-based electrode. Thus, we selected the Devcon-based epoxy polymer for further experiments. The effects of a diluent (i.e., THF) and a plasticizer (i.e., DOA and NPOE) on the response characteristics of the resulting chloride-selective electrodes were also summarized in Table 1, in terms of response slope, detection limit, selectivity, preconditioning period (t_{pre}; the hydration time required by virgin electrodes to reach 0.1 mV/min drift, which is a limiting stability recommended by IUPAC for clinical applications), drift, and membrane impedance.[27] As can be seen, the slope, detection limit, and selectivities over other anions were very similar for all Devcon-based electrodes regardless of the use of a diluent or a plasticizer. However, other potentiometric parameters including preconditioning time, drift, and impedance were significantly

(34) Wegmann, D.; Weiss, H.; Amman, D.; Morf, W. E.; Pretsch, E.; Sugahara, K.; Simon, W. *Mikrochim. Acta* **1984**, *3*, 1−16.

(35) Rothmaier, M.; Schaller, U.; Morf, W. E.; Pretsch, E. *Anal. Chim. Acta* **1996**, *327*, 17−28.

(36) Sutton, P. G.; Braven, J.; Ebdon, L.; Scholefield, D. *Analyst* **1999**, *124*, 877−882.

(37) Goff, T. L.; Braven, J.; Ebdon, L.; Scholefield, D. *Anal. Chem.* **2002**, *74*, 2596−2602.

(38) Wotring, V. J.; Johnson, D. M.; Bachas, L. G. *Anal. Chem.* **1990**, *62*, 1506−1510.

Table 2. Determination of Chloride Levels in Various Control and Biological Samples with the Epoxy Resin-Based Chloride Sensor[a]

sample type	composition of sample (or manufacturer's specification)	chloride value determined, mM			
		this work	Ag/AgCl[b]	ETH 9009[c]	Nova[d]
standard solutions	Cl⁻ 100 mM	100	100		99
	Cl⁻ 100 mM + Br⁻ 10 mM	98	151		124
	Cl⁻ 100 mM + Br⁻ 20 mM	102			
	Cl⁻ 100 mM + SCN⁻ 3 mM	101	118		128
	Cl⁻ 100 mM + Sal⁻ [e] 1 mM	100		102	
	Cl⁻ 100 mM + Sal⁻ [e] 3 mM	99		136	
control sample[f]	Cl⁻ 80 ± 5 mM	79 ± 1	79 ± 1		79 ± 2
normal serum[g]	Cl⁻ 94.4 ± 4.0 mM	96.3 ± 0.9	103.4 ± 3.3		95 ± 2.8
abnormal serum[g]	Cl⁻ 113 ± 4 mM	111 ± 1			113 ± 2
whole blood[h]		101 ± 0.5	111 ± 3		100 ± 1.3

[a] Number of samples: $n = 5$ or 7. [b] Solid-state Ag/AgCl electrode. [c] ETH 9009-doped PVC membrane-based electrode. [d] The polymeric membrane-based electrode installed in Nova Stat Profile Ultra M clinical analyzer (Waltham, MA). [e] Salicylate anion. [f] Blood gas/electrolyte control samples (Model A701-001) from Alko Diagnostic (Holliston, MA). [g] Control human serum samples (Model Suitrol N or A) from Nissui Pharmaceutical (Tokyo, Japan). [h] Samples obtained from a local blood bank.
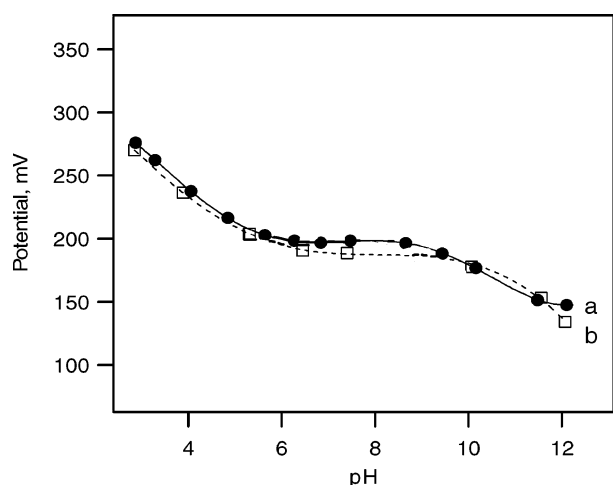

Figure 3. pH response of the epoxy resin-based chloride-selective membranes: (a) Devcon/ THF and (b) Devcon/DOA.

Figure 4. Chloride measurements in various sample solutions with the Devcon/THF-based chloride microsensor assembled in a flow cell cartridge: Cal 1 and 2, commercial standard solutions (S5185 and S5195, respectively) for ABL clinical analyzers. (a) Control sample; (b) normal serum; and (c) whole blood.

different depending on the use of a diluent or a plasticizer during membrane preparation. The addition of THF or DOA in Devcon-based epoxy polymers yielded much stable chloride-selective electrodes with fast hydration times and low membrane resistances. This might be explained by an assumption that a diluent or a plasticizer added retards the cross-linking process between epoxy resins and hardeners and eventually creates micropores or cavities inside the polymer networks.

Figure 2 presents dynamic response toward (a) chloride, (b) bromide, and (c) salicylate, and the calibration curve for chloride observed with the resulting Devcon/THF-based chloride-selective electrode. It showed a fast response time ($t_{95\%}$: 5 s at a 10 to 100 mM chloride change). The pH response properties of (a) Devcon/THF- and (b) Devcon/DOA-based electrodes were shown in Figure 3. Both compositions exhibited small signal changes in the physiological pH range (i.e., pH 6−8), indicating that these chloride-selective electrodes would be useful for the determination of chloride in blood samples. The pH response of epoxy-based electrodes in a low pH region might be due to secondary and tertiary amine sites present in the cured epoxy matrix, which can respond to protons. In addition, interfering responses of quaternary ammonium sites toward buffer anions (i.e., borate, citrate,
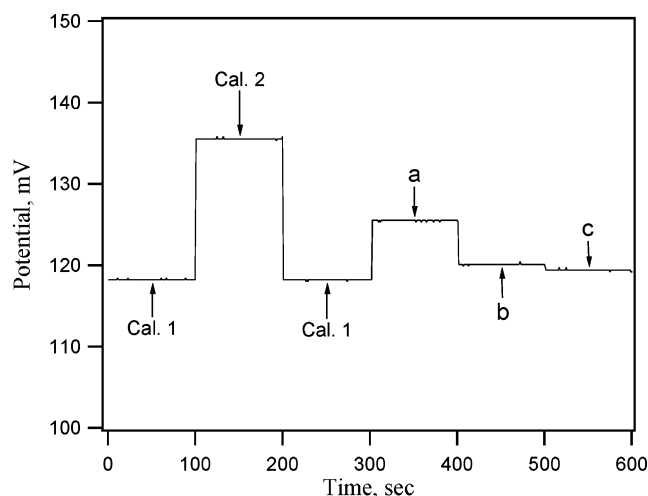
and $H_2PO_4^-/HPO_4^{2-}$) and hydroxide ion (at pH >9) could also affect potentiometric measurements of the electrode.

We have also investigated the long-term stability of the epoxy-based electrodes by checking the calibration curves during a rather long period of time. Between each calibration, electrodes were stored in a buffer solution at room temperature. The response slopes of planar all-solid-state chloride sensors employing epoxy resin-based membranes with and without THF were estimated in the range of 0.1−100 mM (data not shown). The results showed that the chloride sensitivities of both electrodes with and without THF remain stable for at least 250 days. Furthermore, no considerable degradation in the response time and selectivities over lipophilic anions was observed.

Applications of Epoxy Resin-Based Chloride Sensors for Clinical Samples. We first examined the potentiometric response of four different chloride-selective electrodes (i.e., epoxy resin, Ag/AgCl, ETH 9009-based, and Nova electrodes) toward 100 mM chloride with and without typical interfering anions, that is, 10 mM bromide, 3 mM thiocyanate, and 3 mM salicylate. The concentrations of interfering anions added to the chloride solution

are their upper clinical levels. The values measured with the Devcon/THF-based chloride microsensor were compared to the values obtained with other electrodes (see Table 2). It is seen that the Ag/AgCl solid-state electrode, the ETH 9009-doped PVC electrode, and the polymer membrane-based chloride electrode employed in commercial analyzers (i.e., Nova Stat Profile M) resulted in a large error in the presence of interfering species (i.e., bromide, thiocyanate, and salicylate), while the Devcon/THF-based epoxy electrode returned relatively accurate values.

In an attempt to further demonstrate the analytical performance of the epoxy resin (Devcon/THF)-based chloride-sensing membrane, a planar microelectrode was fabricated on a ceramic substrate and was assembled into a stop-flow cell cartridge as described in the Experimental Section. Figure 4 illustrates the results of the experiment performed by injecting aliquots (5 μL) of samples to the sensor cartridge: two commercial calibration solutions, control and serum samples, and a whole blood specimen. In Table 2, the results were compared with those obtained by the Ag/AgCl electrode and the commercial blood gas/electrolyte analyzer (Nova Stat Profile Ultra M). As can be seen in Figure 4, the epoxy resin-based microsensor yielded very fast preconditioning and response properties with stable signal outputs. Moreover, the analytical results of the proposed epoxy sensor system in determining chloride levels in various samples were in excellent agreement with those of the standard desktop chemical analyzer (see Table 2).

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