

Synthesis of Azo-functionalized Calix[4]arenes and Its Application to Chloride-selective Electrode as Ionophores

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Azo-functionalized calix[4]arenes as ionophores for chloride-selective electrode, 5,11,17,23-Tetra-*tert*-butyl-25,27-bis[(*N,N*-dimethyl-aniline-azo-phenylthioureido)ethyl]oxy-26,28-dihydroxycalix[4]arene (**4a**) and 5,11,17,23-Tetra-*tert*-butyl-25,27-bis[(*N,N*-dimethyl-aniline-azo-phenylthioureido)ethyl]oxy-26,28-dimethoxycalix[4]arene (**4b**) were synthesized. The PVC membrane electrode based on azo-functionalized calix[4]arene **4a** with *o*-NPOE exhibits a linear stable response over a wide concentration range (1.5×10^{-4} - 1.0×10^{-1}) with a slope of -52.0 mV/decade and a detection limit of $\log[\text{Cl}^-] = -4.02$. This ionophore-based membrane exhibited improved selectivity for chloride anion compared with classical Hofmeister series.

Key Words : Azo-functionalized calix[4]arene, Cl⁻-ISEs, Neutral ionophore

Introduction

The ligands for complexation of anions need to have comparatively large cavities, which have so far proved difficult to synthesize. Selective complexation of anions is also more demanding than that of cations a result of the higher free solvation energies of anions, the low charge density of anions, and the pH dependence of anion complexation.^{1,2} Accompanied by the development of ion-selective electrodes (ISEs), the selective determination of many anions has a crucial drawback -- the classical Hofmeister series, which is correlated with a preference for hydrophobic anions. Therefore, the need for ionophores with improved selectivities and sensitivities in the field of anion-selective electrodes increases. Several liquid and polymer membrane-based ISEs have been reported for the analysis of the chloride anion.^{3,4} Most of the current ISEs applied to clinical chloride determinations are based on an ion-sensing material of an ion-exchanger type, such as quarternary ammonium salts.⁵⁻⁷ Basically, the selectivity of an ISE based on such as ion-exchanger is ruled by the lipophilicity of the ion.⁸ Chloride-selective electrodes, the impregnation of lipophilic anions into a plasticized anion-exchange membrane,³ an annealed polyion complex membrane,⁹ an organic-inorganic hybrid sol-gel matrix,¹⁰ all-solid-state electrodes with plasticizer-free membrane,¹¹ silicone rubber matrix,¹² polyurethane membranes,¹³ and electropolymerized electrodes¹⁴ have been reported to enhance chloride selectivity.

In the present study, we describe the fabrication and characterization of new ISEs based on two azo-functionalized calix[4]arenes as neutral ionophores. Calixarene's structures have received considerable attention as interesting classes of an ionic and molecular binding host.^{15,16} Recently we reported that lower rim urea-functionalized calix[4]di-quinones show high selectivity as neutral receptors for HSO₄⁻,¹⁷⁻¹⁹ and as ionophores for HSO₃⁻.²⁰ The urea and thiourea groups have been used in the development of neutral ionophores, because the hydrogen bond donors can

provide the anion binding site selectively. The ISE dynamic response is generated by selective complexation of the target ion with neutral ionophores dispersed in a poly(vinyl chloride) (PVC) matrix. PVC membranes based on azo-functionalized calix[4]arenes are investigated here as Cl⁻-selective sensing electrodes. When compared with Hofmeister series obtained from the electrode based on a quarternary ammonium salt, the ISE based on azo-functionalized calix[4]arene **4a** exhibits improved selectivity and longevity for chloride anion.

Experimental Section

Reagents. Azo-functionalized calix[4]arenes (**4a**, **4b**) tested as chloride ionophores were synthesized, and their synthetic procedure is shown in Fig. 1. High molecular weight PVC, 2-nitrophenyl octyl ether (*o*-NPOE), dioctyl sebacate (DOS), tridodecylmethylammonium chloride (TDDMACl) and tetrahydrofuran (THF), which were obtained from Fluka, were used to prepare the PVC membrane electrodes. Analytical grade sodium and potassium salts of tested anions were used. Doubly distilled water in a quartz apparatus was used to prepare all aqueous electrolyte solutions.

Syntheses. **5,11,17,23-Tetra-*tert*-butyl-25,27-bis(cyanomethoxy)-26,28-di-hydroxycalix[4]arene (2a).** A *p-tert*-butylcalix[4]arene (5.0 g, 7.7 mmol), potassium carbonate (4.27 g, 0.031 mol), chloroacetonitrile (2 mL, 0.032 mol), and sodium iodide (4.63 g, 0.031 mol) in acetone (125 mL) were stirred and heated under reflux for 7 hrs. The cooled mixture was filtered and the residue was washed with dichloromethane and the product triturated with MeOH to give 2.8 g (50%) of **2a**, mp >290 °C (decomp.). ¹H NMR (CDCl₃) δ 7.12 and 6.73 (two s, 8H, ArH), 5.56 (s, 2H, ArOH), 4.81 (s, 4H, OCH₂CN), 4.22 and 3.45 (a pair of d, 8H, ArCH₂Ar *J* = 13.2 Hz), 1.33 and 0.88 (two s, 36H, *tert*-butyl).

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(cyanomethoxy)-26,28-di-methoxycalix[4]arene (2b). To a solution of 1.0 g

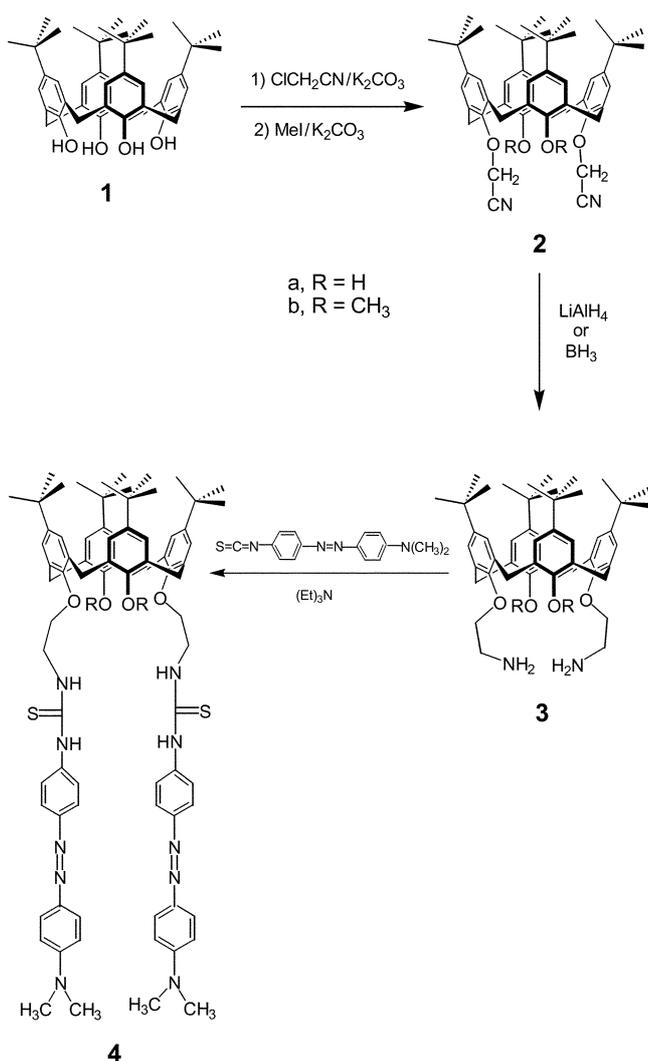


Figure 1. Synthesis of ionophores derived from calix[4]arenes.

(1.38 mmol) of **2a** and 0.66 g (27.5 mmol) of NaH in 60 mL of THF and 6 mL of DMF, 5.12 g (36.1 mmol) of CH₃I was added, and refluxed for 2 hrs. After cooling to room temperature, 10 mL of MeOH was added and stirred for 30 min, then acidified with 2 N HCl solution. The mixture was extracted by CHCl₃ (2 × 100 mL). The solvents were removed and the residue was triturated with MeOH to give 0.79 (76%) of **2b**. mp 231–333 °C. ¹H NMR (CDCl₃) δ 7.12 and 6.50 (s, 8H, ArH), 4.98 (s, 4H, OCH₂CN), 3.82 (s, 6H, OCH₃), 4.40 and 3.28 (a pair of d, 8H, ArCH₂Ar *J* = 13.2 Hz), 1.31 and 0.85 (s, 36H, *tert*-butyl).

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(2-aminoethoxy)-26,28-dihydroxycalix[4]arene (3a). To a vigorously stirred solution of compound **2a** (1.0 g, 1.4 mmol) in diethylether (50 mL), a slurry of LiAlH₄ (0.43 g, 11.3 mmol) was added in portion, and the reaction mixture was refluxed for 5 hrs. Then the reaction flask was immersed into an ice-water bath, benzene (50 mL) and water (2 mL) added to the flask contents, and an organic layer separated out. The solvent was removed to afford compound **3a** as a white solid 0.98 g (96%). mp 143–145 °C. ¹H NMR (CDCl₃) δ 8.31 (broad s,

2H, ArOH), 7.04 and 6.97 (two s, 8H, ArH), 4.24 and 3.43 (a pair of d, 8H, ArCH₂Ar *J* = 12.9 Hz), 4.07 (t, 4H, -OCH₂-), 3.30 (t, 4H, -CH₂N), 1.25 and 1.09 (two s, 36H, *tert*-butyl).

5,11,17,23-Tetra-*tert*-butyl-25,27-bis(2-aminoethoxy)-26,28-dimethoxycalix[4]arene (3b). A 6 mL of 1 M BH₃/THF solution was added to 0.3 g of **2b** under nitrogen atmosphere and refluxed for 2 hrs. The solvents were removed and the residue was treated with 10 mL of 2 N HCl and refluxed for 1 h. After cooling to room temperature, 10% KOH solution was added until the solution became basic, which was then extracted by CHCl₃ (2 × 40 mL). The solvents were removed and the residue triturated with MeOH to give 0.163 g (54.1%) of **3b**. mp >238 °C, dec. ¹H NMR (CDCl₃) δ 7.15 and 6.53 (s, 8H, ArH), 4.38 (t, 4H, OCH₂), 3.88 (s, 6H, OCH₃), 4.21 and 3.25 (a pair of d, 8H, ArCH₂Ar *J* = 12.6 Hz), 3.52 (t, 4H, -CH₂NH₂), 1.33 and 0.82 (s, 36H, *tert*-butyl).

5,11,17,23-Tetra-*tert*-butyl-25,27-bis[(*N,N*-dimethylaniline-azo-phenylthioureido)ethyl]oxy-26,28-dihydroxycalix[4]arene (4a). To a 100 mg (0.137 mmol) of compound **3a** in chloroform (20 mL), 85 mg dimethyl-aniline-azo-phenylisothiocyanate (0.3 mmol) and 30 μL (Et)₃N (0.274 mmol) were added and the mixture was stirred overnight under nitrogen atmosphere. At the end of the reaction, 20 mL of 1 N HCl solution were added and stirred vigorously for 20 min. The separated organic layer was removed from the solvent. The crude product was further purified by column chromatography (eluent *n*-hexane : CHCl₃ : methanol = 8 : 4 : 1) to give 120 mg (67%) of **4a** having an orange color. mp 220 °C. ¹H NMR (CDCl₃) δ 8.0 (t, 2H, -NH), 7.87 and 6.78 (two d, 8H, ArH, *J* = 9.3 Hz), 7.76 (s, 2H, -OH), 7.58 and 7.20 (a pair of d, 8H, ArH, *J* = 8.7 Hz), 7.46 (s, 2H, -NH-), 6.88 and 6.74 (two s, 8H, ArH), 4.16 and 4.01 (m, 8H, -OCH₂CH₂NH-), 3.75 and 3.13 (a pair of d, 8H, -ArCH₂Ar-, *J* = 12.3 Hz), 3.11 (s, 12H, -N(CH₃)₂), 1.23 and 0.97 (two s, 36H, -C(CH₃)₃).

5,11,17,23-Tetra-*tert*-butyl-25,27-bis[(*N,N*-dimethylaniline-azo-phenylthioureido)ethyl]oxy-26,28-dimethoxycalix[4]arene (4b). To a 149 mg (0.95 mmol) of compound **3b** in chloroform (10 mL), 121 mg dimethyl-aniline-azo-phenylisothiocyanate (0.43 mmol) and 54 μL (Et)₃N (0.39 mmol) were added and the mixture was stirred overnight under the nitrogen atmosphere. At the end of the reaction, 20 mL of 1 N HCl solution were added, and stirred vigorously for 20 min. The separated organic layer was removed from the solvent. The crude product was further purified by column chromatography (eluent *n*-hexane : CHCl₃ : methanol = 6 : 3 : 1) to give 193 mg (75%) of **4b** having an orange color. mp 196 °C. ¹H NMR (CDCl₃) δ 10.76 and 8.75 (two s, 4H, -NH), 7.84 and 6.74 (a pair of d, 8H, ArH, *J* = 9 Hz), 7.77 and 7.04 (a pair of d, 8H, ArH, *J* = 8.9 Hz), 4.04, 3.87, 3.74, 3.09 (broad m, 16H, -OCH₂CH₂-, -ArCH₂Ar-), 3.09 (s, 12H, -N(CH₃)₂), 1.25 and 0.97 (two s, 36H, *tert*-butyl).

Preparation of polymeric ion-selective electrodes. The compositions of PVC-based chloride-selective electrodes are summarized in Table 1. The ionophore, plasticizer, additive and PVC were dissolved in an appropriate volume of THF

Table 1. Compositions and sensitivities of chloride-selective electrodes based on azo-functionalized calix[4]arenes

no.	Ionophore (mg)	PVC ^a	<i>o</i> -NPOE ^a	DOS ^a	Add ^b	Slope	LOD
m-1	–	33	66		50 ^c	-46.0	-2.87
m-2	–	33		66	50 ^c	-44.0	-3.34
m-3	4a (1)	33	66		50	-52.0	-4.02
m-4	4a (1)	33		66	50	-42.5	-4.02
m-5	4a (1)	33	66		–	-40.0	-4.33
m-6	4b (1)	33	66		50	-30.0	-2.67
m-7	4b (1)	33		66	50	-37.6	-3.92

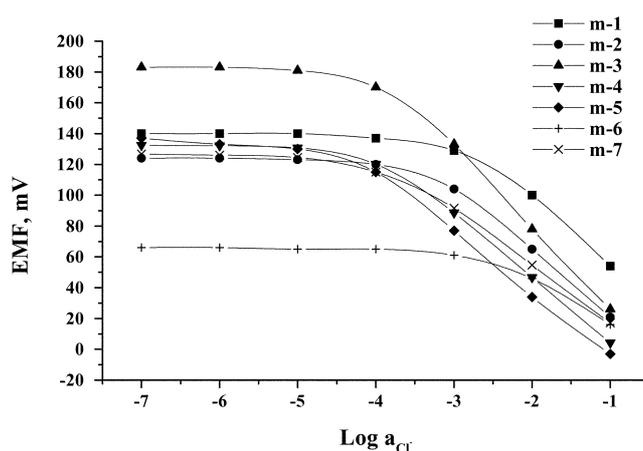
^aIn mg. ^bmol % relative to the ionophore. ^cmol % relative to the ionophore **4a**.

and mechanically stirred. All membrane cocktails were cast in glass rings placed on glass plates for conventional ion-selective electrodes. Solvent from PVC membrane was allowed to evaporate for more than 24 hours at room temperature. The thickness of the resulting membrane was about 0.3 mm.

Potentiometric measurements. The electrochemical properties of chloride-selective electrodes were investigated in the conventional configuration. Small disks were punched from the cast membranes and mounted in Philips electrode bodies (IS-561). For all electrodes, 0.1 M KCl was used as an internal filling solution. The external reference electrode was an Orion sleeve-type double-junction Ag/AgCl reference electrode (Model 90-02). The electrochemical potential was measured by using Kosentech 16-channel potentiometer (KST101-1) coupled to a computer. The dynamic response curves were produced by adding standard solutions of anions to magnetically stirred 0.05 M [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-LiOH buffer solutions. The selectivity coefficients were determined by the separate solution method (SSM). At least three measurements were performed.

Results and Discussion

An anion ionophore should bind selectively with specific anion, and have kinetically fast exchange, sufficient lipophilicity. Azo-functionalized calix[4]arenes (**4a**, **4b**) as chloride ionophores were synthesized in the present study. The potentiometric responses of PVC polymeric Cl⁻-ISEs based on ionophores **4a**, or **4b** were examined for several anions in buffer solutions. The Cl⁻-ISE membrane compositions were optimized to produce the best sensitivity and selectivity toward the chloride anion. The optimization was carried out with variations in the ratio in PVC membrane components, such as PVC, plasticizer, ionophore, additive (TDDMACl), and THF. The polymeric membranes have been investigated to measure the ability of the ionophores to act as neutral carriers in the absence and presence of the ionophore. Electrode membrane containing no specific selective ionophore showed poor response to all anions tested. However, the response of the membranes containing additive ion-exchanger (TDDMACl) and the ionophore gave better

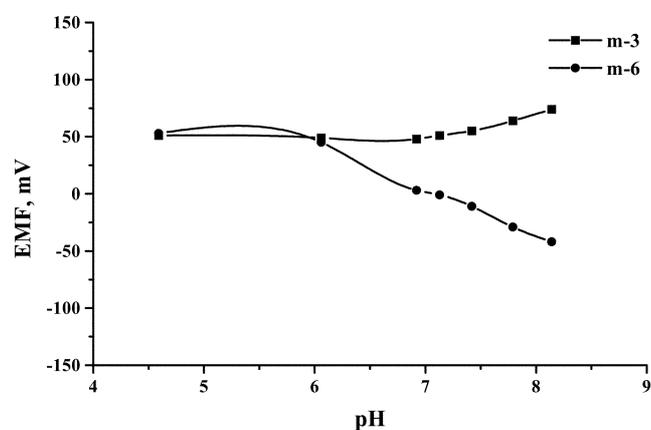
**Figure 2.** The potentiometric responses of the chloride selective electrodes based on different ionophores and plasticizers.

sensitivity and selectivity than those prepared with only TDDMACl, indicating that the novel ionophore improves sensitivity and selectivity to chloride anion in membrane electrodes. Most of the membranes used in this work contained TDDMACl. The potentiometric response of the membranes was evaluated as to the slope and detection limit (DL) towards chloride anion. The potentiometric responses of PVC polymeric membranes containing azo-functionalized calix[4]arenes (**4a**, **4b**) for chloride anion-selective electrode were extensively studied in pH 7.0 solutions. The typical composition was 50 mol% TDDMACl vs. ionophore, 33 mg PVC, 66 mg plasticizer, and 1 mg ionophore **4a**, **4b**. Figure 2 shows the potentiometric responses of PVC membrane electrodes prepared with/without ionophores, and the potentiometric results for chloride are shown in Table 1. The ISE (m-3) based on azo-functionalized calix[4]arene **4a** exhibits a linear stable response over a wide concentration range (1.5×10^{-4} – 1.0×10^{-1}) with a slope of -52.0 mV/decade and a detection limit of $\log[\text{Cl}^-] = -4.02$. Among the membranes prepared from azo-functionalized calix[4]arenes (**4a**, **4b**), the azo-functionalized calix[4]arene **4a** gives good sensitivity and sub-Nernstian slope. These results imply that the azo-functionalized calix[4]arene **4a** having the appropriate cavity may effectively bind with chloride anion in the polymeric membrane. The responses of the membrane to eleven interfering anions were tested under the determined optimal conditions. Using an electrode based on a quaternary ammonium salt (TDDMACl), the potentiometric response follows the Hofmeister series. The selectivity series of the membrane containing ionophore **4a** gives the following as $\text{ClO}_4^- > \text{Salicylate} > \text{I}^- > \text{SCN}^- > \text{Br}^- > \text{NO}_3^- > \text{Cl}^-$, $\text{NO}_2^- > \text{HSO}_3^- > \text{HSO}_4^- > \text{CH}_3\text{COO}^- > \text{H}_2\text{PO}_4^-$. Compared with an electrode based on a quaternary ammonium salt (TDDMACl), this ionophore-based ISE exhibited improved selectivity, responsibility, and detection limit for chloride anion.

It is known that the membrane matrix, *i.e.* polymer, plasticizer and additive, play crucial roles in the functioning of the membrane electrodes. The membranes containing only PVC and plasticizer (DOS or *o*-NPOE) give a

Table 2. Selectivity coefficients of various interfering ions for membrane (m-3) electrodes obtained by SSM

Interfering ion	$K_{Cl^-,j}^{pot}$
ClO_4^-	38
Sal^-	37
I^-	30
SCN^-	5.9
Br^-	3.1
NO_3^-	2.9
NO_2^-	1.0
HSO_3^-	8.1×10^{-2}
HSO_4^-	1.6×10^{-2}
CH_3COO^-	1.2×10^{-2}
$H_2PO_4^-$	3.3×10^{-3}

**Figure 3.** The pH dependence of potentiometric responses of PVC membrane electrodes prepared from azo-functionalized calix[4]arenes (**4a**, **4b**) with *o*-NPOE as a plasticizer.

substandard potentiometric response. Moreover, as can be seen in Table 1, the membranes containing PVC, plasticizer, and additive show some potentiometric response, but with poor sensitivities. The effect of plasticizers in the presence of ionophores was investigated in PVC polymeric membranes containing 1 mg ionophore **4a**, 50 mol% TDDMACl vs. ionophore, 33 mg PVC, and 66 mg plasticizer. Based on the results of Fig. 2 and Table 1, membranes prepared from *o*-NPOE showed the best detection limit, good linear range, and sub-Nernstian slope for chloride anion. Thus, *o*-NPOE was chosen as a proper plasticizer for use in the Cl^- -ISE membranes. The pH dependence of potentiometric response was measured in different pH solutions. Fig. 3 shows the pH dependence of potentiometric responses of PVC membrane electrodes prepared from azo-functionalized calix[4]arenes (**4a**, **4b**) with *o*-NPOE as a plasticizer. The membrane electrode of azo-functionalized calix[4]arene **4a** is independent of the solution pH used. Thus, pH 7.0 buffer solutions were used in this study. The response time of the ionophore-based membrane electrode ranged from less than 10 sec at all concentrations of chloride ion, and these novel prepared electrodes did not display any hysteric effects. The stability of these membrane electrodes was measured as a function of

time. The membranes are stored in 0.05 M HEPES-LiOH pH 7.0 buffer solutions when not in use. The stability in the polymeric membrane may be dependent upon the lipophilicity of an ionophore, which can result in the ionophore bleeding from the membrane. Since azo-functionalized calix[4]arenes (**4a**, **4b**) as ionophores are neutral compounds having high lipophilicity, the membranes based on them should produce slow bleeding of ionophore. After one month, the electrodes were responding at 95% of the initial response. The prolong lifetime of the membrane as the Cl^- -ISE is due to the relatively high lipophilicity of the neutral ionophores tested in the membrane.

Conclusions

Azo-functionalized calix[4]arenes (**4a**, **4b**) tested as chloride ionophores were synthesized and used as ionophores for chloride-selective electrode. The ISE (m-3) based on azo-functionalized calix[4]arene **4a** exhibited a linear stable response over a wide concentration range (1.5×10^{-4} – 1.0×10^{-1}) with a slope of -52.0 mV/decade and a detection limit of $\log[Cl^-] = -4.02$. This ionophore-based ISE exhibited improved selectivity, responsibility, and detection limit for the chloride anion compared with classical Hofmeister series. The membrane electrode was found to be chemically and physically stable, and gave steady potential response within 10 seconds with high reproducibility. The long lifetime of the membrane as the chloride-ISE is due to the relatively high lipophilicity of azo-functionalized calix[4]arenes as neutral ionophores in the membrane.

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