Salicylate Ion-Selective Electrode Based on New Tetranuclear Copper Complexes of *O*-Vannlin-methionine as Neutral Carriers

Guangrong YE, Yaqin CHAI, Yuan RUO,[†] Lu ZHOU, Yan LI, and Lina ZHANG

Chongqing Key Laboratory of Analytical Chemistry, College of Chemistry and Chemical Engineering, Southwest University, Chongqing 400715, P. R. China

A new salicylate-selective PVC membrane electrode based on a new Schiff base tetranuclear copper complex of *O*-vannlin-methionine (Cu(II)₄-TVM) as a neutral carrier is described. This electrode displays a preferential potentiometric response to salicylate and an anti-Hofmeister selectivity sequence in the following order: $\text{Sal}^- > \text{ClO}_4^- > \text{SCN}^- > \text{I}^- > \text{NO}_2^- > \text{NO}_3^- > \text{Br}^- > \text{Cl}^- > \text{SO}_3^{2-} > \text{SO}_4^{2-} > \text{H}_2\text{PO}_4^-$. The electrode exhibits near-Nernstian potential linear range of 1.5×10^{-6} – 1.0×10^{-1} M with a detection limit of 8.0×10^{-7} M and a slope of -56.3 mV/decade in pH 3.0 – 8.0 of phosphate buffer solution at 20°C. Thanks to the tetranuclear copper(II) in the carrier, the electrode has the advantages of simplicity, fast response, fair stability and reproducibility and low detection limit. The response mechanism to the electrodes is discussed by the a.c. impedance technique and the UV spectroscopy technique. The electrode can be applied to analyses of medicine and the results obtained are in fair agreement with the results given by a standard method.

(Received August 23, 2006; Accepted October 10, 2006; Published February 10, 2007)

Introduction

The salicylate is a metabolite of aspirin, in addition, it can be used for sterilizing, and the sodium-salt of salicylic can be used for antisepticing,¹ therefore salicylate and its derivatives require suitable detection in clinical medicine and pharmaceutical analysis. So scientists have reported many methods for determination of salicylic ion in the literature, varying from fluorometry,² chromatography,^{3–5} and spectrophotometry^{6,7} to voltammetric procedures. Most of the methods are tedious and time-consuming. A simple and direct potentiometric method is needed.

The application of ion-selective electrodes is a typical kind of potential analytical method. The electrodes act as an indicator electrode. This is also an important branch of the electrochemical analysis and such mature chemical sensors have been applied very widely.^{8–10} The PVC membrane ion-selective electrode has many advantages such as simple construction, low cost, simple pretreatment and rapid online determination and so on. As a conventional detecting approach to a variety of cations and anions, this kind of electrode is already applied widely in the field of clinical medicine, environmental science, and industrial production and such like.^{11–13} Design, synthesis and application of neutral carriers of high selectivity and sensitivity are important research areas in the ion-selective electrode study fields. Thus research to find electrical active material that can recognize the special target ion with higher performance is needed. This material will be applied to construct the PVC membrane ion-selective electrode.¹⁴ Recently, studies on ion-selective electrodes with anti-Hofmeister selectivity series are an important and interesting project in the fields of electroanalytical chemistry and electrochemistry sensor.^{15,16}

Shortly after the researchers discovered that the Schiff base metallic complexes have the special selective to the specific anion,¹⁷ the study of this sort of electrode has become popular in this field. The scientists have studied mononuclear, and binuclear Schiff base complexes^{18–23} to construct all kinds of electrodes. Up to now, there are however few reports of research using more than binuclear complexes to prepare the electrode.

This paper explores a kind of new Schiff base named tetranuclear copper metallic complexes Cu(II)₄-TVM, as an ionophore of the ion-selective electrode. Such complexes contain tetranuclear copper(II) and exhibit high selectivity to salicylate in the intricate and real sample systems for determining salicylate in medicine, and the electrode has the advantages of simplicity, fast response, fair stability and reproducibility and low detection limit.

Experimental

Apparatus

All potentiometric and pH measurements were made with a pH meter (MP 230, Mettler-Toledo, Switzerland) and a digital ion analyzer (Model pHs-3C, Dazhong Instruments, Shanghai, China). UV-visible absorption spectra were obtained with a UV/Vis spectrophotometer (Lambda 17, Perkin-Elmer, USA). The AC impedance of the electrode membrane was recorded with an impedance measurement unit (IM6e, ZAHNER Elektrik, Germany) equipped with THALES software 6.88. Organometallic complexes synthesized were characterized by elemental analysis with a NHC elemental analyzer (D-6450, Heracus, Germany).

Reagents

PVC of high molecular weight, tridodecylmethylammonium chloride (TOMACl), dibutyl phthalate (DBP) and dioctyl

[†] To whom correspondence should be addressed.
E-mail: yuanruo@swu.edu.cn

Table 1 Composition of membrane phase (wt%) for salicylate-selective PVC membrane electrode

No.	Composition, %				Slope	Linear range
	PVC	Mediator	Additive	Ionophore		
1	32.0	<i>o</i> -NPOE 63.0	—	5.0	54.8	$1.0 \times 10^{-1} - 8.0 \times 10^{-6}$
2	32.0	DOP 63.0	—	5.0	50.2	$1.0 \times 10^{-1} - 3.0 \times 10^{-5}$
3	32.0	DBS 63.0	—	5.0	51.6	$1.0 \times 10^{-1} - 4.0 \times 10^{-5}$
4	32.0	DBP 63.0	—	5.0	46.5	$1.0 \times 10^{-1} - 6.0 \times 10^{-5}$
5	33.0	<i>o</i> -NPOE 64.0	TOMACl 3.0	0.0	—	—
6	32.0	<i>o</i> -NPOE 62.0	TOMACl 2.0	6.0	56.3	$1.0 \times 10^{-1} - 1.5 \times 10^{-6}$
7	33.0	<i>o</i> -NPOE 64.0	NaTPB 3.0	0.0	—	—
8	32.0	<i>o</i> -NPOE 62.0	NaTPB 1.0	5.0	43.3	$1.0 \times 10^{-1} - 1.0 \times 10^{-4}$
9	31.0	<i>o</i> -NPOE 62.0	TOMACl 3.5	3.5	57.1	$5.0 \times 10^{-2} - 9.0 \times 10^{-6}$
10	31.0	<i>o</i> -NPOE 60.0	TOMACl 1.0	8.0	49.8	$3.0 \times 10^{-2} - 7.5 \times 10^{-6}$

Fig. 1 The structure of tetranuclear copper complex of *O*-vannlin-methionine ($R = (CH_2)_2SCH_3$).

phthalate (DOP) were used as received from Shanghai Reagents, China. Potassium or sodium salts of all anions, tetrahydrofuran (THF) and all other chemicals were of highest purity available from Merck, and were used without further purification, except THF, which was distilled before use. The 2-nitrophenyloctylether (*o*-NPOE) was synthesized as described by Horning.²⁴ The ionophore of tetranuclear copper complex of *O*-vannlin-methionine ($Cu(II)_4$ -TVM) (Fig. 1) was synthesized by a literature method²⁵ and used after purification. All of the solutions were prepared using doubly distilled deionized water. The pH adjustments were made with a dilute nitric acid solution or dilute sodium hydroxide solution as required. A stock solution of salicylate was prepared by dissolving the appropriate amount of potassium salicylate in 100 mL of buffer. All of the working solutions were buffered at pH 5.0, using phosphate buffer solution. The observed elemental analysis of the complex was consistent with the theoretical data obtained on the basis of the following structure. Found (calc., %), $Cu(II)_4$ -TVM: C 40.90 (40.95), H 5.02 (4.97), N 3.59 (31.67).

Electrode preparation

The general procedure to prepare the PVC-membrane was to mix thoroughly 95 mg of powdered PVC, 186 mg of plasticizer *o*-NPOE and 4 mg of additive TOMACl in 5 mL of THF. Then to this solution was added 15 mg of ionophore $Cu(II)_4$ -TVM, and its mixture was obtained. A Pyrex tube (3–5 mm in diameter on top) was dipped into the oily mixture for about 10 s so that a semitransparent film of about 0.3 mm thickness was formed.^{26–30} The tube was then pulled out from the mixture and kept at the room temperature for about 24 h. The tube was then

filled with an internal filling solution (1.0×10^{-3} M KSal). The electrode was finally conditioned for 24 h by soaking in a 1.0×10^{-3} M solution of KSal. A silver-silver chloride electrode was used as an internal reference electrode. The ratios of different membrane ingredients were optimized to provide membranes, that resulted in reproducible, noiseless and stable potentials. The potential measurements were carried out at 25°C using saturated calomel electrodes (SCE) as reference electrodes with the following cell assembly:¹⁰ Ag/AgCl | 0.1 M KCl | electrode membrane | sample solution | Hg-Hg₂Cl₂, KCl (satd.).

Results and Discussion

Potential response of the electrodes based on $Cu(II)_4$ -TVM

The proposed membrane electrode based on $Cu(II)_4$ -TVM was found to be more responsive to than those based on the other anions tested, showing good potential response to salicylate ion. Under optimal conditions, the proposed electrode displays a preferential potentiometric response to salicylate and an anti-Hofmeister selectivity sequence in the following order: $Sal^- > ClO_4^- > SCN^- > I^- > NO_2^- > NO_3^- > Br^- > Cl^- > SO_3^{2-} > SO_4^{2-} > H_2PO_4^-$. The electrode exhibits near-Nernstian potential linear range of $1.5 \times 10^{-6} - 1.0 \times 10^{-1}$ M with a detection limit of 8.0×10^{-7} M and a slope of -56.3 mV/decade in pH 3.0–8.0 of phosphate buffer solution at 20°C.

Effect of membrane composition

It is well known that the sensitivity and selectivity depend significantly on its membrane composition and the properties of the solvent mediator employed as well as the plasticizer/PVC ratio used.^{10,31–33} Thus, different aspects of membrane preparation based on $Cu(II)_4$ -TVM for Sal[−] were optimized, the results are given in Table 1. Since in preparation of many PVC membrane-selective electrodes a plasticizer/PVC ratio of 2 will usually result in very suitable performance characteristics,²⁶ this ratio was kept at about 2 in optimization of the ingredients of the Sal[−] ion-selective electrodes proposed.

Since the nature of solvent mediators influences the dielectric constant of the membrane phase, the mobility of the ionophore molecules and the state of ligands,^{34,35} it was expected to play a key role in determining the ion selective characteristics. So various solvent mediators were added in varying amounts to the membranes and the ion-selective characteristics were studied. As seen from Table 1, among four different additives commonly used (electrodes 1–4), *o*-NPOE was found to give the best sensitivity and the widest linear range (Fig. 2). Also, the

Fig. 2 Potentiometric response of ionophore electrode for salicylate incorporating. a) *o*-NPOE and additive TOMACI and various plasticizers, b) *o*-NPOE, c) DOP, d) DBS, e) DBP.

optimization of selectivity of the membrane electrode is known to be highly dependent on the incorporation of an additional component, therefore, sodium tetraphenylborate (NaTPB) or tridodecylmethylammonium chloride (TOMACI) was incorporated into the membranes in order to investigate the mechanism of the membrane and to improve the response properties of the electrode (electrodes 5–8). As seen, the electrode 1 without any ionic additives already exhibits a near-Nernstian response slope towards Sal^- ion. The response of the blank membranes containing additives without ionophore (electrodes 5 and 7) was far from Nernstian. Electrode 6 in the presence of TOMACI as a cationic additive results in the best potentiometric response characteristics with considerably improved selectivity. Significantly deteriorating potentiometric response on electrode 8, especially with decreased selectivity, is observed, during the addition of anion additive NaTPB (Table 1). Moreover, some 6.0% of ionophore $\text{Cu(II)}_4\text{-TVM}$ was chosen as the optimum amount of ionophore in the PVC membrane (electrode 6). Of the various membranes prepared using $\text{Cu(II)}_4\text{-TVM}$ as the ion-active phase, the one with a PVC:*o*-NPOE: $\text{Cu(II)}_4\text{-TVM}$:TOMACI percent ratio of 32.0:62.0:6.0:2.0 exhibited the best working concentration range of 1.0×10^{-1} to 1.5×10^{-6} M with a slope of -56.3 mV/decade of activity (Fig. 2). The limit of detection for this membrane calculated as recommended by IUPAC^{36,37} from the intersection of the two extrapolated segments of the calibration curve was 8.0×10^{-7} M. The static response time of the membrane electrode was found to be less than 15 s for the concentrations of Sal^- ion $< 1.0 \times 10^{-3}$ M.

pH effect on electrode response

The effect of pH of the test solution on the response of the membrane electrodes was examined at two salicylate concentrations. As illustrated in Fig. 3 for 1.0×10^{-4} , 1.0×10^{-2} and 0 M of salicylate, the potentials remain constant from pH of about 3.0–8.0. The pH dependence of the ISE response in the absence of salicylate is also interesting; this proposed electrode has a similar response in that the potentials remain constant from pH of about 3.0–8.0 in suffer solution without any salicylate as shown in Fig. 3. The results show that the sensor is suitable for salicylate determination over a wide pH range of 3.0–8.0. In high pH media, probably OH^- will compete with salicylate ion, as where in acidic media ($\text{pH} < 3.0$), the drift in the potential may be due to the instability of the ionophore

Fig. 3 Effect of pH of test solution on the response of the proposed electrode 1.0×10^{-2} M Sal^- ion (a), 1.0×10^{-4} M Sal^- ion (b) and 0 M Sal^- ion (c).

(complex of the copper with the ligand) due to the oxygen sites' protonation. The working pH range of the electrode can be used (3.0–8.0) near the physiological conditions (pH 7.2–7.6). A pH of 5.0 was then chosen for further experiments.

Selectivity

The selectivity behavior is one of the most important characteristics of a sensor, because it determines the sensor's response for primary ion in the presence of foreign ions. This parameter is measured in terms of potentiometric selectivity coefficient ($\log K_{\text{Sal}^-, \text{jn}}^{\text{pot}}$). In order to determine the selectivity coefficient of the proposed electrode, researchers have evaluated $\log K_{\text{Sal}^-, \text{jn}}^{\text{pot}}$ using suitable solution methods (SSM).^{36,37} In this method, a_A (1.0×10^{-2} M Sal^-) and a_B (1.0×10^{-2} M interfering ion) were taken to measure the selectivity coefficients in the present studies. The near Nernstian slope is -56.3 mV per decade.

The response characteristics of the electrode were improved by addition of a lipophilic salt such as TOMACI to the membrane, and this not only reduced the membrane resistance but also enhanced the response behavior and selectivity and reduced interferences from lipophilic sample anions.^{38,39} Recently, several reports^{36–40} focused on the effects of lipophilic ionic additives on the selectivity of the membrane electrodes, so the addition of ionic sites is highly advisable. The presence of lipophilic ionic sites keeps the total concentration of measuring ion in the membrane phase constant, and can be used to adjust membrane selectivity. The preferential response toward salicylate is believed to be associated with the coordination of salicylate with central copper ion of the carrier and the lipophilicity of the anion. It is well-known that the sensitivity and selectivity obtained for a given ionophore depend significantly on the membrane condition.^{41,42}

In this work, the effect of the lipophilic salts on the selectivity of the $\text{Cu(II)}_4\text{-TVM}$ -doped membrane was studied. The composition of the membrane is shown in Table 1 and its selectivity coefficients are shown in Table 2. For electrode 6, the selectivity coefficient over other anions, especially Sal^- , ClO_4^- , and I^- , is significantly improved in the presence of TOMACI as a cationic additive. The selectivity sequence of the electrode was found to be: $\text{Sal}^- > \text{ClO}_4^- > \text{SCN}^- > \text{I}^- > \text{NO}_2^- > \text{NO}_3^- > \text{Br}^- > \text{Cl}^- > \text{SO}_3^{2-} > \text{SO}_4^{2-} > \text{H}_2\text{PO}_4^-$. On the other hand, the selectivity of electrode 8 was decreased during the addition of anion additive NaTPB, especially over Sal^- , ClO_4^- , or I^- . According to the theoretical models that predict the effect of

Table 2 Selectivity coefficients $\log K^{\text{pot}}_{\text{Sal}^-, \text{jc}^-}$ of $\text{Cu(II)}_4\text{-TVM}$ -based electrodes and similar electrodes

Electrode	$\log K^{\text{pot}}_{\text{Sal}^-, \text{jc}^-}$										
	Sal^-	ClO_4^-	SCN^-	I^-	NO_3^-	NO_2^-	Br^-	Cl^-	SO_3^{2-}	SO_4^{2-}	H_2PO_4^-
Electrode 1	0	-0.56	-0.80	-1.08	-1.90	-2.72	-3.04	-3.71	-3.82	-4.13	-4.45
Electrode 5	0	-1.31	-1.62	-2.42	-3.22	-3.64	-3.75	-4.12	-3.83	-4.42	-4.90
Electrode ^a	0	-0.73	-1.03	-1.31	-2.48	-2.86	-2.87	-2.93	-3.40	-3.73	-4.1
336-Sal ^b	0	2.24	1.04	0.62	-1.82	-0.95	-1.17	-1.96	-3.96	-2.80	—

a. Cited from Ref. 44. b. Cited from Ref. 45.

Table 3 Stability and reproducibility of the salicylate-selective electrode ($n = 5$)

Time/ day	Slope/ mV decade^{-1}	Linear range/ M	Correlation of coefficient
1	-56.3	$1.5 \times 10^{-6} - 1.0 \times 10^{-1}$	0.998
5	-55.8	$1.5 \times 10^{-6} - 1.0 \times 10^{-1}$	0.998
10	-54.6	$4.0 \times 10^{-6} - 1.0 \times 10^{-1}$	0.997
20	-54.0	$5.0 \times 10^{-6} - 1.0 \times 10^{-1}$	0.996
40	-52.0	$5.0 \times 10^{-6} - 1.0 \times 10^{-1}$	0.996
55	-51.0	$8.0 \times 10^{-6} - 1.0 \times 10^{-1}$	0.994
75	-49.5	$8.0 \times 10^{-6} - 1.0 \times 10^{-1}$	0.994
90	-48.5	$1.0 \times 10^{-5} - 1.0 \times 10^{-1}$	0.991
100	-48.1	$1.0 \times 10^{-5} - 1.0 \times 10^{-1}$	0.988

lipophilic ionic additives on the selectivity of the membrane electrodes,⁴³ the result clearly shows that the electrode, compared with those in the literature,^{44,45} displays a highly selective response to salicylate.

The stability and reproducibility

The stability and reproducibility of the electrodes were also tested. The standard deviations of 10 replicate measurements for PVC sensor at 1.0×10^{-2} , 1.0×10^{-3} and 1.0×10^{-4} M salicylate were ± 0.4 , ± 0.5 and ± 0.8 mV, respectively. The stability over a long term of the electrode was studied by periodically recalibrating in standard solutions and calculating the response slope over the range of $1.5 \times 10^{-6} - 1.0 \times 10^{-1}$ M. The electrode was very stable and could be used over a period of at least 3 months. The slopes of the electrode response were reproducible to within 8.2 mV/decade over a period of 3 months (Table 3). The lifetime of solvent polymeric ion-selective electrodes is limited by leaching of the membrane components into the sample solutions. Because ionic sites of high lipophilicity are available, the loss of ionic sites is, in general, not a concern.

Mechanism to thiocyanate response

The electrode based on $\text{Cu(II)}_4\text{-TVM}$ exhibited anti-Hofmeister selective behavior and highly selective response to salicylate mainly due to the strong interactions between the central Cu(II) atom in complexes and Sal^- . It is possible to study the interaction between the central metal of carriers and Sal^- with UV-visible spectroscopy. The UV-vis spectra of the ionophore with and without interacting with Sal^- are shown in Fig. 4. The band maxima of $\text{Cu(II)}_4\text{-TVM}$ complexes correspond to metal-to-ligand charge transfer and $\Pi\text{-}\Pi^*$ transitions within the ligand molecule.⁴⁶ The considerable shift in spectrum in the presence of Sal^- is due to coordination of the central metal with the analyte.

The AC impedance spectra of the electrode based on $\text{Cu(II)}_4\text{-TVM}$

Fig. 4 UV absorption spectra of chloroform solution of $\text{Cu(II)}_4\text{-TVM}$ (—) and $\text{Cu(II)}_4\text{-TVM}$ treated with 0.1 M Sal^- solution (---). Fig. 5 Impedance plots for the membranes containing a $\text{Cu(II)}_4\text{-TVM}$ carrier. $C_{\text{Sal}^-} = 1.0 \times 10^{-1}$, 1.0×10^{-3} , $1.0 \times 10^{-5} \text{ M}$ (from inner to outer).

TVM at various concentrations of Sal^- in pH 5.0 phosphonate buffer solution were recorded after conditioning in sodium salicylate solution for 4 h. The working electrode was an Ag/AgCl foil with 0.5 cm^2 area. A saturated calomel electrode (SCE) and a Pt foil with 0.5 cm^2 area were used as reference electrode and counter electrode, respectively. The frequency range used was $10^{-2} - 10^6 \text{ Hz}$ and the AC amplitude was 25 mV at 20°C . Well-defined semicircle bulk impedance at high frequencies and warburg impedance at low frequency interval were observed (Fig. 5), and the bulk resistance decreased with increasing the concentration of Sal^- . The results indicated that the transfer process of salicylate across the PVC membrane was controlled by the diffusion process.⁴⁷

Preliminary applications

The proposed electrode here reveals fairly good response characteristics for Sal^- ion. The practical utilization of the

Table 4 Determination of aspirin in pharmaceuticals

Tablet	Potentiometry, w% ($n = 5$)	Titrimetry, ^a w%
Aspirin	86.68 $\pm$ 0.30	88.25 $\pm$ 0.25
APC	71.08 $\pm$ 0.32	71.40 $\pm$ 0.28

a. Ref. 48.

Table 5 Comparison of results of electrode and spectrophotometric methods for the determination of salicylate concentration (mM) in urine samples

Method	Measurement No.				
	1	2	3	4	5
Electrode	0.68	0.85	0.78	1.21	0.75
Spectrophotometry ^a	0.64	0.86	0.76	1.24	0.79

a. Ref. 49.

Cu(II)₄-TVM electrode has been demonstrated through the analytical determination of salicylate in human urine and in drugs. Samples were diluted 1:10 and adjusted to pH 5.0 with H₃PO₄ and NaOH solutions. The calculated results of ionic strength of samples are 0.006, 0.004 and 0.002 M in Aspirin, APC and urine samples, respectively. A titration method and spectrophotometry for the assay of salicylate were used as the reference methods.^{48,49} The results obtained by the proposed electrode agreed fairly well with those obtained by traditional titration methods without any prior treatment of the samples, thereby reflecting the utility of the proposed electrode. The results are shown in Tables 4 and 5.

Conclusions

The results obtained from the above mentioned study reveal that a potentiometric PVC-based membrane sensor based on Cu(II)₄-TVM functions as an excellent Sal⁻ selective sensor. One can determine this ion in the presence of considerable concentrations of common interfering ions. The electrode was very easy to prepare and showed high sensitivity and selectivity, a wide range, a low detection limit, and very fast response time. The electrode could be applied to determination of content of Sal⁻ in human urine and in drugs without need for preconcentration or pretreatment steps.

Acknowledgements

Financial support of this work was provided by the National Natural Science Foundation of China (20675064) and the Natural Science Foundation of Chongqing City, China (CSTC-2004BB4149, 2005BB4100) and High Technology Project Foundation of Southwest University (XSGX 02).

References

1. A. Relman, *N. Engl. J. Med.*, **1988**, 318, 245.
2. S. Adams and J. Miller, *J. Pharmacol.*, **1978**, 30, 81.
3. A. M. Molokhia, H. L. A. L. Shora, and S. A. L. Rhaman, *J. Liquid Chromatogr.*, **1985**, 820, 93.
4. M. Carlson and R. D. Thompson, *J. Liquid Chromatogr.*, **1987**, 10, 997.
5. T. Dadgar, J. Climax, R. Lambe, and A. T. Darragh, *J. Chromatogr.*, **1985**, 315, 324.
6. U. Saha and K. Baksi, *Analyst*, **1985**, 110, 739.
7. P. Trinder, *Biochem. J.*, **1954**, 301, 57.
8. K. Kavallieratos, A. Danby, G. J. Van Berkel, M. A. Kelly, R. A. Sachleben, B. A. Moyer, and K. Bowman-James, *Anal. Chem.*, **2000**, 72, 5258.
9. Q. Qian, G. S. Wilson, K. Bowman-James, H. Girault, *Anal. Chem.*, **2001**, 73, 497.
10. M. Shamsipur, T. Poursaberi, M. Rezapour, M. R. Gangali, M. F. Mousavi, V. Lippolis, and D. R. Montesu, *Electroanalysis*, **2004**, 16, 1336.
11. M. M. Ardakani, M. S. Jalayer, J. Safari, Z. Sadeghi, and H. R. Zare, *Anal. Biochem.*, **2005**, 341, 259.
12. S. S. M. Hassan, M. H. AbouGhalia, A. G. E. Amr, and A. H. K. Mohamed, *Anal. Chim. Acta*, **2003**, 482, 9.
13. F. Sancenon, A. Benito, J. M. Loris, R. Marnez-Manez, T. Pardo, and J. Soto, *Helv. Chim. Acta*, **2002**, 85, 1505.
14. E. Bakker, P. Bühlmann, and E. Pretsch, *Chem. Rev.*, **1997**, 97, 3083.
15. E. Bakker, P. Bühlmann, and E. Pretsch, *Electroanalysis*, **1999**, 11, 915.
16. R. P. Buck and E. Lindner, *Anal. Chem.*, **2001**, 73, 88.
17. R. Yuan, Y. Q. Chai, D. Liu, D. Gao, J. Z. Li, and R. Q. Li, *Anal. Chem.*, **1993**, 65, 2572.
18. A. P. Thoma, A. Viviani-Nauer, S. Arvanitis, W. E. Morf, and W. Simon, *Anal. Chem.*, **1977**, 49, 1567.
19. A. B. Descalzo, D. Jimenez, M. D. Marcos, R. Martinez-Manez, J. Soto, L. E. Haskouri, C. Guillem, D. Beltran, P. Amoros, and M. V. Borrachero, *Adv. Mater.*, **2002**, 14, 966.
20. R. Yuan, X. L. Wang, L. Xu, Y. Q. Chai, Z. Y. Sun, X. Q. Huang, Q. F. Li, Q. Zhao, and L. Zhou, *Electrochem. Commun.*, **2003**, 5, 717.
21. Y. Umezawa, M. Kataoka, W. Takami, E. Kimura, T. Koike, and H. Nade, *Anal. Chem.*, **1988**, 60, 2392.
22. M. Kataoka, R. Naganawa, K. Odashima, Y. Umezawa, E. Kimura, and T. Koike, *Anal. Lett.*, **1989**, 22, 1089.
23. R. Naganawa, H. Radecka, M. Kataoka, K. Tohda, K. Odashima, Y. Umezawa, E. Kimura, and T. Koike, *Electroanalysis*, **1993**, 5, 731.
24. E. C. Horning, "Org. Synth. Coll.", ed. W. John, **1955**, Vol. III, Academic Press, New York, 140.
25. Y. H. Qiao, M. Yu, M. Sun, and F. M. Liao, *Chin. J. Appl. Chem.*, **2001**, 20, 192.
26. M. M. Ardakani, M. S. Jalayer, J. Safari, Z. Sadeghi, and H. R. Zare, *Anal. Biochem.*, **2005**, 341, 259.
27. M. K. Amini and S. Shahrokhian, *Anal. Chim. Acta*, **1999**, 402, 137.
28. P. C. Hauser, *Anal. Chim. Acta*, **1993**, 278, 227.
29. S. Nomura, *Analyst*, **1995**, 120, 503.
30. M. K. Amini and S. Shahrokhian, *Anal. Chem.*, **1999**, 71, 2502.
31. N. A. Chaniotakis, S. B. Park, and M. E. Meyerhoff, *Anal. Chem.*, **1989**, 61, 566.
32. E. Bakker, E. Maliowska, K. D. Schiller, and M. E. Meyerhoff, *Talanta*, **1994**, 41, 881.
33. J. J. Li, X. J. Pang, D. Gao, and R. Q. Yu, *Talanta*, **1995**, 42, 1775.
34. D. Midgley, *Anal. Chim. Acta*, **1986**, 182, 91.
35. R. S. Hutchins and L. G. Bachas, *Anal. Chem.*, **1995**, 67, 1654.
36. P. Bühlmann, E. Pretsch, and E. Bakker, *Chem. Rev.*, **1998**,

- 98, 1593.
37. G. R. Ye, Y. Q. Chai, R. Yuan, and J. Y. Dai, *Anal. Sci.*, **2006**, 22, 579.
38. M. Huser, P. M. Gehrig, W. E. Morf, W. Simon, C. Lindner, J. Jeney, K. Toth, and E. Pungor, *Anal. Chem.*, **1991**, 63, 1380.
39. S. Kamata, A. Bhale, Y. Fukunaga, and H. Murata, *Anal. Chem.*, **1988**, 60, 2464.
40. S. Amemiya, P. Bühlmann E. Pretsch, B. Rusterholz, and Y. Umezawa, *Anal. Chem.*, **2000**, 72, 1618.
41. T. Katsu and Y. Mori, *Talanta*, **1996**, 43, 755.
42. N. N. Leyzerovich, N. V. Shvedene, Y. N. Bilkova, L. G. Tomilova, and I. V. Pletnev, *Electroanalysis*, **2000**, 3, 246.
43. Y. Umezawa, K. Umezawa, and H. Sato, *Pure Appl. Chem.*, **1995**, 67, 507.
44. Y. Q. Chai, A. L. Sun, R. Yuan, J. Y. Dai, L. Xu, and G. F. Gui, *Anal. Lett.*, **2005**, 38, 45.
45. D. Liu, G. L. Shen, and R. Q. Yu, *Analyst*, **1996**, 121, 1495.
46. A. Abbaspour, M. A. Kamyabi, A. R. Esmailbeig, and R. Kia, *Talanta*, **2002**, 57, 859.
47. L. Xu, R. Yuan, Y. Q. Chai, and X. L. Wang, *Anal. Bioanal. Chem.*, **2005**, 381, 781.
48. Pharmacopeia Committee of the Ministry of Health of China, "*Pharmacopeia in China*", **2000**, Beijing Chemistry Industry Press, 80.
49. T. G. Whiston and G. W. Cherry, *Analyst*, **1962**, 87, 819.
-