

Valence Discriminative Detection of Metal Cations by a Chromotropic Acid-grafted Glassy Carbon Electrode

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Covalent grafting of chromotropic acid (CA) moiety to a glassy carbon electrode was performed by electrochemical reduction of the corresponding diazonium cation generated *in situ*. Grafted electrodes were subjected to the electrochemical detection of multivalent cations using $\text{Fe}(\text{CN})_6^{3-/4-}$ as an electrochemical probe molecule. In the absence of metal cations, redox reaction of the anionic probe was depressed by electrostatic repulsion between the probe and negatively charged CA moieties on the electrode surface. Binding of tetravalent cations such as Zr(IV) and Hf(IV) and trivalent cations such as Al(III) and lanthanide(III) to the CA moiety on the electrode surface resulted in an increase in the current intensity of the probe due to a decrease in electrostatic repulsion. Highly selective detection of the tetravalent cations was achieved in the micromolar concentration range at pH 1.0.

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Introduction

Grafting of thin organic ligand layers onto electrode surfaces is a useful method for the modification of electrodes, or the construction of functionalized electrodes with specific chemical and physical properties. In recent years, electrode grafting using aryldiazonium salts or cations has been widely employed on carbon, metal, and semiconductor surfaces.¹ The advantage of the method derives largely from the formation of covalent bonding between the surface atoms and the modifier molecules, giving stable and robust molecular layers.² Consequently, surfaces grafted in this manner have been investigated in various applications, including corrosion protection,³ sensor fabrication,⁴ catalytic electrodes,⁵ and molecular electronics.⁶ It should be also emphasized that the surface coverage of the grafted layer can be regulated by changing electrochemical grafting conditions, such as the concentration of the diazonium precursor and the number of potential cycling in cyclic voltammetry or the duration time in constant-potential electrolysis. This kind of feature is a great advantage over self-assembled monolayers by alkane thiols that form spontaneously from a solution phase and reach full coverage immediately.⁷

Chromotropic acid (CA) is often used for the spectrophotometric determination of several cations such as titanium,⁸ aluminum,⁸ zirconium,⁹ and other metal ions.¹⁰ It was reported that the complexation between CA moiety and several metal ions occurs by the displacement of two protons from each hydroxyl group to form a charged complex in the aqueous solution.¹¹ Since the sulfo groups of CA moiety are fully ionized in the pH region,¹²

the grafting of CA on the glassy carbon (GC) electrodes results in the generation of negative charges at the ligand layer-solution interface on the electrode surface. If metal ions combine to the CA moiety on the electrode surface, the change in the negative charge density depends on the valency of the metal cation.

In the present study, we investigated the grafting of GC electrodes with CA functionalities and the voltammetric response of an anionic probe such as $\text{Fe}(\text{CN})_6^{3-/4-}$, which is electrochemically subject to the redox reaction on the electrode, in order to construct an electrochemical metal cation sensor.

Experimental

Reagents and chemicals

Chemicals were of reagent-grade, and were used without further purification. Zr(IV) and Hf(IV) solutions were prepared by dissolving corresponding oxychloride octahydrate salt in hydrochloric acid and standardized by the EDTA titration with xylenol orange. Standard solutions of lanthanoid(III), such as lanthanum(III), gadolinium(III) and lutetium(III), were prepared by dissolving a known amount of each oxide (more than 99.9% purity) in hydrochloric acid and diluting with water. Other metal stock solutions were prepared by diluting respective standard solutions for atomic absorption spectrometry with hydrochloric acid. All aqueous solutions were prepared using water treated by a Milli-Q system (Millipore Corp., Bedford, MA).

Apparatus

An Ag/AgCl (sat. KCl) electrode and a platinum wire were used as reference and counter electrodes, respectively. GC electrodes ($\phi 3$ mm) were purchased from BAS (Tokyo, Japan) and used as a working electrode. Before each experiment, GC electrodes were polished successively by 1.0 and 0.05 μm alumina slurries with Milli-Q water on polishing cloths. After

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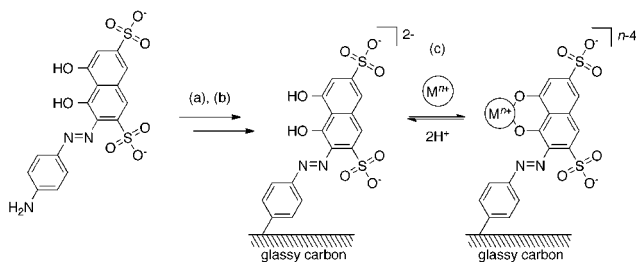


Fig. 1 Schematic illustration of the grafting of the GC electrode by the electrochemical reduction of *in situ* generated PV diazonium cation. (a) Five equiv. NaNO_2 , 1 M HCl, 4°C, (b) potential cycling between +0.6 and -0.8 V vs. Ag/AgCl. (c) Complexation of the metal ion with CA moiety on the electrode surface and the change in apparent charge of the CA complex.

polishing, the electrodes were rinsed with water several times and sonicated in water. Electrode grafting and further electrochemical measurements were conducted using an electrochemical analyzer (Model 650A, BAS, Japan) with a standard three-electrode configuration. A UV/Vis spectrophotometer (V-630, JASCO) was used for the measurements of absorption spectra of CA derivatives. A pH meter (Model F-52, Horiba) equipped with a combination glass electrode (9611-10D, Horiba) was calibrated by standard buffer solutions (pH 6.86, 4.01, and 1.68 at 25°C).

Electrode grafting procedure

Electrode grafting was performed by electrochemical reduction of an *in situ* generated diazonium cation¹³ as follows (Fig. 1). Pontacyl Violet 4BSN (PV) as a precursor, a CA derivative having an aromatic amine moiety with an azo linkage, was dissolved in a 1 M HCl solution at 4°C, then an ice-cold aqueous NaNO_2 (5 equiv.) solution was added to form a diazonium cation of PV. After stirring of the mixture for 10 min at 4°C, a GC electrode was immersed into the mixture and the potential cycling (+0.6 to -0.8 V vs. Ag/AgCl (sat. KCl)) was carried out from the positive potential limit with a scan rate of 0.1 V s⁻¹. Several grafting experiments under different conditions were performed at a series of potential cycle numbers (*n*) ranging from 1 to 30.

Results and Discussion

Grafting of glassy carbon electrode

The electrochemical reduction of an *in situ* generated diazonium cation of PV at a GC electrode was investigated by cyclic voltammetry. Figure 2 shows the first three cyclic voltammograms (CVs) for diazotized PV. The first cycle presents two reduction peaks at -0.11 and -0.75 V, which decreased and totally disappeared during the following cycles. This change in voltammogram was already observed in previous studies for the reduction of isolated diazonium salts dissolved in acetonitrile and attributed to the growth of a grafted and passivating layer on the electrode surface.¹⁴⁻¹⁶ The mechanism was shown to involve the intermediate aromatic radical obtained upon one-electron reduction of the diazonium cation; this radical reacting with the carbon surface to form a covalent bond. The formation of an organic layer significantly limits further reduction of diazotized PV, although it is not completely inhibited.¹⁶⁻¹⁸ The intensity of the peak at -0.11 V, observed during the reduction of diazotized PV at the most positive

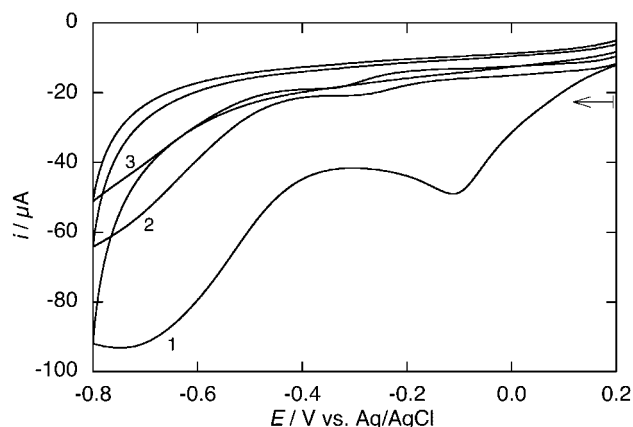


Fig. 2 CVs for the reduction of 1 mM PV in the presence of 5 mM NaNO_2 recorded at a scan rate of 0.1 V s⁻¹. CVs are obtained in a 1 M HCl solution at 4°C. The scans were started at the positive potential limit, and three consecutive cycles are shown.

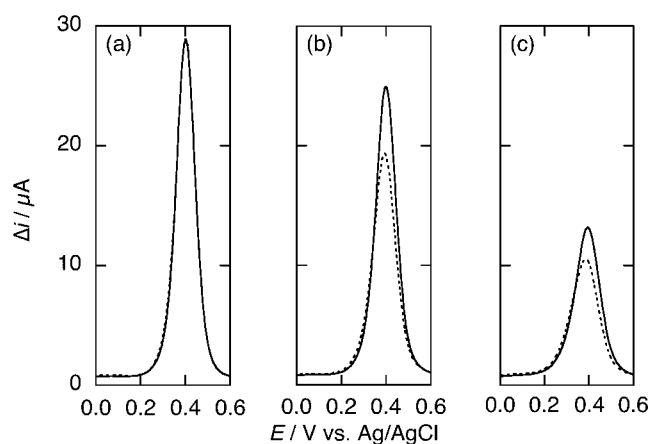


Fig. 3 SWVs of 1 mM $\text{K}_3\text{Fe}(\text{CN})_6$ in 0.1 M HCl with (solid line) and without (dotted line) 30 μM Zr(IV) at CA/GC electrodes prepared with *n* = 1 (a), 3 (b), and 5 (c), respectively.

potential, is attributed to the reduction of the azo group of PV because a similar reduction peak was observed in the absence of NaNO_2 (figure not shown). Furthermore, the CA layer was not formed on the electrode surface when the potential sweep was reversed at -0.3 V during the grafting procedure. The second peak of the first sweep at more negative potential corresponds to the reduction of diazonium cations to a radical as well as the subsequent formation and growth of the grafted CA layer.

Electrochemical response of $\text{Fe}(\text{CN})_6^{3-/4-}$ at CA-grafted GC electrodes

Figure 3 (dotted lines) shows square wave voltammograms (SWVs) of $\text{Fe}(\text{CN})_6^{3-/4-}$ in 0.1 M HCl solutions at CA-grafted GC (CA/GC) electrodes prepared with a series of potential cycles with *n* ranging from 1 to 5. In the case of *n* = 1 (Fig. 3(a)), a clear SWV peak at +0.40 V was observed and the peak current intensity of 29 μA was consistent with that of a bare GC electrode. The peak current intensity decreased in the CA/GC electrodes prepared at *n* = 3 and 5 (Figs. 3(b) and 3(c)), reflecting an increase in the amount of grafted CA moiety on the electrode surface. These observations indicate that the

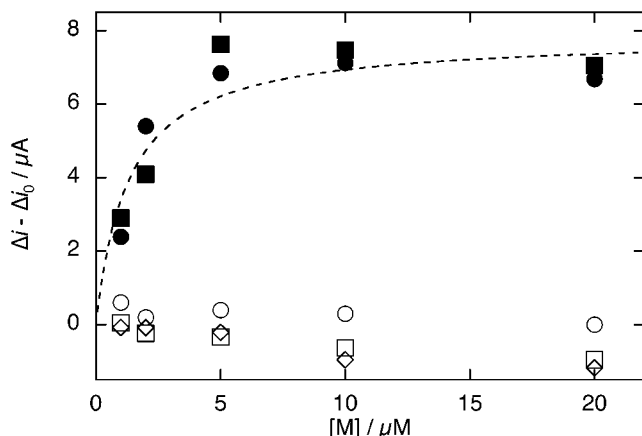


Fig. 4 Changes in SWV peak current intensity of 1 mM $\text{Fe}(\text{CN})_6^{3-/4-}$ at CA/GC electrodes as a function of the concentration of metal ions at pH 1.0. Zr(IV) (●), Hf(IV) (■), Al(III) (○), La(III) (□), Lu(III) (◇). A dotted line shows the calculated Langmuir-type isotherm curve for Zr(IV).

amount of grafted CA increased gradually with an increase in n , and the electrostatic repulsion between $\text{Fe}(\text{CN})_6^{3-/4-}$ and the surface CA groups with the negative charge were strengthened by increasing n . It is noteworthy that CA/GC electrodes prepared with $n > 10$ gave almost complete depression of the SWV signal with a current intensity of less than 1 μA (figure not shown). Although the redox current may be expected with respect to the azo group of PV, we could not directly determine the surface concentration of CA on the grafted electrode due to the lack of a significant current response for the azobenzene-modified GC electrode.¹⁹ It is, however, expected that a fully covered mono- or multilayer was formed on the CA/GC electrode with $n > 10$ and the redox reaction of $\text{Fe}(\text{CN})_6^{3-/4-}$ was completely hindered. On the other hand, sub-monolayer formation or suitable grafting were achieved in the case of $n = 3$ and 5 to indicate moderately depressed SWV signals of $\text{Fe}(\text{CN})_6^{3-/4-}$.

Figure 3 (solid lines) shows the effect of Zr(IV) cation on the current intensity in SWV. The suppressed current intensity (shown by dotted lines) in the absence of the Zr(IV) cation was recovered in the presence of Zr(IV). This is called “signal-on” response of the CA/GC electrode upon the addition of metal cations. It is evaluated as a recovery in the current intensity of $\text{Fe}(\text{CN})_6^{3-/4-}$ upon the addition of metal cation, *i.e.*, $\Delta i_p - \Delta i_0$ where Δi_p and Δi_0 represent SWV peak currents in the presence and absence of 30 μM of Zr(IV), respectively. The $\Delta i_p - \Delta i_0$ values for the four CA/GC electrodes with $n = 1, 3, 5$ and 10 are $-0.1, +6.6, +2.9,$ and $+0.4 \mu\text{A}$, respectively. The value of $\Delta i_p - \Delta i_0$ depends on the number of potential cycling during the grafting procedure. In the case of $n = 1$, addition of Zr(IV) does not affect the current intensity, indicating the significantly low surface coverage of CA on the electrode surface. Both Δi_p and Δi_0 were completely suppressed for $n > 10$ due to the dense packing or multilayer formation of the CA moiety that prevents access of the probe to the electrode surface. Under the given experimental conditions, the CA/GC electrode with $n = 3$ and 5 was found to give clear “signal-on” type responses. As described later in detail, the Zr(IV) cation decreased the negative charge density on the CA group and allowed $\text{Fe}(\text{CN})_6^{3-/4-}$ to approach the electrode surface that results in the recovery of the current intensity. The largest recovery is obtained with $n = 3$ due to the

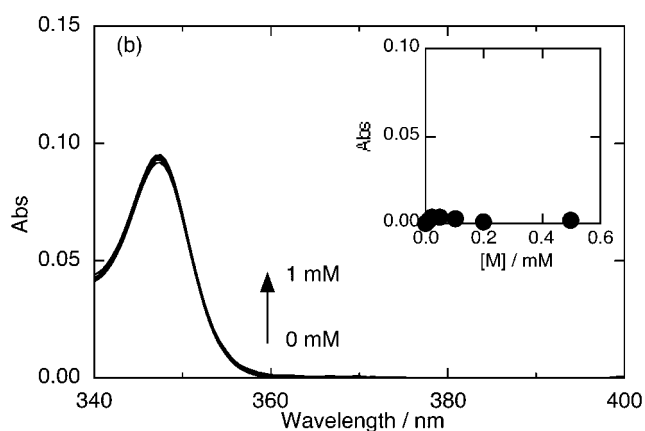
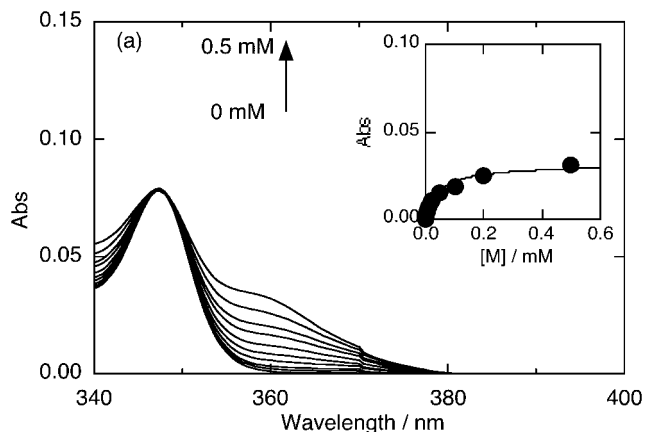


Fig. 5 UV/Vis spectra of 10 μM CA in 0.1 M HCl (pH 1.0) upon the addition of (a) Zr(IV) and (b) Al(III). $[\text{M}] = 0.001$ to 0.5 mM. (Inset) Changes in absorbance at 360 nm against the concentration of metal cation. The solid line shows the values calculated from K' of the 1:1 complex.

suitable surface coverage of the CA moiety for the desirable change in charge density upon complexation with Zr(IV) and other metal cations. The optimal grafting conditions for the preparation of the electrode were obtained with $n = 3$ and used for the following experiments.

Selective response of tetravalent cations at pH 1.0

Figure 4 shows responses to tetravalent cations, Zr(IV) and Hf(IV), in the concentration range from 1 to 20 μM . The current recovery is recognized for 1–5 μM and reaches a plateau value at around 10 μM , with some deviations at higher concentrations. Assuming the formation of 1:1 complex of metal(IV) ions and the CA moiety at CA/GC electrode surface, responses to Zr(IV) and Hf(IV) can be explained on the basis of Langmuir adsorption isotherms. According to the analysis of the response curves by Reinhoudt and coworkers,²⁰ the adsorption constants ($\log a$) of tetravalent cations to the surface CA layer are estimated to be 5.8 ± 0.2 and 5.9 ± 0.3 for Zr(IV) and Hf(IV), respectively. On the other hand, almost no change was observed for trivalent cations, Al(III), La(III), and Lu(III), over the concentration range examined.

To elucidate the selectivity of the CA/GC electrode toward the metal ions in the electrochemical detection, the complexation ability of CA for Zr(IV) and Al(III) at pH 1.0 was investigated by spectrophotometric measurement. Figure 5 shows UV/Vis

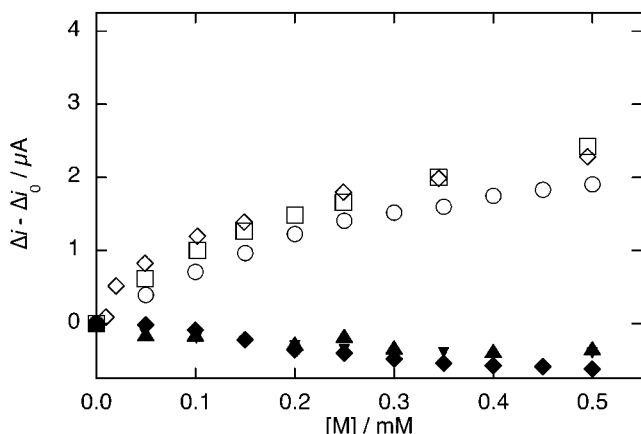


Fig. 6 Changes in SWV peak current intensity as a function of the concentration of metal ions at pH 4.0. Al(III) (○), La(III) (□), Lu(III) (◇), Be(II) (◆), Mg(II) (▲), Sr(II) (▼).

spectra of CA (10 μ M) in 0.1 M HCl. In the absence of metal ions, a maximum absorbance of CA is observed at 347 nm. As shown in Fig. 5(a), a new absorption band appears at 360 nm upon the addition of Zr(IV) in the concentration range of 0.001 to 0.2 mM, whereas no spectral change is observed for Al(III). From the UV/Vis spectra, the conditional stability constant ($\log K'$) of a 1:1 complex of Zr(IV) and Hf(IV) with CA at pH 1.0 were calculated to be 4.2 ± 0.1 and 3.9 ± 0.2 , respectively. It can be concluded that the difference in the electrochemical response shown in Fig. 4 is derived from the complexation ability of CA at pH 1.0. The optimum concentration range of Zr(IV) in the spectrophotometric detection with a CA derivative was reported to be 4 - 12 ppm (*ca.* 44 - 131 μ M).²¹ The observed responses in Fig. 4 are highly selective to tetravalent cations over trivalent cations, and the response range of 1 - 5 μ M for Zr(IV) is superior to that of spectrophotometric detection by one order of magnitude.

Discrimination of trivalent cations from divalent cations at pH 4.0

As shown in Figs. 4 and 5(b), the CA moiety did not respond to trivalent cations both electrochemically and spectrophotometrically at pH 1.0. According to the literature,²² optimum pH range for Al(III) determination with CA was reported to be from 3 to 4 in the spectrophotometric or spectrofluorometric measurement. Therefore, the pH value of the sample solution was varied from 1 to 4 to improve the response of the trivalent cations. Figure 6 shows the SWV results for trivalent cations, Al(III), La(III), and Lu(III) and divalent cations, Be(II), Mg(II), and Sr(II). The response of the trivalent cations was obviously observed and readily discriminated from divalent cations below 0.5 mM range at pH 4.0. The change in the SWV intensity of the trivalent cations was used to determine the adsorption constants of cations to the surface CA layer using Langmuir-type isotherm. The $\log a$ values of trivalent cations are also calculated to be 3.5 ± 0.1 , 3.5 ± 0.1 , and 3.8 ± 0.1 for Al(III), La(III), and Lu(III), respectively. In the response of divalent cations, a slight decrease in the current intensity is observed for a set of experiments. This phenomenon can not be clearly explained, but may be associated with the interaction between $\text{Fe}(\text{CN})_6^{3-4-}$ and the divalent cations in the solution phase²³ or changes in the molecular conformation and electronic structure of CA moiety on the electrode surface upon the complexation with metal

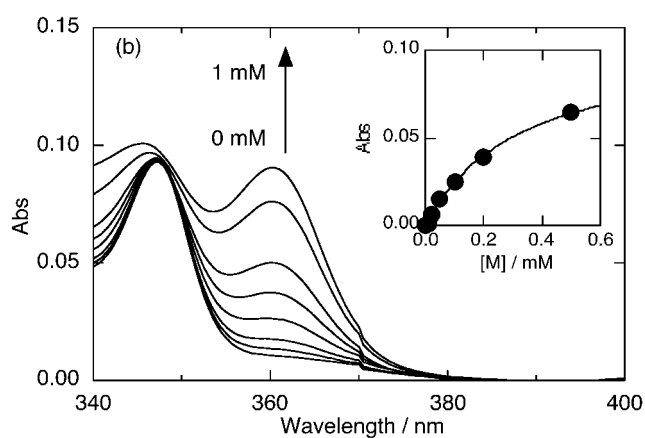
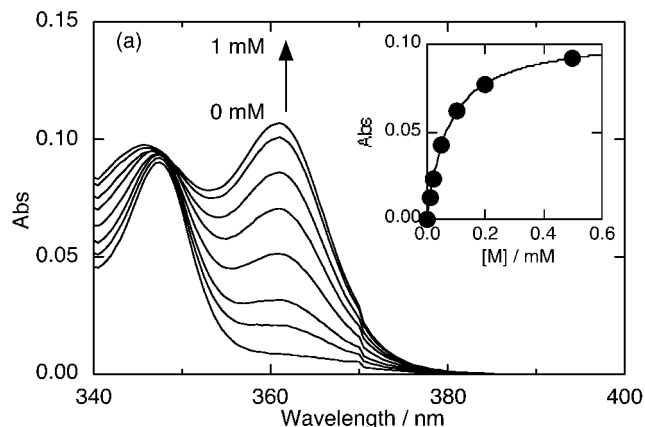


Fig. 7 UV/Vis spectra of 10 μ M CA in 0.1 M KCl (pH 4.0) upon the addition of (a) Al(III) and (b) Be(II). [M] = 0.01 to 1.0 mM. (inset) Changes in absorbance at 361 nm against the concentration of metal cations. The solid line shows the values calculated from K' of the 1:1 complex.

cations.

The UV/Vis spectra of CA at pH 4.0 exhibit a distinct change upon the addition of both Al(III) and Be(II) as shown in Fig. 7, and thus the wavelength at the maximum absorbance of CA is varied from 347 to 361 nm. The shapes of the spectra resemble each other and the effect of the metal concentration is quite similar for both Al(III) and Be(II). Indeed, 1:1 stability constants ($\log \beta$) of Al(III) and Be(II) with CA were reported to be 17.2 and 16.7, respectively.²⁴ It should be emphasized that the CA moiety do react for both Al(III) and Be(II) at pH 4.0, whereas the electrochemical response of Al(III) is clearly discriminated from that of Be(II) (Fig. 4). This strongly demonstrates that the change in current intensity by the addition of metal ions is ascribed to the change in the surface charge density of CA/GC electrode.

Conclusions

We demonstrated here, in combination with the CA-metal cation complexation on the surface of an electrode, that the CA/GC electrode selectively discriminates valency of cations with significant changes in electrochemical response with the aid of a negatively charged probe. The CA moiety was successfully

grafted on the GC electrode surface by electrochemical reduction of *in situ* generated diazonium cation containing the CA group. Grafted electrodes were capable of complexation with several metal cations in an aqueous solution at proper pH. Electrochemical response of $\text{Fe}(\text{CN})_6^{3-/4-}$ at the CA/GC electrode was utilized to evaluate the complexation between electrochemically inactive metal cations and the CA moiety on the electrode surface. From these experiments, we conclude that regulating the surface coverage of CA is achieved by the number of potential cycling during the grafting procedure and is an important factor affecting current response for the electrochemical detection of metal cations. The “signal-on” type response observed is responsible for the change in the charge density on the CA/GC electrode surface. This provides evidence that arylradical methods may hold promise in constructing chemical- and biosensor. Further examination of various types of chelating ligand may lead to a new design of amperometric ion sensors; such studies are now in progress in our laboratory.

Acknowledgements

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