Development and application of a fluorescent sensor for potassium ions based on a calix[6]arene ionophore and a novel cationic dye

Guomei Zhang[a]; Yinghui Li[a]; Shaomin Shuang[a]; Chuan Dong[a]; Martin M. F. Choi[b]; Winghong Chan[b]
[a] Center of Environmental Science and Engineering Research, School of Chemistry and Chemical Engineering, Shanxi University, Taiyuan, People's Republic of China [b] Department of Chemistry, Hong Kong Baptist University, Hong Kong SAR, People's Republic of China

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis
Taylor & Francis Group

Development and application of a fluorescent sensor for potassium ions based on a calix[6]arene ionophore and a novel cationic dye

Guomei Zhang[a], Yinghui Li[a], Shaomin Shuang[a], Chuan Dong[a]*, Martin M.F. Choi[b] and Winghong Chan[b]*

[a]Center of Environmental Science and Engineering Research, School of Chemistry and Chemical Engineering, Shanxi University, Taiyuan 030006, People's Republic of China; [b]Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong SAR, People's Republic of China

A plasticised poly(vinyl chloride) optode membrane incorporated with a calix[6]arene hexaester, a H^+-selective chromoionophore (3,3′,5,5′-tetramethyl-*N,N*-dibenzylbenzidine, a novel synthetic cationic dye) and a lipophilic potassium tetrakis(4-chlorophenyl)borate was used as a sensing device for the indirect optical determination of potassium ions. It exhibited a reversible response to the potassium ion in 0.05 mol/l HCl buffer media in the concentration range from 1×10^{-6} to 1×10^{-2} mol/l. The linear range was from 1.53×10^{-5} to 3.20×10^{-3} mol/l. The proposed optode sensor exhibited a fast response of less than 1 min, good repeatability ($n = 7$, RSD = 3.62%) at 5×10^{-5} mol/l, and long-term stability with 92% of its initial sensitivity after 1 month of storage. The selectivity of the potassium-selective membrane allows its application for the detection of the potassium concentration in real sample analysis. The result was satisfactory compared with atomic absorption spectrometry.

Keywords: optode membrane; potassium ion; calix[6]arene hexaester supramolecular; ion-exchange mechanism

1. Introduction

Calix[*n*]arenes, which are cyclic oligomers of phenol–formaldehyde condensates, have emerged as one of the most important supramolecular hosts since their OH groups on the lower rim can be selectively functionalised (*1–4*). Calixarenes that have a 3D structure associated with versatile recognition properties can form very stable complexes with metallic or organic ions and neutral molecules as well (*5, 6*). The size and geometry of calixarenes have been determined by the functionalisation of the upper rim, the lower rim or both (*7–9*). This is the reason for calixarenes to be superior to crown ethers and cyclodextrins and to have high selectivity for various guest molecules (*10, 11*). Calixarenes are frequently selected as host molecules in sensor research due to their well-defined frame and chemically stable intermolecular cavity of tunable size and coordination selectivity (*12–14*). Special applications in molecular switches have also been reported (*15*). Moreover, considerable interest has been focused on the preparation of calixarene ionophore based on optodes (*16–18*).

Optical sensors have attracted the attention of many researchers because of their small size, ease of operation and freedom from electrical interference. Another advantage of the optical sensor versus electrical methods for studying the multiple weak interactions is that the potentiometric response of ion-selective electrode (ISE) membranes is, in most cases, related to the phase boundary ion activity on the sample side of the sample–membrane interface (*19*). In the optode systems, a cation-selective neutral ionophore is used to recognise a given cation, and a proton-selective dye is used to provide absorbance or fluorescence signals. The optode systems share a common ion-exchange response principle (*20, 21*). It is well known that the sensing principle of optode membranes of an ion-exchange system is based on a reversible mass transfer of the analyte from the sample into the bulk of sensing layer, which is very much like that of a process in biomembranes (*22*). Until now, most of the optodes have been based on absorbance measurements since proton-selective chromo-ionophores are used (*16, 23*). However, the fluorescent ion-exchange optode reveals the possibility to develop highly selective and sensitive optode systems. On the basis of the aforementioned reasons, it is our objective to design a novel fluorescent optode based on ion-exchange mechanisms to synthesise 3,3′,5,5′-tetramethyl-*N,N*-dibenzylben-zidine (TMB) as a fluorescent indicator for the detection of cationic species.

Potassium contents are related to renal diseases (*24*). These diseases restrict patients from a diet containing a large amount of potassium. From the potassium determination, medical information concerning physical

*Corresponding authors. Email: dc@sxu.edu.cn; whchan@hkbu.edu.hk

conditions of the patient can be obtained. When potassium concentration in human serum becomes higher than 9 mmol/dm^3, the heart often stops (25). Hence, accurate, easy and rapid sensing of potassium ions is very essential.

Current research for determination of potassium is mostly limited to electrochemical determinations (26–28) and absorption optode systems (17, 29). In this work, we fabricate a new fluorescent ion-exchange optode for potassium ion determination. The optode membrane contains an ionophore (calix[6]arene hexaester) as the host selective to the potassium ion, a novel chromoionophore, TMB, selective to the hydrogen ion and a lipophilic potassium tetrakis(4-chlorophenyl)borate (KTpClPB) as the anionic site. Owing to the specific host–guest interaction between the calixarene ionophore and the potassium metal ion, the construction of the sensor for potassium determination can be achieved using the proposed optode-membrane system. The proposed potassium ion sensor shows fast response and high selectivity. It was successfully applied to determine the potassium ion concentration in real samples and the results were satisfactory.

2. Experimental

2.1 Chemicals and reagents

Bis(2-ethylhexyl) sebacate (DOS), high molecular weight poly(vinyl chloride) (PVC) and KTpClPB were purchased from Fluka (Buchs, Switzerland). Calix[6]arene hexaester and ethyl bromoacetate were obtained from Aldrich (Milwaukee, WI, USA). Quality control serum samples were obtained from Ciba Corning (Irvine, CA, USA).

All solutions were prepared with distilled water. Inorganic and organic chemicals were of the analytical-reagent grade and used without further purification.

2.2 Synthesis of TMB

The cationic dye TMB was synthesised according to the following procedures. A fresh distilled solution of 0.83 mmol benzylaldehyde was added to a stirred solution of 3,3′,5,5′-tetramethylbenzidine (80 mg, 0.33 mmol) in 10 ml of absolute ethanol. The mixture was allowed to stir for 4 h at room temperature. The mixture was ice-cooled and the precipitate was collected by suction filtration. The crude TMB was washed with a small amount of cold ethanol. A dry, two-necked flask was charged with 8 ml of dry tetrahydrofuran (THF) and 44 mg of lithium aluminium hydride. TMB in 5 ml of dry THF was added dropwise to the reaction flask. The mixture was stirred at room temperature overnight. The unreacted lithium aluminium hydride is cautiously hydrolysed by dropwise addition of water. After the completion of hydrolysis, the reaction mixture was extracted with ether and purified by silica gel column chromatography (eluent petroleum ether/ethyl acetate 8:2) to afford TMB (73%). The product was characterised by ^{1}H NMR: (400 MHz, CDCl$_3$), 2.323 (s, 12H, 4CH$_3$), 3.218 (s, 2H, 2CH$_2$), 7.225 (s, 4H, aromatic), 7.364 (m, 10H, aromatic) and ^{13}C NMR: (100 MHz, CDCl$_3$), 18.66, 52.96, 127.14, 127.26, 127.97, 128.56, 129.91, 134.87, 140.42, 144.73. Anal. calcd for C$_{30}$H$_{32}$N$_2$: C, 85.67; H, 7.67; N, 6.66. Found: C, 85.39; H, 7.72; N, 6.73.

2.3 Optode membranes fabrication

The optode membrane M1 was obtained by dissolving 25 mg of PVC, 50 mg of DOS, 1.8 mg (3.57 μmol) of anionic site (KTpClPB), 4.1 mg of calix[6]arene hexaester and 1.5 mg of TMB in 1 ml of freshly distilled THF. On a quartz-glass plate (diameter 35 mm; thickness 0.3 mm), 0.2 ml of the mixture was pipetted, which was mounted on a home-made spin (rotating frequency 600 rpm) aluminium alloy rod. After a spinning time of 5 s, a reproducible and thin (approximately 4 μm) membrane was obtained onto the quartz plate.

In a similar manner, membranes M2 and M3 were prepared from the described home-made spin. The thickness of the membranes was evaluated by the interference pattern method (28).

2.4 Apparatus

Fluorometric measurements were performed on a fluorescence spectrophotometer (Hitachi F-4500, Tokyo, Japan) with a specially designed flow-through measuring cell as shown in Figure 1. The solution pH values were recorded on an Orion Model 231 combined pH meter. All measurements were made at a constant temperature of 20 ± 1°C.
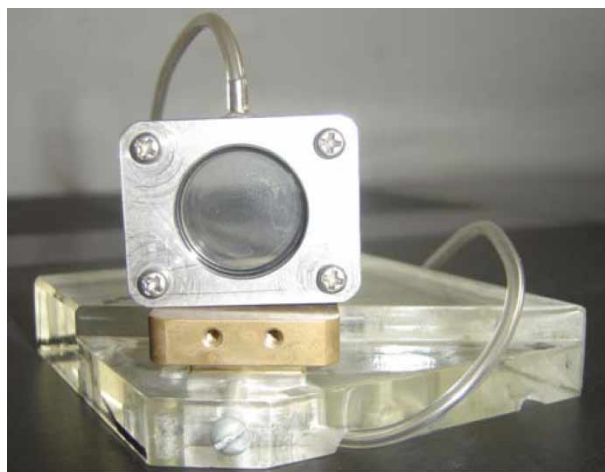

Figure 1. Picture of the flow cell.

2.5 Experimental procedure

A membrane cast on a quartz plate and a 35-mm diameter PVC plate were mounted in the special flow-through measuring cell. The cell was positioned in the fluorescence spectrophotometer at an optimum position such that the measuring emission intensity was minimised from the scattering light of the excitation source. About 3 ml of a sample solution was introduced into the cell and the fluorescence intensity was measured. Before use, the membrane was washed with HCl solution until the fluorescence intensity was stabilised.

3. Results and discussion

3.1 Theoretical consideration of the optical sensor response

The optode membrane described here belongs to the class of ion-exchange systems as described by Morf et al. (*20*). In this system, the optode membranes contain neutral calix[6]arene hexaester (calix, C) as the selective host for alkali metal ion (M$^+$), a cationic dye molecule, TMB (indicator, IND), as a proton-sensitive fluorescing compound, which was designed to bring about a large change in fluorescence spectra by deprotonation in the definite pH range; in addition, a lipophilic salt, KT*p*ClPB as the anionic site (R$^-$; Figure 2).

Figure 3 shows the fluorescence spectra of the membrane M1, which were obtained after equilibrium with a 0.05 mol/l HCl solution containing different concentrations of the potassium ion. A considerable increase in the fluorescence intensity was observed at the excitation wavelength 300 nm and emission wavelength 358 nm as the concentrations of the potassium ion
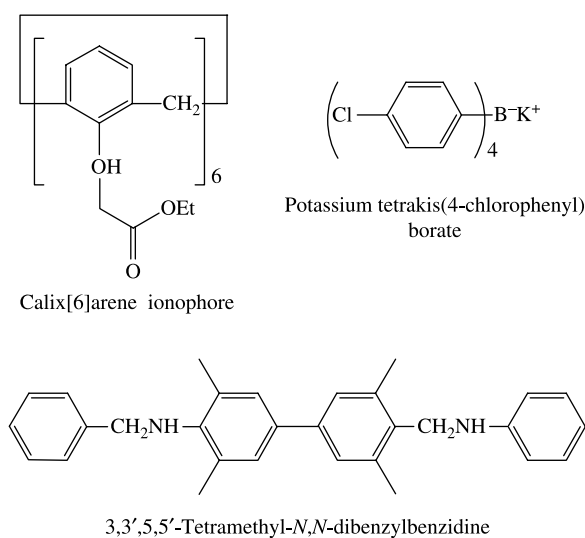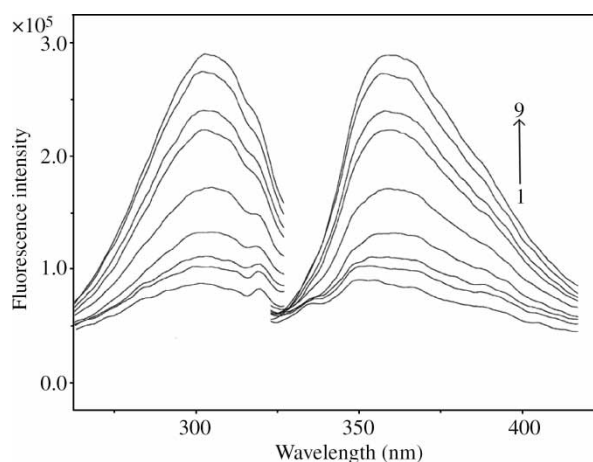

Figure 3. Fluorescence spectra of the optode membrane M1 after equilibration with 1, pH 1; 2, 0.05 mol/l; 9, pH 8.5, 0.2 mol/l Tris–HCl buffer, and with 0.05 mol/l buffer solutions containing different concentrations of KCl (M): 3, 1×10^{-6}; 4, 1×10^{-5}; 5, 1×10^{-4}; 6, 1×10^{-3}; 7, 1×10^{-2}; and 8, 1×10^{-1}.

increase. It is obvious that a complex was formed between the calixarene and the potassium ion.

Assuming that a 1+1 calix–K$^+$ complex is formed, the overall equilibrium between the polymeric membrane phase (m) and the aqueous sample solution (aq) governed by the equilibrium constant K_{exch} can be described as:

$$M^+_{(aq)} + INDH^+_{(m)} + C_{(m)} \xrightleftharpoons{K_{exch}} M^+C_{(m)} + IND_{(m)}$$
$$+ H^+_{(aq)}. \tag{1}$$
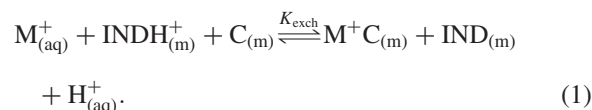
The synthesis of the novel dye molecule TMB (IND) is very much key to the experiment because the signal of the sensor is from TMB. The response of the TMB in the sensor has been attributed to the changes in the fluorescence signal caused by the change in the micro-environment of the TMB. The extraction of potassium ion from a watery phase into a lipophilic membrane phase requires the release of a positive charge H$^+$ from TMB, which leads to a change in the fluorescence intensity of the system. As TMB itself is a H$^+$-selective chromoionophore, it transfers from the membrane phase to the watery phase.

According to the derivation reported elsewhere, a response function for the metal ions is given below (*16*):

$$\frac{[M^+]}{[H^+]} K_{exch} = \frac{\{[R^-]_T - (1 - \alpha)[IND]_T\}}{\{[C]_T - [R^-]_T + (1 - \alpha)[IND]_T\}} \frac{\alpha}{1 - \alpha}, \tag{2}$$

where $[C]_T$, $[R^-]_T$ and $[IND]_T$ are the respective total concentrations of calix[6]arene hexaester, KT*p*ClPB and the cationic dye TMB in the membrane preparation. In addition, the term α in Equation (2) can be defined as the

Calix[6]arene ionophore

Potassium tetrakis(4-chlorophenyl) borate

3,3′,5,5′-Tetramethyl-*N*,*N*-dibenzylbenzidine

Figure 2. Structure of active materials in the optode membrane preparation.

Table 1. Composition of optode membrane casting solutions and the response behaviour of their optode membranes in 0.05 mol/l HCl.

Entry	TMB (μmol)	Calix[6]arene (μmol)	KTpClPB (μmol)	Response slope, α concentration (per decade)	Dynamic range (M)
M1	3.57	3.57	3.57	0.26	1.53×10^{-5}–3.20×10^{-3}
M2	3.57	7.14	7.14	0.38	1.21×10^{-5}–7.32×10^{-4}
M3	3.57	7.14	3.57	0.41	8.56×10^{-6}–2.42×10^{-4}

Note: PVC 25 mg and DOS 50 mg in 1 ml THF for all membranes.

ratio of concentration of the unprotonated dye relative to the total dye present in the membrane phase, viz.

$$\alpha = \frac{[IND]}{[IND]_T}. \tag{3}$$

In fact, the measured fluorescence intensity, F, is directly related to the membrane response, α:

$$\alpha = \frac{F - F_0}{F_1 - F_0}, \tag{4}$$

where F_1 and F_0 are the limiting fluorescence intensity for $\alpha = 1$ (fully unprotonated cationic dye) and $\alpha = 0$ (fully protonated cationic dye), respectively. The factor α, here, can also be named as the relative fluorescence intensity.

3.2 Response behaviour of different membrane formulations

As revealed by Equation (2), the response of the optode membrane is highly dependent on its composition. To study the composition effect, membranes M1–M3 of different formulations were fabricated (Table 1). For specific membrane composition of M1–M3, the response function denoted by Equation (2) can be simplified to Equations (5)–(7), respectively.

For M1,

$$\frac{[M^+]}{[H^+]} K_{exch} = \frac{\alpha^2}{(1 - \alpha^2)}, \tag{5}$$

where $[R^-]_T = [IND]_T = [C]_T$.

For M2,

$$\frac{[M^+]}{[H^+]} K_{exch} = \frac{\alpha(1 + \alpha)}{(1 - \alpha)^2}, \tag{6}$$

where $[R^-]_T = 2[IND]_T = [C]_T$.

For M3,

$$\frac{[K^+]}{[H^+]} K_{exch} = \frac{\alpha^2}{(2 - \alpha)(1 - \alpha)}, \tag{7}$$

where $2[R^-]_T = 2[IND]_T = [C]_T$.

The response of different optode membranes is displayed in Figure 4. The curve fitting for the experimental points was
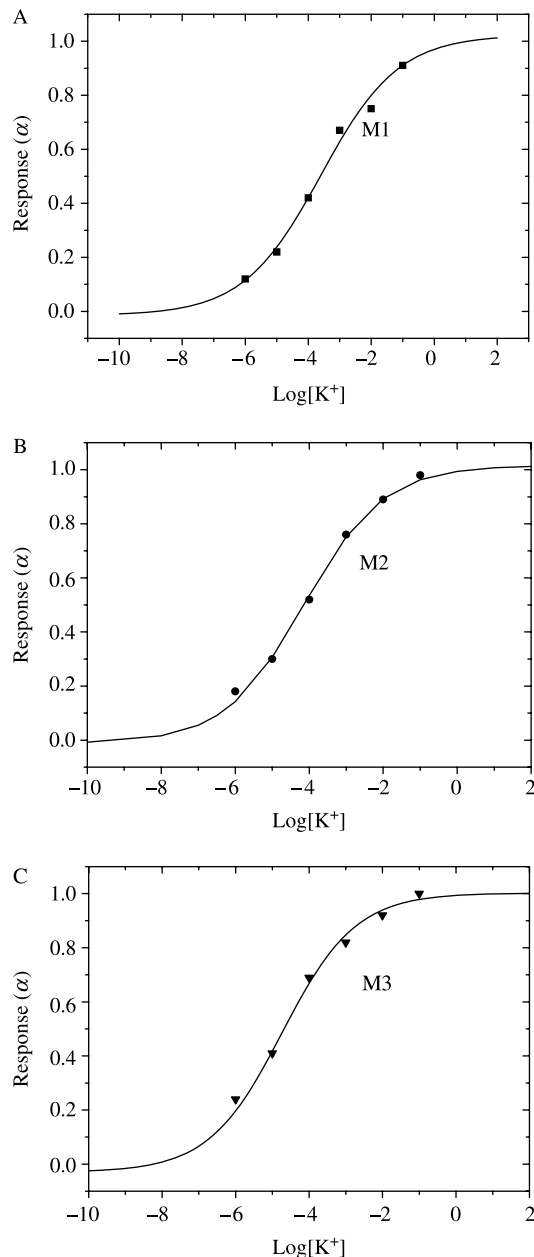
Figure 4. Responses of different optode membranes M1–M3 at 358 nm as a function of log[K$^+$] in 0.05 mol/l HCl. A, M1; B, M2; C, M3.

calculated from Equation (5) to Equation (7) with $K_{exch} = 0.736$. Good agreement between the theoretical prediction curves and the experimental data was found for all membrane systems. Certainly, these are the advantages of the optical detection associated with the optode membrane method over the more conventional ion-selective electrode method.

Therefore, the 1+1 nature of the complex between calix[6]arene ionophore and the potassium ion was confirmed. The response slope and dynamic working range of the membrane may be modified by controlling the relative amount of components in the membrane preparation depicted in Table 1. This flexibility allows us to formulate a unique ion selectivity of the optode membrane suitable for the particular requirement of an analytical method. It was found that a molar ratio of TMB/KTpClPB/calixarene = 1:1:1 is required to get a good response to the potassium ion and increasing the amount of KTpClPB and calixarene could only prolong the lifetime of the sensor. The result is understandable if calixarene competed with the potassium ion for the borate during the detection process. An excess amount of KTpClPB then compensates its loss in the acidic medium. Thus, membrane M1 was used in the subsequent real sample analysis.

3.3 Reproducibility, reversibility and response time

It would be desirable for an optical sensor to have good reproducibility and reversibility. Figure 5 shows the fluorescence intensity response at $\lambda_{ex/em} = 300/358$ nm recording for sensing membrane to the potassium ion solution of different concentrations. An increase in the fluorescence intensity of TMB at 358 nm was apparent, attributing to the formation of the calix–potassium ion complex. It also indicated that the sensor has good
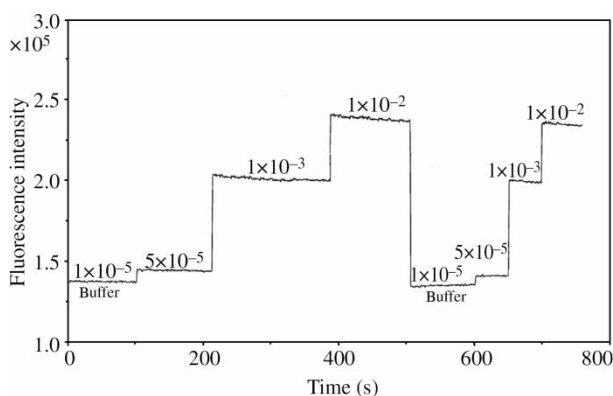
Figure 5. Membrane response of potassium-selective optode membrane M1 versus time at 358 nm after a concentration of K$^+$ change between (mol/l) (1) 1×10^{-5} and (2) 5×10^{-5}; (2) and (3) 1×10^{-3}; (3) and (4) 1×10^{-2}; (4) and (1) 1×10^{-5}; (1) and (2) 5×10^{-5}; (2) and (3) 1×10^{-3}; (3) and (4) 1×10^{-2}.

reversibility. The concentration of HCl solution can affect the reproducibility of the optode membrane. A higher concentration of HCl leads to a shorter recovery time because a higher concentration of H$^+$ speeds up the protonation of TMB. The reproducibility of the optical signals was evaluated by alternatively recording the fluorescence intensity when the membrane was exposed to repeated concentration step changes between 5×10^{-5} and 5×10^{-4} mol/l of the potassium ion in 0.05 mol/l buffer media. The mean fluorescence intensity values with RSD were found to be 3.62% (5×10^{-5} mol/l K$^+$, $n = 7$) and 2.45% (5×10^{-4} mol/l K$^+$, $n = 7$), respectively. The results indicate that the sensor has good reproducibility.

The response time ($t_{95\%}$) of the sensor depends on the thickness of the sensing membrane and concentration of the analyte. When the thickness of the membrane reaches the order of millimetres, no obvious response was observed; thus, it is necessary to prepare a very thin, homogeneous and reproducible PVC-based membrane. The changes of the membrane thickness in the range of 4 ± 0.2 μm caused no significant increase or decrease in the response time. Figure 5 shows the typical response of the optode membrane M1 to the potassium ion after equilibration with 0.05 mol/l HCl buffer solutions containing different concentrations of the potassium ion. It takes less than 1 min to reach the $t_{95\%}$ value after a concentration step change from 1×10^{-5} to 5×10^{-5} mol/l or from 5×10^{-5} to 1×10^{-3} mol/l of the potassium ion in 0.05 mol/l HCl buffer media.

3.4 Short-term stability and lifetime

To study the stability of the optode membrane, the response value of the membrane M1 in contact with 1×10^{-4} mol/l K$^+$ in 0.05 mol/l HCl buffer media was recorded over a period of 10 h. From the fluorescence intensity values taken every 30 min, a mean fluorescence value of 1.70×10^5 and an SD of 1×10^{-4} mol/l K$^+$ ($n = 21$, RSD = 4.62%) were obtained. Under the usual laboratory conditions, the decomposition was low, and this shows that the optode membrane has good short-term stability. The optode membrane is mainly subject to the leaching of active components from the membrane to the aqueous solution on which the lifetime of the membrane depends. The fluorescence intensity of the optode membrane M1 at $\lambda_{ex/em} = 300/358$ nm dropped by 8% during the measurement of sample solutions over a month. Apparently, the lifetime of the sensing membrane is acceptable for continuous analytical applications.

3.5 Selectivity

The unique cationic activity defined by the six phenoxyacetate groups of calix[6]arene hexaester matched

(15) Atwood, J.L., Davies, J.E.D., Mac Nicol, D.D., Vögtle, F., Lehn, J.M., Eds.; *Comprehensive Supramolecular Chemistry*; Pergamon Press: New York, 1996.

(16) Choi, M.M.F.; Wu, X.J.; Li, Y.R. *Anal. Chem.* **1999**, *71*, 1342–1349.

(17) Chan, W.H.; Lee, A.W.; Kwong, D.W.; Tam, W.L.; Wang, K.M. *Analyst* **1996**, *121* (4), 531–534.

(18) Kawabota, Y.; Mashiro, T.; Kitazaki, Y.; Imasaka, T. *Sens. Actuators B* **1995**, *29*, 135–139.

(19) Mathison, S.; Bakker, E. *Anal. Chem.* **1998**, *70*, 303–309.

(20) Morf, W.E.; Seiler, K.; Lehmann, B.; Behringer, C.; Hartmann, K.; Simon, W. *Pure Appl. Chem.* **1989**, *61*, 1613–1618.

(21) Seiler, K.; Simon, W. *Anal. Chim. Acta* **1992**, *266*, 73–87.

(22) Murkovic, I.; Lobnik, A.; Mohr, G.J.; Wolfbeis, O.S. *Anal. Chim. Acta* **1996**, *334*, 125–132.

(23) Bakker, E.; Lerchi, M.; Rosatzin, T.; Rusterholz, B.; Simon, W. *Anal. Chim. Acta* **1993**, *278*, 211–225.

(24) Harrison, T.R., Adams, R.D., Bennett, I.L., Resnik, W.H., Thorn, G.W., Wintrobe, M.M., Eds.; *Principles of Internal Medicine*, 5th ed.; McGraw-Hill: New York, 1966.

(25) Komaba, S.; Arakawa, J.; Seyama, M.; Osaka, T.; Satoh, I.; Nakamura, S. *Talanta* **1998**, *46*, 1293–1297.

(26) Chan, W.H.; Lee, A.W.M.; Kwong, D.W.J.; Liang, Y.Z.; Wang, K.M. *Analyst* **1997**, *122*, 657–661.

(27) Zhan, D.P.; Xiao, Y.J.; Yuan, Y.; He, Y.B.; Wu, B.L.; Shao, Y.H. *J. Electroanal. Chem.* **2003**, *553*, 43–48.

(28) Giannetto, M.; Mori, G.; Pappalardo, S.; Parisi, M.F. *Ann. Chim. (Rome)* **2002**, *92*, 1099–1107.

(29) Rosatzin, T.; Holy, P.; Seiler, K.; Rusterholz, B.; Simon, W. *Anal. Chem.* **1992**, *64*, 2029–2035.