

The Potentiometric Performance of Mercury (II) Ion-Selective Electrode Based on Tetracycline Antibiotics

Jonggyu Baek[†] and Insook Rhee Paeng^{*}

Department of Chemistry, Seoul Women's University, Seoul 139-774

[†]Department of Chemistry, Yonsei University, wonju 220-710

(Received January 28, 2008 : Accepted February 5, 2008)

Abstract : Poly (vinylchloride) (PVC) membrane electrodes based on neutral carrier, tetracycline was prepared as an active sensor for Hg(II) ion, and tested in different contents of the potassium tetrakis (4-chlorophenyl) borate (KTp-CIPB) as lipophilic salt. Bis (2-ethylhexyl) sebacate (DOS), bis(1-butylpentyl) adipate (BBPA), 2-nitrophenyl octyl ether (NPOE) and dibutyl phthalate (DBP) were used as diverse plasticizing solvent mediators. This electrode shows excellent potentiometric response characteristics and display good linearity with $\log [Hg^{2+}]$ versus EMF response, over a range of concentrations between 10^{-7} and 10^{-3} M. With 30.8 mV/decade Nernstian slope, the detection limit was 6.9×10^{-9} M and the response time was less than 20s. The proposed electrode yields very good selectivity for mercury (II) ion over many cations such as alkali, alkaline earth, transition and heavy metal ions. And it shows a very stable potential values in a wide pH range. This reliable electrode prepared was kept at least a month without considerable alteration in their response to Hg (II) ion.

Keywords : Tetracycline, Antibiotics, Mercury (II) ion, Ion-selective electrode.

1. Introduction

Mercury (Hg) ion is a toxic heavy metal ion for human body. Especially, it causes harmful effects on human nervous system even though the incident exposure of Hg. Thus, many methods for the detection of Hg(II) ion have been developed. Among them, the ion-selective electrode (ISE) has been known as a sensitive, fast, reproducible and accurate analytical method. The carrier based ISEs are one of the most important groups of chemical sensors. And a PVC-based membrane electrode is a typical good example, which can be easily prepared by small amount of electro-active compounds, (ionophore and lipophilic salts) into solvent polymeric matrix and plasticizer.^{1,2)} Each has the role for developing successful sensors and the selectivity is mostly relied on the molecular recognition ability of ionophores.

The first neutral carrier-based Hg(II)-ISE was developed in 1986 by Lai et al.³⁾ based on 1, 4-dithia-12-crown-4 ionophore. Crown ether series^{4,5)} or neutral carrier^{6,7)} have been reported in the literature. However, it still has had an effort to find better Hg(II) ion selective ionophores because developed Hg (II)-PVC based membrane electrodes had limitation such as poor response time, long equilibration time, short life time, narrow range of working pH and interferences from various metal ions, especially Ag(I) and/or Fe(III) ion.

Several groups showed that metal binding antibiotics is very attractive choice as cation selective ionophores.⁸⁻¹⁰⁾ Stability constants of metal complexes with tetracycline (TC: Fig. 1)

were reported at 1950s by Albert and coworkers.^{11,12)} The ability of TC antibiotics binding to metal ions has obtained much attention since then. And thus, TCs could be very good candidate as an excellent ionophores for Mg(II) and Ca(II) ion, because TCs have very good affinity toward aforementioned cations. Recently, we reported the TC based membrane electrode formulated with common PVC composite exhibited potentiometric responses to both cations as employing appropriate pasticizers.¹³⁾ Especially, TC based membrane electrode shows better responses to Hg(II) ions.

Here, we study to develop and optimize a new Hg(II) ISE based on TC antibiotics as a neutral carrier. Also, we propose the interactions between Hg(II) ions with TC antibiotics according to UV-Vis and ¹H NMR spectroscopy.

2. Experimental

2.1. Reagents and Apparatus

Tetracycline (TC) was purchased from Sigma (Saint Louis, Mo, USA). Poly(vinyl chloride) (PVC), potassium tetrakis(4-chlorophenylborate) (KTpCIPB) and plasticizers such as bis (2-ethylhexyl) sebacate (DOS), 2-nitrophenyl octyl ether (NPOE), dibutyl phthalate (DBP) and bis(1-butylpentyl) adipate (BBPA) were supplied from Fluka (Ronkonkoma, NY, USA). Solvents and all other chemicals including nitrate salts of cations examined were analytical reagent grade. Solutions were prepared using de-ionized water. (Millipore, Billerica, MA, USA)

*E-mail: irpaeng@swu.ac.kr

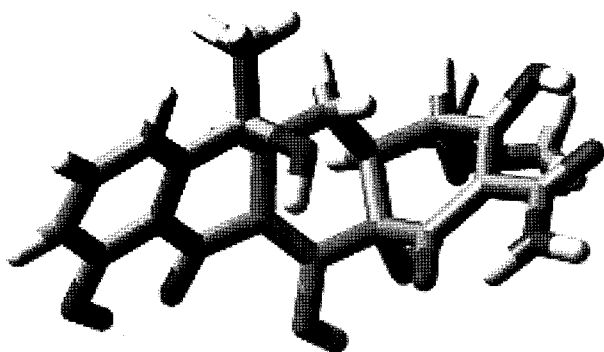
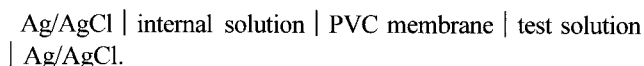


Fig. 1. Spatial structure of tetracycline.

2.2. Preparation and evaluation of polymer membranes and electrodes

PVC matrix membranes were prepared according to the method reported previously.¹⁴⁾ The ISE membrane was prepared by mixing 1 wt% of TC, 33 wt% PVC and 66 wt% plasticizer, resulting in 200.0 mg dissolved in 1 mL THF. The solution was cured to produce an elastic membrane. A small disk of 5.5 mm diameter was cut out from the PVC membrane and then mounted into the end of electrode body (IS-561; Glasblaserei Möller, Zürich, Switzerland).

The potentiometric behavior of the membrane electrode was evaluated with conventional ISE configuration. For all electrodes, a solution of 10^{-3} M $\text{Hg}(\text{NO}_3)_2$ was employed as the internal filling solution. The external reference electrode was an Orion sleeve-type double junction $\text{Ag}/\text{AgCl}/\text{KCl}$ (saturated) electrode (Model 90-02). The electrochemical cell for the electromotive force (EMF) measurement is as follows:



Selectivity coefficients were studied according to the separate solution method at an interfering cation concentration of 1.0 mM.¹⁵⁾ The response to pH was investigated by titrating an universal buffer of pH 2.8 (11.4 mM boric acid; 6.7 mM citric acid; 10.0 mM sodium dihydrogen phosphate) with small aliquots of LiOH and simultaneously monitoring the pH of the sample solution with a combination glass pH electrode.

2.3. Spectroscopic measurements

UV-Vis spectra were obtained using Perkin Elmer UV-visible spectrophotometer (LX20-000B). ^1H NMR spectra were obtained using a Bruker spectrometer working at 400 MHz for proton in $\text{DMSO}-d_6$ solvent. The concentration of TC was 10.0 mM.

3. Results and Discussion

3.1. Spectroscopic evaluation

The spectroscopic technique has been used to assess the strength of ion-carriers. UV-visible spectrophotometric studies have been carried out to investigate the interaction between ionophore (TC) with $\text{Hg}(\text{II})$ ion and it revealed that TC forms stable complex with $\text{Hg}(\text{II})$ ion. Fig. 2 shows the absorption

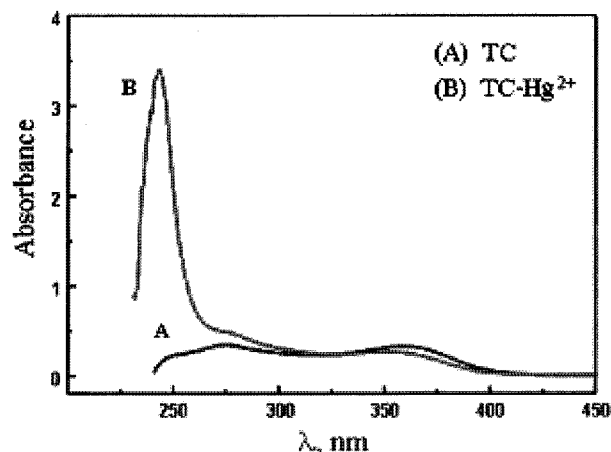


Fig. 2. UV-visible spectra of tetracycline (A) and with $\text{Hg}(\text{II})$ ion (B).

spectra of TC (10.0 mM) and its complex with $\text{Hg}(\text{II})$ ion (10.0 mM) in $\text{H}_2\text{O} : \text{DMSO}$ (9 : 1) solution. $\text{Hg}(\text{II})$ itself possesses negligible absorption between 200 - 400 nm spectral regions and TC shows two small absorption bands at 275.0 and 362.0 nm. However, a distinct and very strong band at 255 nm was observed when $\text{Hg}(\text{II})$ ion-containing solution was added to the TC. It means that the preferred coordination was formed between the target cation, $\text{Hg}(\text{II})$ and the carrier, TC.

The binding ratio of the complex, TC (host, H) and $\text{Hg}(\text{II})$ ion (guest, G), was determined by the Job's plot,¹⁶⁾ in which stoichiometric composition of the complex was decided as the ratio of complex formation at a maximum spectroscopic result. Fig. 3 shows the Job's plot and the maximum value of $[\text{HG}]$ complex was obtained when $[\text{H}]/([\text{H}]+[\text{G}]) = 0.5$. This suggests that this complex was formed as an associate of 1 : 1 composition.

3.2. Binding Model

The sites of possible chelating terminals at TC has been known as (a) C10-C11 ketophenol,¹⁷⁾ (b) C11-C12-diketone,¹⁸⁻²⁰⁾ (c) C4 dimethylamine and C3 or C12a hydroxyl,²¹⁾ (d) C11-C3 tricarbonyl methane,^{18,22)} and (e) multidentate combination of C11-C12-diketone and C1-C3 tricarbonyl methane achieved through folding the molecule along the C4a-C12a

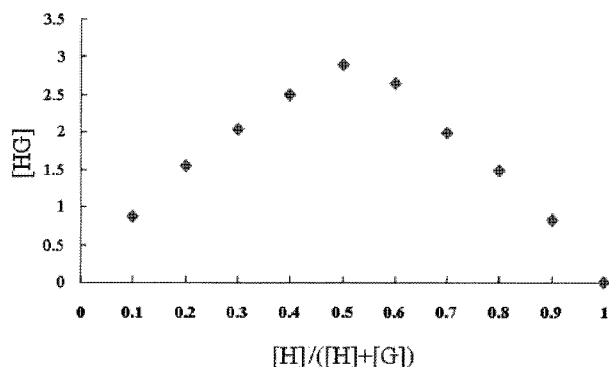


Fig. 3. Job's plot for complex of tetracycline (host: H) with $\text{Hg}(\text{II})$ ion (guest: G).

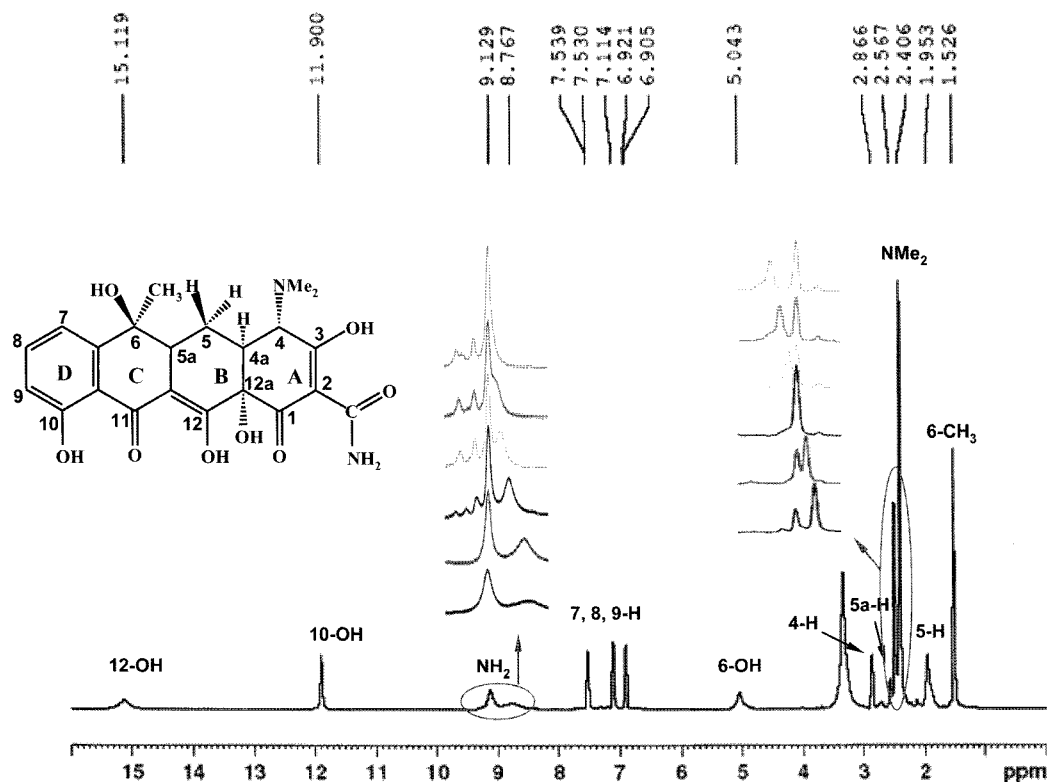


Fig. 4. ¹H NMR (400MHz at T=296K) study of tetracycline adding Hg(II) ion in DMSO-d₆. The colored traces represented 0.0, 1.0, 2.0, 3.0, 4.0 and 4.5 eq of Hg(II) ion, respectively, to TC from bottom to top.

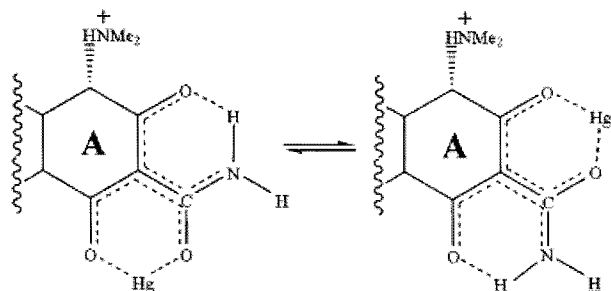


Fig. 5. A proposed binding structure of Hg(II) ion with tricarbonylmethane functional group of the ring A in tetracycline.

axis.²³⁾ Fig. 4 shows the ¹H NMR study of TC having 0.0 eq to 4.5 eq of Hg(II) in DMSO-d₆. The colored traces in Fig. 4 represented 0.0, 1.0, 2.0, 3.0, 4.0 and 4.5 eq of Hg(II) ion from bottom to top, respectively, to TC. With increasing of Hg(II) ion, significant downfield shifts occurred for protons in NMe₂ and amide, and proton in amide is split to several peaks. In this ¹H NMR results, we could suggest that the binding model TC with Hg(II) ions in membrane is mainly a tricarbonylmethane function of ring A in TC. From those result, a binding structure of Hg(II) ion with TC is proposed in Fig. 5.

3.3. Composition of membranes and characteristics of the Hg(II) ISE

Hg(II) ISE prepared with different composition of membranes was evaluated. Table 1 listed those compositions and

summary of the potentiometric performances of them. PVC-based membrane electrode is prepared by small amount of ionophores and lipophilic salts into solvent polymeric matrix and plasticizer. Each components have the role for developing successful sensors. And thus, the potentiometric response slope and selectivity coefficient of the Hg(II) ion depend on the compositions of the membrane components. The effects of lipophilic additive, (KTpCIPB) and plasticizers (DOS, NPOE, DBP and BBPA) in the Hg(II) ISE were investigated for the optimization of the potentiometric response of the sensor. E1, PVC membrane containing ionophore, DOS and no lipophilic additive shows a slope of 17.8 mV/decade and a narrow linear range of 10⁻⁶~ 10⁻⁴ M. Addition of 50 mol % of KTpCIPB to membrane composition, E2, increases response slope to 30.8 mV/decade which is Nernstian slope. With 100 mol% of KTpCIPB, E3, it shows super-Nernstian slope of 34.6 mV/decade. In the solution study as shown in this section 3.1, TC shows one to one complex with Hg(II) ion. However, TC could yields multi-dentate performance with increasing mobility of Hg(II) ion in membrane due to ionic site created by 100 mol% of lipophilic additives. Additionally, we can not exclude the possibility of an adduct of Hg(II) ion with anionic site in membrane to become [Hg(anion)](I) ion. It may cause the super-Nernstian slope. Further studies are needed to obtain the decisive conclusion.

It is well known that the type of plasticizer also influences the dielectric constant of the membrane phase and the state of the ligands.²⁴⁾ Four PVC matrix membranes containing the plasticizer DOS (ε = 4.0), BBPA (ε = 4.0), DBP (ε = 6.44)

Table 1. Composition of membranes and their potentiometric response properties of Hg(II) ISE

# of electrode	Plasticizer	Lipophilic additive (KTpCIPB : mol %)	Slope (mV/decade)	Linear range (M)	Detection limit (log [M])
E1	DOS	0	17.8	$10^{-6} \sim 10^{-4}$	6.13
E2	DOS	50	30.8	$10^{-7} \sim 10^{-3}$	8.16
E3	DOS	100	34.6	$10^{-6} \sim 10^{-3}$	6.20
E4	NPOE	50	44.1	$10^{-6} \sim 10^{-3}$	7.37
E5	DBP	50	37.7	$10^{-6} \sim 10^{-3}$	7.29
E6	BBPA	50	33.4	$10^{-6} \sim 10^{-3}$	7.10

Table 2. The potentiometric responses of Hg(II) ISEs

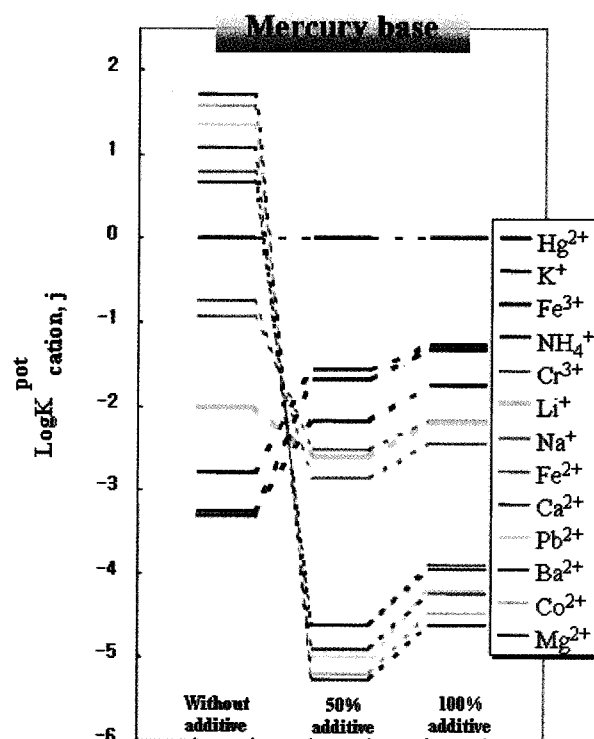
Ionophore	Response time (s)	Linear range (M)	$\log K_{\text{Hg}^{2+}, J}^{\text{Pot}}$			Ref.
			Fe ³⁺	Mg ²⁺	Ag ⁺	
1,4-Dithia-12-crown-4	60	$1.0 \times 10^{-6} \sim 1.0 \times 10^{-2}$	1.5	2.9	3.6	3
Pentathia 15-crown-5	20	$2.5 \times 10^{-5} \sim 1.0 \times 10^{-1}$	3.4	-	0.0	4
1-(2-Hydroxy-1,2-diphenylethylidene)-thiosemicarbazide	-	$2.0 \times 10^{-6} \sim 1.0 \times 10^{-2}$	3.46	3.66	0.8	6
<i>N,N</i> -Dimethylformamide-salicylacylhydrazone	< 30	$6.2 \times 10^{-7} \sim 8.0 \times 10^{-2}$	2.88	3.08	0.54	7
Tetracycline	< 20	$1.0 \times 10^{-7} \sim 1.0 \times 10^{-3}$	1.69	5.27	2.05	This work

and NPOE ($\epsilon = 23.1$) were prepared separately and showed the dependence of the characteristic responses on the membrane composition in E2, E4, E5 and E6, respectively. The data revealed that the E2 membrane electrode with DOS and 50 mol% lipophilic additive, exhibited the best response slope of 30.8 mV/decade and low detection limit (6.9×10^{-9} M) for Hg(II) ions among the electrodes tested in this experiment. The result for E2 was comparable or better to membrane electrodes based on Hg(II) selective ionophores published previously. (Table 2).

3.4. Potentiometric Selectivity Coefficients

The selectivity is obviously one of the most important characteristics of a sensor. They measured changes of potential in the presence of the target and the interfering ions together. In most cases, the ligand structure and cation size, complex formation or extractability is the major determining factor for selectivity.²⁵⁾

The selectivity coefficients of the sensor towards different cationic species were evaluated by using separate solution method (SSM). The properties of ISE based on neutral ionophores are more influenced by the ionic sites in membranes. As seen in Fig. 6, selectivity coefficient of membrane with different amount of ionic sites (KTpCIPB) was compared with other interfering ions. The inference of alkali metal ions (Li⁺, Na⁺ and K⁺), alkaline earth metal ions (Mg²⁺, Ca²⁺ and Ba²⁺), transition metal ions (Fe²⁺, Pb²⁺, Fe³⁺, Cr³⁺ and Co²⁺) and other cations (NH₄⁺) were studied. In case of divalent cations (Fe²⁺, Ca²⁺, Pb²⁺, Ba²⁺, Co²⁺ and Mg²⁺), the incorporation of 50 mol% KTpCIPB leads to the increasing of Hg(II) selectivity but, 100 mol% to rather the decreasing membrane selectivity. This could be explained that too much ionic site loose the superiority of the ion mobility of Hg(II) ion compare to the other cations, because the mobility of the other cations also improved significantly with high concentration of additive.

**Fig. 6. Selectivity coefficients of membrane with various amounts of ionic additive (KTpCIPB).**

3.5. Influence of pH

In order to investigate the pH effect on the potential response of the Hg(II) ISE, the potential was measured at the presence of 1.0×10^{-3} M Hg(II) ion concentration over the pH range 1.5–11.5 with E2 and the result is depicted in Fig. 7. Potential value was kept from pH 3.0 to 6.5 and 7.5 to 11.5. Small potential changes were found at below pH 3.0 and between 6.5 and 7.5. It shows this electrode can be

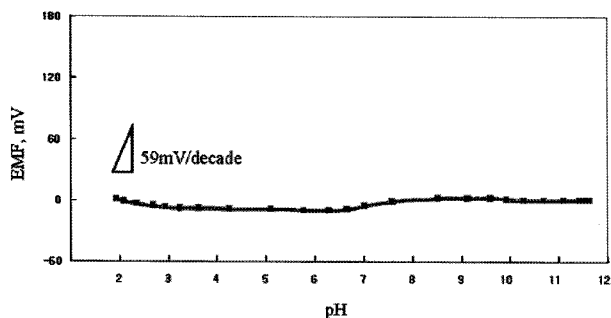


Fig. 7. The influence of pH on the potentiometric response of the optimized Hg(II) ISE (E2).

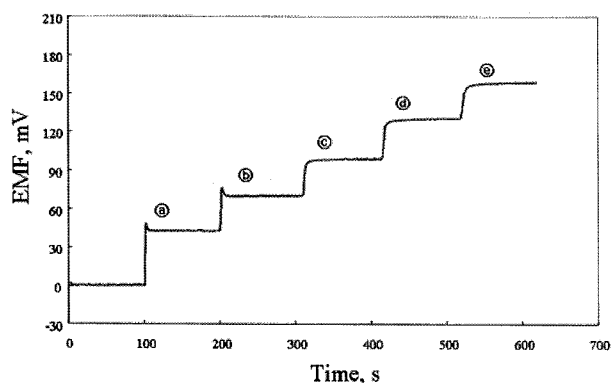


Fig. 8. Dynamic response of Hg(II) ISE (E2) based on tetracycline for step changes in concentration of mercury(II) ion; a) 1.0×10^{-7} M, b) 1.0×10^{-6} M, c) 1.0×10^{-5} M, d) 1.0×10^{-4} M and e) 1.0×10^{-3} M.

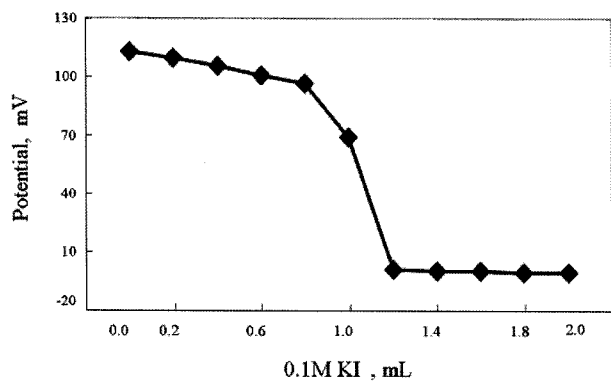


Fig. 9. Potentiometric titration curve for 200.0 mL of 0.050 M Hg(II) solution with 0.10 M KI titrant using the mercury electrode based on tetracycline.

applied to Hg(II) samples even at alkaline pH without buffer solution and it is superior to the other Hg(II) ISE published previously which is working only at acidic pH.

3.6. Response time and life time

The dynamic response time is an important factor of an ISE and it was recorded from 1.0×10^{-7} to 1.0×10^{-3} M in the sample solution with E2 sensor (Fig 8.). The response time

was less than 20s at various concentrations, and the potentials remained constant for more than 10 min. The lifetime of the electrode membrane prepared was kept at least a month without a considerable change in their response to Hg(II) ion. The electrodes were stored in 1.0×10^{-3} M Hg(II) ion solution when it is idle.

3.7. Analytical applications

ISE based on TC was successfully applied as indicator electrodes to the potentiometric titration of Hg(II) ion solution with potassium iodide (KI). It shows a distinct end point of titration curve for the titration of 200 mL of 5.0×10^{-2} M Hg(NO₃)₂ with 0.1 M KI titrant (Fig. 9). Before the end-point, the potential shows a usual logarithmic change with the volume of titrant added, while the potential response after the end-point remains almost constant, which is due to the absent of Hg(II) ion in the solution.

Acknowledgements

This work was supported by a special research grant from Seoul Women's University (2007).

References

1. E. Bakker, P. Buhlmann, E. Pretsch, *Chem. Rev.* **97**, 3083 (1997).
2. P. Buhlmann, E. Pretsch, E. Bakker, *Chem. Rev.* **98**, 1593 (1998).
3. M. T. Lai; J. S. Shih, *Analyst*, **111**, 891 (1986).
4. V. K. Gupta; S. Jain; U. Khurana, *Electroanalysis* **9**, 478 (1997).
5. Lu, J.; Tong, X.; He, X. *J. of Electroanal. Chem.* **540**, 111 (2003).
6. L. Xu; R. Yuan; Y. Q. Chai, *Chem. Lett.* **34**, 440 (2005).
7. G. Ye, T. Chai, R. Yuan, J. Dai, *Analytical Sciences* **22**, 579 (2006).
8. W. E. Morf, W. Simon, *Helv. Chim. Acta*, **54**, 2683 (1971).
9. M. Schafer, G. M. Sheldrick, I. Bahner, H. Lackner, *Angew. Chem. int.* **37**, 2391 (1998).
10. I. R. Paeng, *Anal. Sci. Tech.* **18**, 491 (2005).
11. A. Albert, *Nature(London)*, **172**, 201 (1953).
12. A. Albert; C. W. Rees, *Nature (London)*. **177**, 433 (1956).
13. J. Baek, J.-S. Kim, I. R. Paeng, K.-J. Paeng, *Bull. Kor. Chem. Soc.*, **29**, 165 (2008).
14. S. S. Park, S. O. Jung, S. M. Kim, J.-S. Kim, *Bull. Kor. Chem. Soc.* **17**, 405 (1996).
15. P. Richard, E. L. Buck, *Pure & Appl. Chem.* **66**, 2527 (1994).
16. P. Job, *Ann. Chim.* **9**, 113 (1928).
17. F. Benet; J. E. Goyan, *J. Pharm. Sci.* **55**, 1184 (1966).
18. J. L. Colaizzi; A. M. Knevel; A. N. Martin, *J. Pharm. Sci.* **54**, 1425 (1965).
19. K. H. Ibsen; M. R. Urist, *Proc. SOC. Exp. Biol. Med.* **109**, 797 (1962).
20. L. A. Mitscher; A. C. Bonacci; B. Slater-Eng; A. K. Hacker; T. D. Sokoloski Antimicrob, *Agents Chemother.* **11**, 1 (1969).
21. J. T. Doluisio; A. N. Martin; *J. Med. Chem.* **6**, 16 (1963).
22. W. A. Baker, P. M. Brown, *J. Am. Chem. Soc.* **88**, 1314 (1968).
23. A. H. Caswell; J. D. Hutchison, *Biochem. Biophys. Res. Commun.* **43**, 625 (1971).
24. M. H. Mashhadizadeh; I. Sheikhshoae, *Talanta* **60**, 73 (2003).
25. A. Lewenstam; A. Hulanicki, *Selec. Electrode Rev.* **12**, 161 (1990).