Epinephrine-Selective Electrode Based on Lipophilic 1,3-Bisbridged Cofacial-calix[6]crown-5-ether

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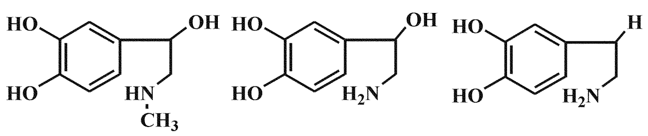
The potentiometric response of electrode no. 4 based on 1,3-bisbridged cofacial-calix[6]crown-5-ether (IV) gave a sub-Nernstian (45.0 mV/decade) response and the best detection limit (-log a_{ep} = 4.73) towards epinephrine. The responses are decreasing in the order of epinephrine > K^+, dopamine > NH_4^+ > norepinephrine > Na^+. It is remarkable that the proposed electrode shows the reasonable selectivity to epinephrine against other catecholamine neurotransmitters (dopamine and norepinephrine) as well as alkali metal ions.

Key Words : Epinephrine, Ion-selective electrode, Cofacial-calix[6]crown-5-ether, Catecholamine, Ionophore

Introduction

Liquid membrane type ion-selective electrodes (ISEs) have provided one of the most powerful sensing methods because it is possible to select various sensory elements according to the shape and size of the target ion.[1] Although the organic cation ISEs based on synthetic or natural ionophores have been investigated actively, there are still limited studies of ISEs for neutral organic guests. The selectivity of these electrodes is controlled by the lipophilicity of guest.[2-4] In addition to liquid membrane type ISEs, calixarenes have received considerable attention as an interesting class of ionic and molecular binding hosts,[5] and studied that various functionalized calixarenes were selective host molecules for cations as well as anions.[6-13] Many macrocycles including related calixarenes have also been synthesized for organic amine recognition and ISEs which have received considerable attention for clinical and environmental analysis.[14-25] The well-defined structure of the calixarene cavity was investigated for an inclusion of amine guests. Actually calix[6]arene hexaesters, homooxacalix[3]arene triester, and para-1-adamantylcalix[8]arene have been shown to display selectivities for primary amines such as dopamine.[26-29] Calix[6]arene has a sufficiently large cavity, so that it can accommodate amine guests, whereas that of calix[4]arene is too small for ordinary amine guests. In this study, poly(vinyl chloride) (PVC) polymeric membrane electrodes were prepared from lipophilic 1,3-bisbridged cofacial-calix[6]-crowns as catecholamine selective ionophores. Lipophilic 1,3-bisbridged cofacial-calix[6]crowns used in this study seem to provide an effective binding site towards catecholamines and large cations. Unlikely ionophores such as calix[6]arene hexaesters, homooxacalix[3]arene triester, and para-1-adamantylcalix[8]arene, 1,3-bisbridged cofacial-calix[6]crown-5-ether (IV) shows the enhanced selective response towards epinephrine of secondary amine rather than dopamine of primary amine. This paper discusses the construction and evaluation of an epinephrine sensitive and selective membrane electrode.



epinephrine norepinephrine dopamine

Experimental Section

Reagents. Five 1,3-bisbridged cofacial-calix[6]crowns tested as epinephrine hosts are shown in Figure 1. They were synthesized in our laboratory.[30] High molecular weight PVC, dioctyl sebacate (DOS), 2-nitrophenyl octyl ether (*o*-NPOE), potassium tetrakis(*p*-chlorophenyl)borate (KT*p*ClPB) and

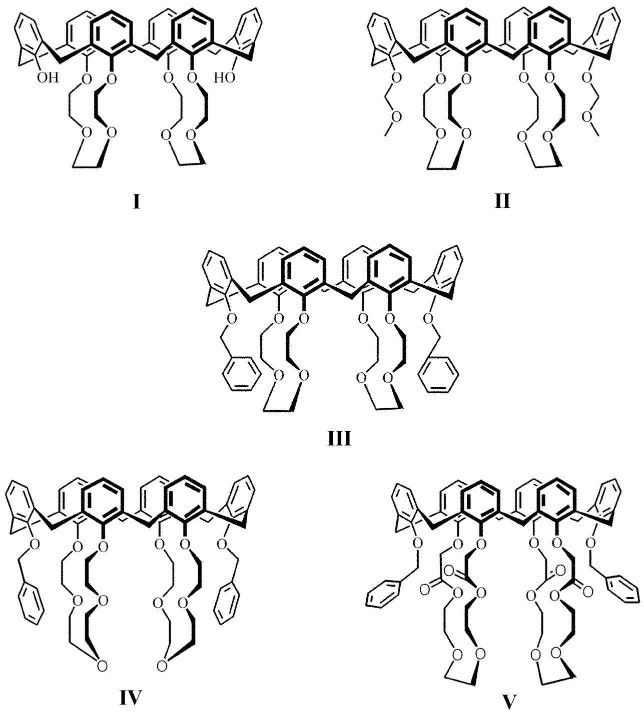


Figure 1. Structures of 1,3-bisbridged cofacial-calix[6]crowns **I-V** used in this study.

Table 1. Compositions of PVC-based organic amine-selective membranes[a]

Electrode no.	Ionophore	PVC	*o*-NPOE	KT*p*ClPB[b]	Ionophore
1	I	33	66	50	1
2	II	33	66	50	1
3	III	33	66	50	1
4	IV	33	66	50	1
5	V	33	66	50	1

[a]In wt %. [b]Mol % relative to the ionophore.

tetrahydrofuran (THF), which were obtained from Fluka, were used to prepare the PVC membranes. Analytical grade hydrogen chlorides of dopamine, epinephrine, and norepinephrine were used. Doubly distilled water in a quartz apparatus was used to prepare all aqueous electrolyte solutions.

Preparation of polymeric ion-selective electrodes. Table 1 summarizes the compositions of the epinephrine-selective electrodes employed in this study. The typical composition of PVC-based epinephrine-selective electrodes was 33 mg PVC, 66 mg plasticizer, 1 mg ionophore, and 50 mol% additive (KT*p*ClPB). The ionophore, plasticizer, and PVC were dissolved in an appropriate volume of THF 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 at least 24 hours at room temperature.

Evaluation of the electrode performance. The electrochemical properties of the epinephrine-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 using 16-channel potentiometer coupled to a computer. The dynamic response curves were produced by adding standard solutions of cations under the magnetically stirred buffer solution (0.1 M Tris-HCl, pH 7.2). The selectivity coefficients were determined by matched potential method (MPM). The detection limits were estimated by the intersection of two linear lines, the one extrapolated from a high concentration range and the other parallel to the x-axis drawn through the mean potential value of the lowest metal ion concentration used in the plot of the potential change and the concentration of epinephrine. At least, three-time measurements were performed, and the data were determined from the plot. All measurements were performed at room temperature.

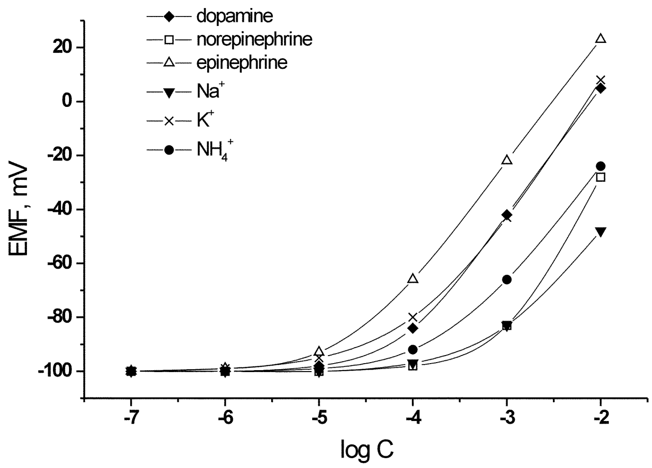


Figure 2. The potentiometric responses of the electrode no. 4 prepared from **IV** towards catecholamines and alkali metal ions in 0.1 M Tris-HCl buffer solutions (pH 7.2).

Results and Discussion

Potentiometric response. The potentiometric response of PVC polymeric electrodes for epinephrine as a secondary amine based on ionophores I-V was examined for catecholamines, alkali metals, and ammonium ions in buffer solutions. The membrane compositions were optimized to produce the best sensitivity and selectivity towards epinephrine. The optimization was carried out with varying the ratio in PVC membrane components such as PVC, plasticizer, ionophore, additive (KT*p*ClPB), and THF. Figure 2 shows the potentiometric response curves of electrode no. 4 based on 1,3-bisbridged cofacial-calix[6]crown-5-ether (IV) towards catecholamines and alkali metal ions in pH 7.2 (0.1 M Tris-HCl) buffer solutions. Membrane potentials were increased with increasing concentration of guest, indicating that potentiometric response occurs by the complexation between neutral ionophore and cationic guest. The responses gave the different trend compared with those obtained from ion exchanger. The responses were decreasing

Table 2. Electrochemical properties of PVC-based amine-selective membranes

Electrode no.	Ionophore	Slope (mV/decade)	Detection Limit (log a)	$\log k_{ep+,j}$	
				j = dopamine	j = norepinephrine
1	I	29.0	-3.28	0.60	0.40
2	II	41.0	-3.39	1.05	0.40
3	III	29.0	-3.28	1.40	0.60
4	IV	45.0	-4.73	-0.45	-1.15
5	V	37.0	-3.49	0.90	0.45

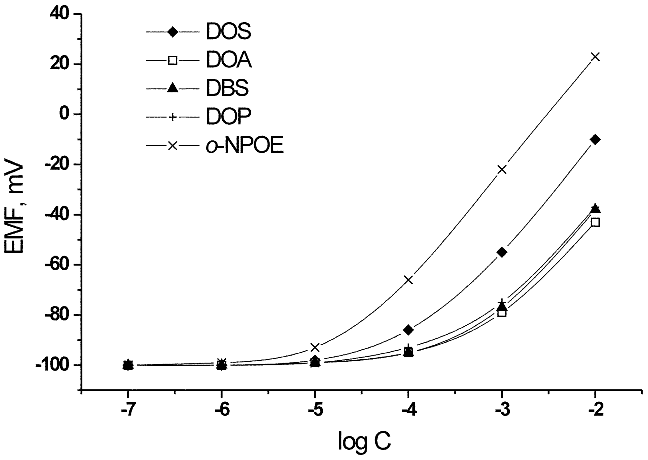


Figure 3. The epinephrine calibration curves for the electrodes based on **IV** with different plasticizers in 0.1 M Tris-HCl buffer solutions (pH 7.2).

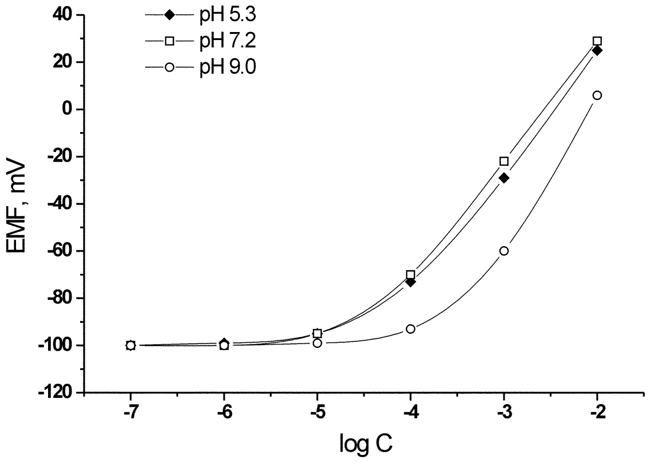


Figure 4. The potentiometric responses of electrode no. 4 prepared from **IV** towards epinephrine at different pH values in 0.1 M Tris-HCl buffer solutions.

in the order of epinephrine > K^+, dopamine > NH_4^+ > norepinephrine > Na^+. The membrane containing no specific selective ionophore gave negligible response to all cations tested. The polymeric electrodes were studied to measure the ability of the ionophores to act as neutral carriers in the absence and presence of ion exchanger. The response of the electrodes without KT*p*ClPB showed worse sensitivity and selectivity than those containing additive ion-exchanger. Most of electrodes with 50 mol% KT*p*ClPB were used in this work. The slope and detection limit for membranes were determined in accordance with IUPAC recommendations.[23] The electrochemical properties of PVC polymeric electrodes for catecholamines are listed in Table 2. Of all electrodes based on 1,3-bisbridged calix[6]crowns, a typical electrode (no. 4) displays a sub-Nernstian (45.0 mV/decade) response to epinephrine in epinephrine solutions over the linear range of 1×10^{-4}-1×10^{-2} M, and the best detection limit of (-log a_{ep} = 4.73) to epinephrine.

The effect of plasticizer was investigated in PVC polymeric electrodes containing 1 mg ionophore IV, 50 mol% KT*p*ClPB vs. ionophore, 33 mg PVC, and 66 mg plasticizer. Plasticizers used are DOS, DOA, DBS, DOP, and *o*-NPOE. Figure 3 illustrates the epinephrine calibration curves for their electrodes in 0.1 M Tris-HCl pH 7.2 buffer solutions. Polymeric membrane fabricated from *o*-NPOE showed the best detection limit, the best linear range, and sub-Nernstian slope for epinephrine. *o*-NPOE was chosen as a proper plasticizer for epinephrine-selective electrode.

The pH effect on the potentiometric response of the novel electrodes prepared with ionophore IV (66 mg *o*-NPOE, 33 mg PVC, 50 mol% KT*p*ClPB, and 1 mg ionophore IV) is studied in 0.1 M Tris-HCl buffer solutions at different pH values, and shown in Figure 4. The higher response for epinephrine in pH 5.3 and pH 7.2 rather than pH 9.0 indicates that the membrane responds to the protonated amine more effectively, and so the preference in neutral and acidic condition is caused by hydrogen bond between amine and ionophore. The pH 7.2 buffer solution was chosen as a proper pH of buffer solution for epinephrine-selective electrode.

Selectivity of the polymeric electrodes. The selectivities of the new membrane electrodes were evaluated by matched potential method. The selectivity coefficients towards epinephrine were determined against the interfering catecholamines. The selectivity coefficients ($pk^{pot}_{ep^+,j}$) with respect to the corresponding chloride of the interfering ion tested are also summarized in Table 2. From this, it can be considered that only ionophore IV is selective towards epinephrine against the interfering catecholamines, but other ionophores are selective towards dopamine among catecholamines. The electrode containing 1,3-bisbridged cofacial-calix[6]crown-5-ether gave the selectivity coefficients [$k^{pot}_{ep^+,j}$ = 0.45 (vs. dopamine), 1.15 (vs. norepinephrine), 0.35 (vs. K^+), 1.05 (vs. NH_4^+), and 1.60 (vs. Na^+)]. An interesting feature of this ionophore is the selectivity to epinephrine against other catecholamine neurotransmitters (dopamine and norepinephrine), and also against alkali metal ions such as K^+, Na^+, and NH_4^+. Although the selectivity coefficient of epinephrine against dopamine is relatively low value, this electrode can be selective to epinephrine over dopamine. The selectivity of epinephrine for the electrode containing ionophore IV is better than those of dopamine and norepinephrine. There were many reports for the superior detection of dopamine over epinephrine,[26-29] but this is a first example for the superior detection of epinephrine over dopamine and norepinephrine. The potentiometric response and selectivity towards epinephrine over dopamine are very important in a biological viewpoint.

Conclusion

The potentiometric response of 1,3-bisbridged calix[6]crown based (I-V) polymeric electrodes was investigated for epinephrine as a secondary amine. The proposed electrode (No. 4) based on 1,3-bisbridged cofacial-calix[6]crown-5-ether (IV) gave a sub-Nernstian (45.0 mV/decade) response and the best detection limit (-log a_{ep} = 4.73) towards epinephrine. The responses are decreasing in the order of epinephrine > K^+, dopamine > NH_4^+ > norepinephrine > Na^+.

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