

Evaluation of the Associate Constant of a Phospholipid – Ca²⁺ Complex Using a Phospholipid Monolayer Adsorbed at an Aqueous|1,2-dichloroethane Interface

Yumi Yoshida,^{*,a} Kohji Maeda,^a and Osamu Shirai^b

^aDepartment of Chemistry, Kyoto Institute of Technology, Matsugasaki, Sakyo, Kyoto 606-8585, Japan

^bDivision of Nuclear Engineering Science, Research Reactor Institute, Kyoto University, Kumatori, Osaka 590-0494, Japan

Received: November 15, 2004; In Final Form: April 21, 2005

The complex formation of Ca²⁺ with a phosphatidylcholine adsorbed at the interface between water and 1,2-dichloroethane, WIDCE, was evaluated to elucidate the adsorption of ions on biomembrane surfaces. An electrochemical method combined with interfacial tension measurement was adopted for evaluation of the complex formation of the phospholipid and the ion. The association constant for the complex formation of Ca²⁺ with the lipid was three orders of magnitude higher than those of monovalent cations with the lipid. The present result suggests that the strong complex formation of highly hydrophilic metal cations with a lipid membrane results in a higher affinity to biomembrane surfaces.

1. Introduction

The evaluation for binding of an ion to a phospholipid membrane surface is very important in understanding the adsorption behavior of an ion on a biomembrane surface or a bacterial surface. The binding of an ion to a bilayer lipid membrane, BLM, has been investigated by NMR measurement, IR spectroscopy, electrophoresis, the ion-selective electrode method, the monolayer-radiotracer technique, neutron diffraction and so on.¹ Results obtained by these techniques reflect not only the complex formation of an ion with the polar head group of a phospholipid but also the hydrophobic interaction caused by breaking the water structure. For example, the binding strength of anion with a BLM corresponded to the hydrophobicity of the anions;¹ e.g. tetraphenylborate > ClO₄⁻ > I⁻ > SCN⁻ > NO₃⁻ > Br⁻ > Cl⁻ > SO₄²⁻.

An electrochemical method combined with interfacial tension measurement has been proposed to evaluate the complex formation of a phospholipid with an ion.^{2,3} In the measurement, the associate constant of a phospholipid with an ion is estimated from the interfacial potential for desorption of the phospholipid monolayer at the aqueous/organic interface created by the transfer of the ion associated with the phospholipid. The electrochemical method has the advantage of avoiding the effect of the hydrophobic interaction.³ We have estimated the association constants of a phospholipid with various monovalent cations.³

In the present work, we attempted to apply the method to evaluate the complex formation of a phospholipid with a more hydrophilic cation such as Ca²⁺.

2. Experimental

Chemicals. The phospholipid employed was L- α -phosphatidylcholine dioleoyl, PC, of analytical grade (SIGMA, Lot No. 32k5202). Bis(triphenylphosphoranylidene)ammonium tetrakis [3,5-bis(trifluoromethyl)phenyl] borate, BTPPA⁺TFPB⁻, was obtained as a precipitate by mixing a methanol solution of BTPPA⁺Cl⁻ with a methanol solution of Na⁺TFPB⁻, and the precipitate was purified by recrystallization based on the

temperature dependence of the solubility of the salt in ethanol. Na⁺TFPB⁻ was synthesized according to the procedure described previously.^{4,5} Tetraphenylarsonium sulfate, (TPhAs⁺)₂SO₄²⁻, was prepared by the titration of TPhAs⁺Cl⁻ (Aldrich) with Ag₂SO₄ in an aqueous solution.⁶ The salt of tetraphenylborate with TPhAs⁺, TPhAs⁺TPhB⁻, was prepared according to the method of previous studies.⁶ The 1,2-dichloroethane, DCE, was purified according to the method described in the literature⁷ and then saturated with distilled water.

All other chemicals used were of reagent grade quality.

Measurement of the interfacial tension at a polarized WIDCE interface. The interfacial tension, γ , at the polarized WIDCE interface was measured by the Wilhelmy vertical plate technique⁸ applying the potential difference, E , at the WIDCE interface.³ Figure 1 shows the cell used for the measurement of γ . The WIDCE interface was formed in a glass beaker (35 mm diameter). A glass plate (width $l = 23.85 \pm 0.05$ mm, thickness $d = 0.3 \pm 0.01$ mm, Kyowa Interface Science Co., Ltd.) silanized

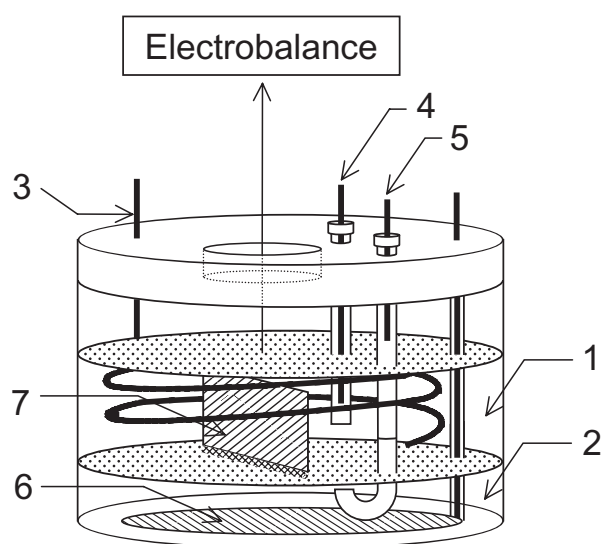


Figure 1. Cell for the measurement of interfacial tension at a polarized WIDCE interface: (1) Aqueous phase, (2) DCE phase, (3) a Pt wire used as a counter electrode in W, (4) an Ag|AgCl electrode used as a reference electrode in W, (5) TPhAsE used as a reference electrode in DCE, (6) a Pt net used as a counter electrode in DCE, (7) a silanized glass plate (From Ref. 3)

*Corresponding author. E-mail: yyoshida@kit.jp. FAX: +81-75-724-7518.

with dimethyldichlorosilane was placed on the WIDCE interface, and connected to an electrobalance.

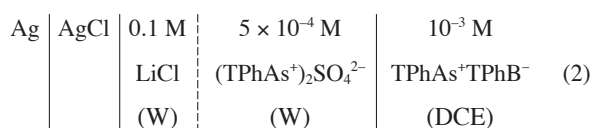
The γ value was estimated from the detaching force of the glass plate from the WIDCE interface:

$$\gamma = \Delta W / (2l + 2d), \quad (1)$$

where ΔW is the weight difference between the glass plate in contact with the interface and the same plate detached from the interface.

Ca^{2+} was added to W as a chloride salt. The pH of W was not controlled by a buffer and was confirmed to be in the range 6–7. 0.01 M ($M = \text{mol dm}^{-3}$) $\text{BTPPA}^+\text{TFPB}^-$, which is a supporting electrolyte, was added with PC to the DCE.

The interfacial potential difference between the W and DCE, E , was applied as the potential of W with reference to DCE using two reference electrodes of an $\text{Ag}|\text{AgCl}$ electrode in W and a tetraphenylarsonium ion-selective electrode, TPhAsE , in DCE, and two counter electrodes of a Pt wire in W and a Pt net in DCE. The configuration of TPhAsE is given by eq 2.



In the presence of PC, the E was held at 0.2 V until the decrease in γ stopped before starting the potential sweep. The adsorption equilibrium of PC was achieved at 0.2 V, where strong adsorption of PC was observed.³ The potential dependence of γ was measured by changing E from a negative to a positive potential.

E was related to the Galvani potential difference at the WIDCE interface, $\Delta\phi$, as,

$$E = \Delta\phi + E_{\text{ref}}, \quad (3)$$

where E_{ref} is the potential of the reference electrodes employed. The E_{ref} of TPhAsE was 0.350 V with reference to the standard potential³ where the Gibbs free energy for the transfer of an ion from W to DCE, ΔG_{tr} , is recognized to be zero based on Parker's extrathermodynamic assumption.⁹ Using eq 3, experimental data for E was converted to $\Delta\phi$ and is shown as thus hereafter.

Voltammetry for the Ca^{2+} transfer at a micro WIDCE interface. The Ca^{2+} transfer at the WIDCE interface was measured as a voltammogram by scanning E applied at a micro WIDCE interface and measuring the current for the ion transfer.^{10, 11} The cell consisted of W and DCE separated by a polyester film 16 μm thick with a micro hole 50 μm in diameter.¹¹ The WIDCE interface was formed at the micro hole. The supporting electrolyte added to DCE was $\text{BTPPA}^+\text{TFPB}^-$.

All electrochemical measurements were carried out at 25 ± 0.5 °C.

Apparatus. The potentiostat/galvanostat for voltammetry at the micro WIDCE interfaces and that for the γ measurement were Model HECS-318 (Huso Electro Chemical System) and Model HA 1010MIS (Hokuto Denko Co.), respectively. The function generator and X-Y recorder used were Model HB-111 (Hokuto Denko Co.) and Type 3086 (Yokogawa Electric Works, Ltd.), respectively. The electrobalance and the electrobalance lift were HD-60 (A&D Company, Ltd.) and SYLB-7K (Sanyu Tech., Ltd.).

3. Results and Discussion

Adsorption of a PC monolayer at the WIDCE interface.

Figure 2 shows the γ value at the WIDCE interface measured as a function of $\Delta\phi$. In the absence of PC in DCE, the potential

dependence of γ showed the ideal electrocapillary curve of a quadratic function, which indicates the formation of an electrical double layer near the interface. Therefore, E was applied exactly at the WIDCE interface. When PC was added to DCE, the γ value decreased to approximately 7 mN m^{-1} at a $\Delta\phi$ of -0.15 V, and remained constant at $\Delta\phi$ values between -0.3 and 0.05 V. In this potential range, the difference in γ between the presence and absence of PC was significant. When the $\Delta\phi$ was shifted to more a positive potential than 0.05 V, the γ value increased steeply, and the difference in γ between the presence and absence of PC became insignificant. These results indicate that the adsorbed PC was released from the interface at a more positive potential than 0.05 V as described in the literature.^{2, 3, 12-17} The threshold of $\Delta\phi$ for lipid desorption, 0.05 V, is defined as $\Delta\phi_{\text{desorb}}$ hereafter.

Figure 3 shows a plot of γ at -0.15 V as a function of the bulk concentration of PC in DCE. When the concentration of PC was in the range between 2×10^{-6} and 2×10^{-5} M, γ decreased at the constant rate of 14 mN m^{-1} with the concentration of PC, indicating that the adsorption of PC at the WIDCE

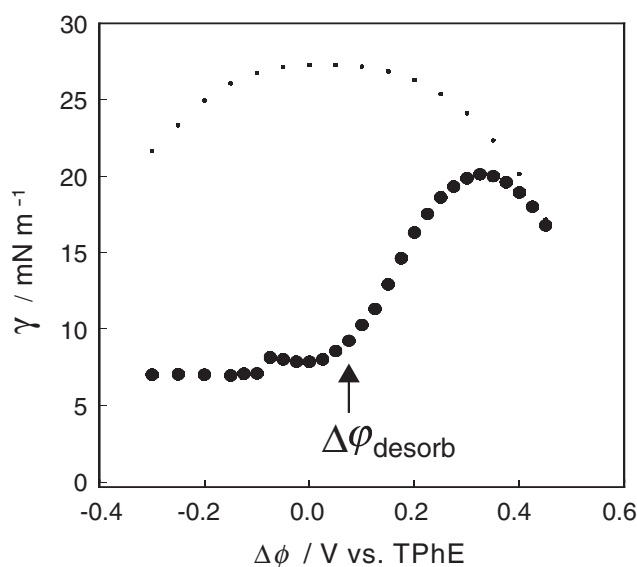


Figure 2. The potential dependence of the interfacial tension in the presence (●) or absence (○) of 10^{-5} M PC at the interface between DCE containing 0.01 M $\text{BTPPA}^+\text{TFPB}^-$ and W containing 0.1 M CaCl_2 .

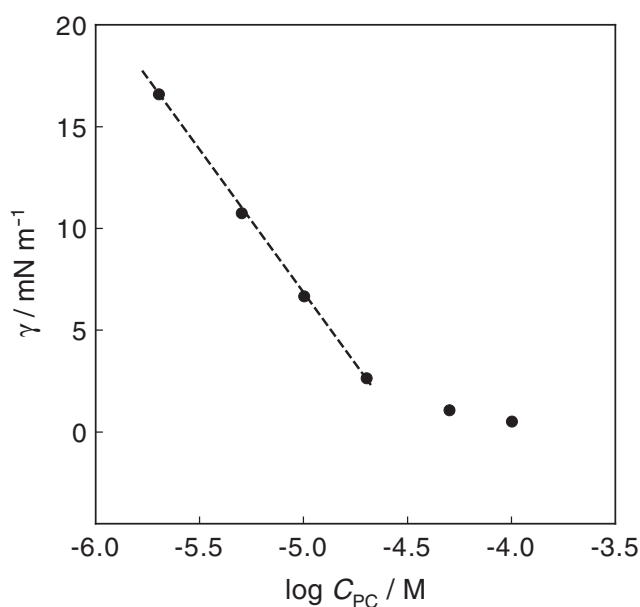


Figure 3. The interfacial tension at -0.15 V as a function of the logarithmic concentration of PC, $\log C_{\text{PC}}$, in DCE.

interface was saturated. The surface excess concentration relative to both solvents, Γ_{PC} , was calculated to be $2.45 \mu\text{mol m}^{-2}$ based on the slope, and was consistent with the reported value of $2.31 \mu\text{mol m}^{-2}$ for a phospholipid monolayer in the presence of NaCl in W.³ The γ decreased at lower rate when the concentration of PC was higher than 5×10^{-5} M, indicating that PC was present the critical aggregation concentration.

Voltammograms for the transfer of Ca^{2+} facilitated by a PC at the WIDCE interface. Previous studies have suggested that the desorption of PC at $\Delta\phi > \Delta\phi_{\text{desorb}}$ is caused by the complex formation of a cation with PC followed by transfer across the interface.^{2, 3, 13, 18} In order to confirm the complex formation of Ca^{2+} with PC, voltammograms for the transfer of Ca^{2+} were measured at the micro WIDCE interface.

Figure 4 shows voltammograms for the transfer of Ca^{2+} in the presence of PC in DCE. In the absence of PC in DCE, the Ca^{2+} transfer from W to DCE was observed as the positive current at 0.45 V in the voltammogram (line 1). When PC was added to the DCE, a new positive current appeared in the voltammograms at negative potentials greater than 0.45 V (line 2–5). The new positive current increased with the increase of the concentration of PC in DCE and shifted by about -0.060 V for a tenfold increase in the Ca^{2+} concentration. Considering the previous study of ion transfer facilitated by a neutral ligand,¹⁹ the new positive current is found to be due to the Ca^{2+} transfer facilitated by the addition of PC to DCE. The facilitation of the Ca^{2+} transfer by the addition of PC results from the complex formation of PC with Ca^{2+} .

The potential range allowing the Ca^{2+} transfer facilitated by PC was greater than 0.05 V. The potential range agreed with that observable for desorption of the PC monolayer from the interface, $\Delta\phi > 0.05$ V ($\Delta\phi_{\text{desorb}}$), as shown in Figure 2. Therefore,

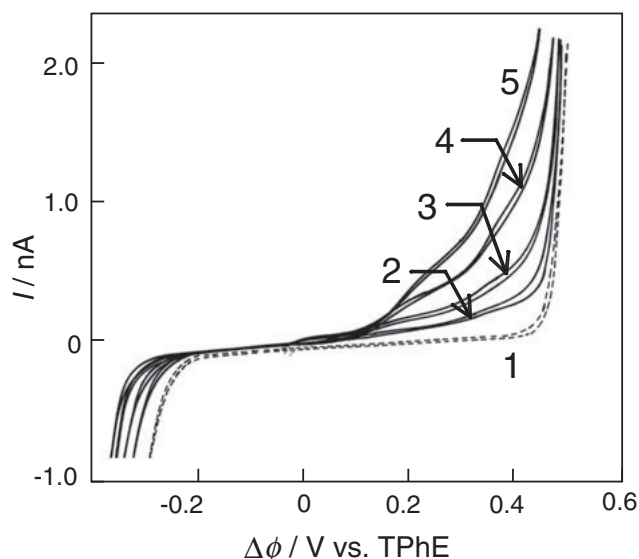


Figure 4. Voltammograms measured at micro interfaces between W containing 0.1 M CaCl_2 and DCE containing 0.01 M BTTPA⁺TFPB⁻ and (1) 0, (2) 10^{-4} , (3) 2×10^{-4} , (4) 5×10^{-4} , and (5) 10^{-3} M PC. Scan rate was 1 mV s^{-1} .

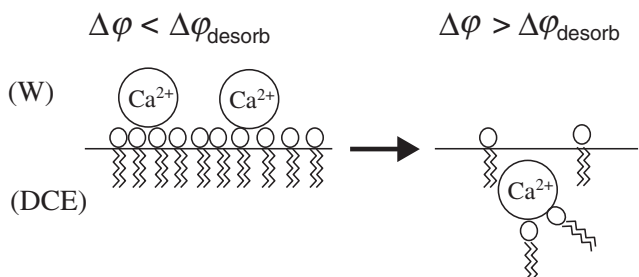


Figure 5. Potential dependence of the PC adsorption at the WIDCE interface.

the Ca^{2+} transfer facilitated by the complex formation with PC causes desorption of the PC monolayer at $\Delta\phi > \Delta\phi_{\text{desorb}}$. The process of the adsorption-desorption behavior of PC in Figure 2 is illustrated in Figure 5.

An association constant of Ca^{2+} with a PC monolayer adsorbed at the WIDCE interface. The adsorption-desorption behavior of PC follows from transfer of the complex of PC with a hydrophilic cation, M^z . The behavior can be expressed as eq 4.^{2, 3, 18}



Here, the subscripts (O) and (ads) denote the species in the bulk organic phase and the adsorbed species at the interface, respectively. Samec et al. proposed a theory for the potential dependence of γ by assuming the reaction in eq 4 and using two Frumkin isotherms for adsorption of PC and MPC^z .² The theory suggests that the onset of the increase in interfacial tension such as $\Delta\phi_{\text{desorb}}$ is determined mainly by the transfer potential for M^z , $\Delta\phi_{\text{M}}^{0'}$, the bulk concentration of hydrophilic salts, MX, in W, C_{MX}^0 , and the association constant in O, $K_{\text{ass}} = C_{\text{MPC}^z(O)} / C_{\text{PC}(O)}C_{\text{M}(O)}$.

$$\Delta\phi_{\text{desorb}} \approx \Delta\phi_{\text{M}}^{0'} - (RT/zF) \ln(K_{\text{ass}}C_{\text{MX}}^0 D_{\text{MPC}}^{1/2} D_{\text{PC}}^{-1/2}), \quad (5)$$

where D is the diffusion coefficient.

The K_{ass} value was evaluated from $\Delta\phi_{\text{desorb}}$ in Figure 2. By using eq 5, the $\log K_{\text{ass}}$ of Ca^{2+} with PC was calculated to be $19.3 \text{ mol}^{-1} \text{ dm}^3$. In calculation, the following points were assumed: the lipid/ion stoichiometry of the complex was 1:1, and D of CaPC^{2+} was equal to that of PC. The value of $\Delta\phi^0$ for Ca^{2+} was estimated to be 0.59 V based on $\Delta\phi^0$ for Mg^{2+} ,²⁰ the potential difference between the positive current for the transfer of Ca^{2+} and that for the transfer of Mg^{2+} in the voltammogram recorded in the absence of PC.

Figure 6 shows the K_{ass} of PC with cations estimated from $\Delta\phi_{\text{desorb}}$. The values of K_{ass} for monovalent cations other than Ca^{2+} are the values reported in our previous work³ which was estimated according to a procedure similar to the present work. The $\log K_{\text{ass}}$ of PC with Ca^{2+} was higher by 3 times than those of PC with monovalent cations. It has been reported that the association of BLM with a metal cation is stronger with the increase of the surface charge density of the metal cation.¹ The complex formation between cations and the PC monolayer adsorbed at the interface demonstrates a trend similar to that reported in the study of BLM.

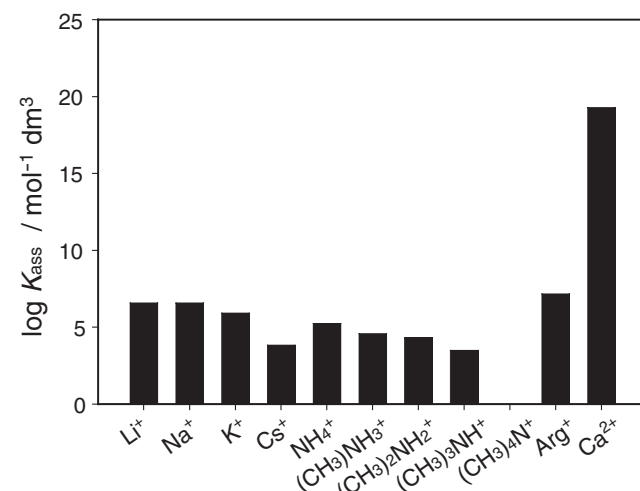


Figure 6. The association constants, K_{ass} , of PC with the cations in DCE evaluated from the onset of the increase in the potential dependence of γ , $\Delta\phi_{\text{desorb}}$. The K_{ass} of cations other than Ca^{2+} were calculated in Reference 3. Arg⁺ indicates the arginine cation. The value of K_{ass} for $(\text{CH}_3)_4\text{N}^+$ was too small to determine.

4. Conclusion

An associate constant for the complex formation of a phospholipid with Ca^{2+} was evaluated from the interfacial potential for desorption of the phospholipid monolayer from the W|DCE interface. Based on this method, the $\log K_{\text{ass}}$ of Ca^{2+} with PC was calculated to be $19.3 \text{ mol}^{-1} \text{ dm}^3$: higher by 3 times than those of PC with monovalent cations. The affinity of the PC monolayer adsorbed at the W|DCE interface to a metal cation is almost similar to that of BLM. Therefore, localization of metal cations on the BLM surface may be responsible for the complex formation of the cation with PC.

Acknowledgements. This study was partly supported by Grants-in-Aid for Scientific Research (No. 16350043 and No. 16550072) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

- (1) S. A. Tatulian, *Phospholipids Handbook*, Marcel Dekker, Inc. New York (1993), ed. G. Cevc, Chapter 14 and references cited therein.
- (2) Z. Samec, A. Trojánek, and H. H. Girault, *Electrochem. Commun.* **5**, 98 (2003).
- (3) Y. Yoshida, K. Maeda, and O. Shirai, *J. Electroanal. Chem.* **578**, 17 (2005).
- (4) H. Nishida, N. Takada, M. Yoshimura, T. Sonoda, and H. Kobayashi, *Bull. Chem. Soc. Jpn.* **57**, 2600 (1984).
- (5) S. R. Bahr and P. Boudjouk, *J. Org. Chem.* **57**, 5545 (1992).
- (6) S. Kihara, M. Suzuki, K. Maeda, K. Ogura, S. Umetani, M. Matsui, and Z. Yoshida, *Anal. Chem.* **58**, 2954 (1986).
- (7) K. M. Kadish and J. E. Anderson, *Pure Appl. Chem.* **59**, 703 (1987).
- (8) L. Wilhelmy, *Ann. Physik.* **119**, 177 (1863).
- (9) J. Parker, *Chem. Rev.* **69**, 1 (1969).
- (10) G. Taylor and H. H. J. Girault, *J. Electroanal. Chem.* **208**, 179 (1986).
- (11) H. Ohde, A. Uehara, Y. Yoshida, K. Maeda, and S. Kihara, *J. Electroanal. Chem.* **496**, 110 (2001).
- (12) S. G. Chesniuk, S. A. Dassie, L. M. Yudi, and A. M. Baruzzi, *Electrochimica Acta* **43**, 2175 (1998).
- (13) Y. Yoshida, H. Yoshinaga, N. Ichieda, A. Uehara, M. Kasuno, K. Banu, K. Maeda, and S. Kihara, *Anal. Sci. (suppl.)* **17**, i1037 (2001).
- (14) H. H. Girault and D. J. Shiffrin, *J. Electroanal. Chem.* **179**, 277 (1984).
- (15) T. Kakiuchi, M. Nakanishi, and M. Senda, *Bull. Chem. Soc. Jpn.* **61**, 1845 (1988).
- (16) T. Kakiuchi, T. Kondo, and M. Senda, *Bull. Chem. Soc. Jpn.* **63**, 3270 (1990).
- (17) D. Grandell, L. Murtoimäki, K. Kontturi, and G. Sundholm, *J. Electroanal. Chem.* **463**, 242 (1999).
- (18) V. Mareček, A. Lhotský, and H. Jänchenová, *J. Phys. Chem. B* **107**, 4573 (2003).
- (19) H. J. Lee, C. Beriet, and H. H. Girault, *J. Electroanal. Chem.* **453**, 211 (1998).
- (20) Y. Yoshida, Z. Yoshida, H. Aoyagi, Y. Kitatsuji, A. Uehara, and S. Kihara, *Anal. Chim. Acta* **452**, 149 (2002).