Determination of Hg_2^{2+} Ions Using a Modified Glassy Carbon Electrode with 2,2':6':2"-Terpyridine

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Received June 28, 2001

A glassy carbon electrode (GCE) modified with 2,2':6':2"-terpyridine (2,2':6':2"-TPR) using a spin coating method was applied for the highly selective and sensitive analysis of a trace amount of Hg_2^{2-} ions. Various experimental parameters, which influenced the response of the 2,2':6':2"-TPR modified electrode to Hg_2^{2+} ions, were optimized. The linear sweep and differential pulse voltammograms for the 2,2':6':2"-TPR modified electrode deposited with Hg show a well-defined anodic peak at +0.65 V (*vs.* Ag|AgCl). After a 25 min preconcentration time in an Hg_2^{2-} ion solution (0.1 M acetate buffer, pH 5.0), differential pulse voltammetry (DPV) with 2,2':6':2"-TPR modified electrode shows a linear response between 1.0×10^{-6} M and 2.0×10^{-7} M. The least-square treatment of these data produce an equation of I [μA] = 0.031 + 0.005 C with r = 0.980 (n = 5). The detection limit of this electrode with linear sweep voltammetry and differential pulse anodic voltammetry were 2.0×10^{-6} M and 8.0×10^{-8} M, respectively. The presence of Pb, Fe, Cd, Ti, Ni, Co, Mg, Al, Mn, and Zn did not interfere in the analysis of the Hg_2^{2-} ion. The 2,2':6':2"-TPR modified GCE has been successfully applied in determination trace amounts of Hg in a human urine sample.

Keywords : Hg_2^{2+} ions, Modified glassy carbon electrode, 2,2':6':2"-Terpyridine, Spin coating method, Voltammetric techniques.

Introduction

Heavy metals have received more attention in the environmental, pharmaceutical and biomedical analysis.[1-5] Among those analysis, the detection of mercury has been the subject of an increasing number of reports. Various methods for the determination of the total mercury content have been reported, including the atomic absorption spectrometry, atomic emission spectrometry, X-ray fluorescence, and inductively coupled plasma mass.[6-9] However, these techniques often require much times to treat, enrichment of the sample, and expensive instrumentations.

Recently, numerous voltammetric techniques[10-16] particularly stripping voltammetry with chemically modified electrodes (CMEs) have been used also widely for the trace analysis of heavy metal ions, because they have some advantages of little or no sample pretreatment and low-cost instrumentation. The stripping method often employs CMEs to selectively preconcentrate the analyte on the surface *via* chemical reactions. Thus, using CMEs with the stripping method gives a versatile preconcentration range with little interference compared to conventional stripping voltammetric techniques. Another advantages of using CMEs are exchanging the sample solution with a clean electrolyte before the stripping step, so that one may effectively bypass the host of electroactive species interferences. To determine the metal ions with a CME, the deposition of the test ions can be made on the modified electrodes that react with the test ions through the complexation reaction or adsorption on the electrode surface. This usually forms insoluble complexes on the electrode in aqueous solution. We have been studied on the determination of Hg^{2+} ions with the carbon paste electrode (CPE) containing *l*-sparteine,[17] a simultaneous determination of Pb^{2+}, Cu^{2+} and Hg^{2+} ions with the CPE containing humic acid,[18] and a simultaneous determination of Hg^{2-} and Ag^{+} ions with the CME containing glyoxyal bis(2-hydroanil).[19]

2,2':6':2"-terpyridine (2,2':6':2"-TPR) has been previously known as a complex agent that forms a complexes with heavy metal ions.[20-23] However, there is no report on the application of 2,2':6':2"-TPR as a modifier to determine mercury in voltammetric techniques. In our preliminary work, the 2,2':6':2"-TPR modified GCE had an electrochemical response for the for 1.0×10^{-5} M Hg_2^{2-} ion at +0.65 V, but the response for the 1.0×10^{-5} M Hg^{2-} ion couldn't be observed in the same experimental conditions. These results indicated that the 2,2':6':2"-TPR modified GCE have higher specific reaction toward an Hg_2^{2+} ions than an Hg^{2-} ions in an aqueous solution. Thus, we studied a method to selectively determine the Hg_2^{2+} ion in an aqueous solution using the 2,2':6':2"-TPR modified GCE by voltammetric techniques.

In the present study, we report the result of the applicability of 2,2':6':2"-TPR modified GCE for determining of Hg_2^{2+} ions with the stripping voltammetry. The effects of analytical parameters (*e.g.*, the effect of pH, preconcentration temperature, preconcentration time, and interference), which affect electrode reactions and analysis processes, were studied using the linear sweep voltammetry (LSV) and differential pulse voltammetry (DPV). This method has been evaluated by analyzing Hg in a human urine sample.

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Experimental Section

Reagents and Apparatus. 2,2':6',2"-TPR was purchased from Aldrich Co. and used without further purification. Metal solutions were prepared from metal salts from Aldrich Co. and diluted as required. Mercuric nitrate and hydroxylamine hydrochloride were used for preparing the Hg_2^{2+} ion solution. Another reagents were of an analytical grade and did not undergo further purification unless otherwise specified. A 0.1 M acetate buffer solution (pH 5.0) served as a supporting electrolyte. All experimental solutions were prepared in doubly distilled water obtained from a Milli-Q water purification system (Millipore).

A Model CV-50W and a Pine Instrument AFRDE4 bipotentiostat/galvanostat were used for voltammetric experiments. Voltammograms were recorded on a Kipp & Zonnen BD90 X-Y recorder. A conventional three-electrode cell involving a glassy carbon electrode (GCE) modified with 2,2':6':2"-TPR as a working electrode, Ag|AgCl (KCl-saturated) as a reference electrode, and Pt wire as a counter electrode were used. All experimental solutions were deaerated with a nitrogen gas for at least 10 min and maintained under the nitrogen atmosphere while voltammetric measurement. During the preconcentration of Hg_2^{2+} ions, the sample solution was stirred without any potential applied.

Fabrication of 2,2':6',2"-TPR Modified GCE. Prior to coat the 2,2':6':2"-TPR on the GCE by the spin-coating technique, we pretreated the GCE as follows: GCE was polished with a alumina slurry (0.05 μm) on a polishing cloth and washed with deionized water followed by ultrasonication. After polishing, the electrochemical pretreatment was performed by a potential step applying +0.85 V for 5 min and -1.4 V for 1 min in 0.1 M H_2SO_4. Then, spin-coating using a homemade rotator with the spin speed of 600 rpm performed the modification of GCE with 2,2':6':2"-TPR. A 1.0×10^{-3} M of 2,2':6',2"-TPR in chloroform solution was used with the amount of four drops of 10 μL.

Analytical Procedure. The stock solution (1.0×10^{-3} M) of Hg_2^{2+} ions, which was made by dissolved five times excess NH_2OH-HCl in the Hg^{2+} solution, was diluted to an adequate concentration and used immediately before each measurement. After preconcentration of Hg_2^{2+} ions was carried out in a 0.1 M acetate buffer solution (pH 5.0) containing Hg_2^{2+} ions for 25 min at an open circuit, the 2,2':6':2"-TPR modified GCE was taken out from the preconcentration solution and then washed with a distilled water thoroughly. Then, the 2,2':6':2"-TPR modified GCE transferred to a separate measuring cell containing a fresh 0.1 M acetate buffer (pH 5.0) solution. In every voltammetric measurement, an initial potential of -0.5 V was applied for one minute to reduce Hg_2^{2+}-complex to Hg^0 on the electrode surface. After electrochemical reduction, the potential sweep started with the scan rate of 100 mV s^{-1} for LSV and 5 mV s^{-1} for DPV, respectively.

Determination of Mercury in Standard Urine Sample. The standard urine sample (SRM 2670, NIST, USA) was taken in 2.5 mL in a pyrex beaker. It was then decomposed in a 5 mL boiling concentrated nitric acid. All mercury ions in the sample solution were converted into Hg_2^{2+} ions through a treatment using five times the excess hydroxylamine hydrochloride. The 2,2':6':2"-TPR modified GCE was immersed in the sample solution to preconcentrate the test ion by stirring for 25 min. After concentration the sample ion on the GCE, the electrode was taken out of the sample solution, and then washed thoroughly with deionized water. Stripping voltammograms were recorded in the blank solution of a 0.1 M acetate buffer solution.

Results and Discussion

Electrochemical Behaviors of 2,2':6':2"-TPR Modified GCE. Figure 1 shows the linear sweep voltammograms (LSVs) recorded for the 2,2':6':2"-TPR modified GCE in a 0.1 M acetate buffer solution (pH 5.0) after the preconcentration in a blank solution (curve-a) and after (curve-b) the preconcentration in a 1.0×10^{-4} M Hg_2^{2+} solution, respectively. As shown in Figure 1-a, the 2,2':6':2"-TPR modified GCE showed no electroactivity in the potential range between -0.3 V and 1.0 V. However, the LSV (Figure 1-b) of the CME after the preconcentration of Hg_2^{2+} ions had taken place for 25 min, shows well-defined anodic peaks at +0.65 V and +0.5 V. The standard reduction potential of Hg_2^{2+} and Hg^{2+} to Hg^0 was +0.792 V and +0.854 V (*vs.* NHE), respectively.[24] Thus, the first small anodic peak (+0.50 V) corresponds to the oxidation of a reduced Hg^0 to Hg_2^{2+} species. The second (+0.65 V) corresponds to the oxidation of a reduced Hg^0 to Hg^{2+} ions.[19] Moreover, the anodic peak current observed at +0.65 V was directly proportional to the concentration of Hg_2^{2+} ions and preconcentration time. These results indicate that the preconcentration of Hg_2^{2+} ions

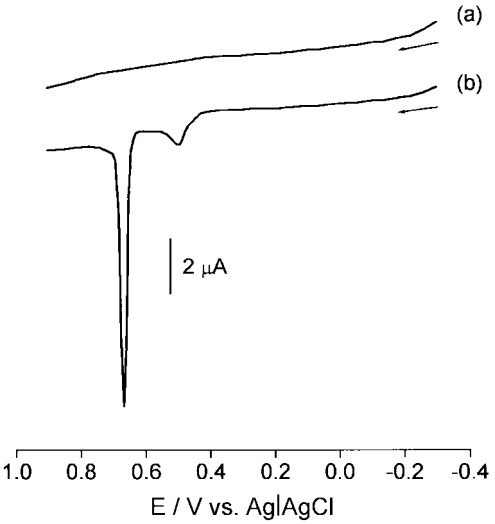

Figure 1. Linear sweep voltammograms for the 2,2':6':2"-TPR modified GCE in a 0.1 M acetate buffer solution (pH 5.0): (a) after dipping the 2,2':6':2"-TPR modified GCE in a blank solution, and (b) after dipping the 2,2':6':2"-TPR modified GCE in a 1×10^{-4} M Hg_2^{2+} solution. (Temperature: 25°C, preconcentration time: 25 min, and scan rate was 100 mV s^{-1}).

is occurring on the 2,2':6':2''-TPR modified electrode surface. Thus, we used this anodic peak as the analytical signal. Moreover, the anodic peak currents observed at +0.65 V were directly proportional to the square root of the scan rate. This indicates that the oxidation process of the mercury ions was diffusion controlled from the CME surface into the bulk solution. However, the same experiment in a 1.0×10^{-4} M Hg^{2+} solution yielded no stripping peaks of Hg^0. This means that the Hg^{2+} ion did not respond to the 2,2':6':2''-TPR modified GCE. We may suggest the analysis mechanism of mercury as follows:

Hg_2^{2+} + TPR (on the GCE) → Hg_2^{2+}-TPR complex
: Preconcentration step
Hg_2^{2+}-TPR complex (on the GCE) → Hg^0 (on the GCE) + TPR : Reduction step
Hg^0 (on the GCE) → $Hg_2^{2+} + e^-$ (+0.5 V).
→ $Hg^{2+} + 2e^-$ (+0.65 V) : Stripping steps

Analytical Conditions for Hg_2^{2+} Ions. In order to determine the optimum conditions for the analysis of Hg_2^{2+} ions with the 2,2':6':2''-TRP modified GCE, we investigated various experimental parameters, such as the preconcentration time, temperature, and pH of the media, which affect to analytical sensitivity.

Figure 2 shows a variation of the anodic peak current according to the pH of the preconcentration solution. Maximum peak height was achieved when the preconcentration was done at pH 5.0. It may be explained that the interaction between Hg and 2,2':6':2''-TPR are weakened at pH < 5, because the organic ligand undergoes protonation. Above the optimum pH, Hg_2^{2+} could be changed to produce $Hg(OH)_2$[18,26] by disproportionation and then it interferes the accumulation. Similar results were reported for studying the effect of the pH on the voltammetric determination of

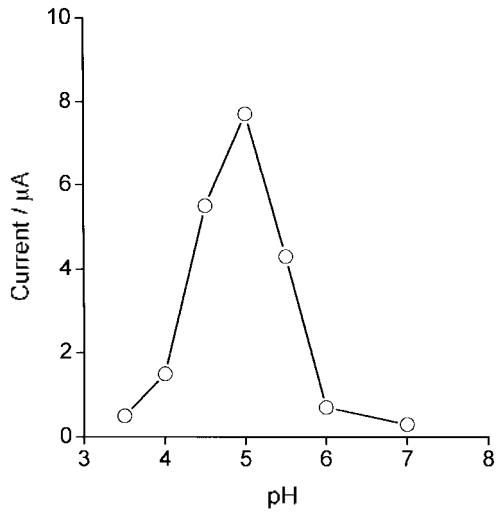

Figure 2. Effect of the pH on the stripping peak current of Hg_2^{2+} ions using the 2,2':6':2''-TPR modified GCE in a 0.1 M acetate buffer solution (pH 5.0) containing 1×10^{-4} M Hg_2^{2+} ions. (Preconcentration time: 25 min; temperature: 25°C; and scan rate: 100 mV s^{-1}).

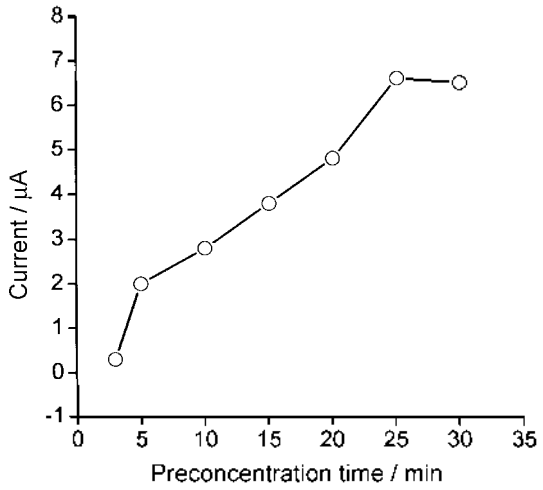

Figure 3. Effect of the preconcentration time on the stripping peak current of Hg_2^{2+} ions using the 2,2':6':2''-TPR modified GCE in a 0.1 M acetate buffer solution (pH 5.0) containing 1×10^{-4} M Hg_2^{2+} ions. (Temperature: 25°C; and scan rate: 100 mV s^{-1}).

mercury by other modifier.[19,25]

The dependence of the anodic peak current on the concentration time in a 1.0×10^{-4} M Hg_2^{2+} solution (pH 5.0) is shown in Figure 3. The stripping peak gradually increased and the peak height was constant after 25 min. A limiting value of the current for the longer accumulation is due to the reaching saturation of the complexation reaction between 2,2':6':2''-TPR and Hg_2^{2+} ions.

We also obtained a plot of variation for the stripping current according to the change of the preconcentration temperature. The 2,2':6':2''-TRP modified GCE was dipped into the 1.0×10^{-4} M Hg_2^{2+} solution for 25 min at various temperatures. As the temperature of the preconcentration solution increased from 15 °C to 40 °C, the anodic stripping current increased. Maximum stripping current was observed at 25 °C. These indicate that the Hg_2^{2+}-2,2':6':2''-TPR complex formed on the CME surface is most stable at 25 °C. Above this temperature, the complex should be more soluble or unstable in the adsorbed state. This makes a decrease in the magnitude of the anodic stripping current at higher temperatures above 25 °C. At lower temperatures less than 25 °C, the complex formation reaction should be not easy than that of above 25 °C.

The interference effect were investigated for several metal ions, which were expected to disturb the determination of test ions through their complex formations with 2,2':6':2''-TRP. Metal ions tested in this experimental were Mn^{2+}, Ca^{2+}, Ba^{2+}, Zn^{2+}, Ti^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, Cr^{3+}, Al^{3+}, Mg^{2+}, Pb^{2+}, and Ag^+ ions. Among these ions, only coexistence of Ag^+ ion (1.0×10^{-5} M) in the test solution showed a decrease of the analytical current corresponding to the Hg_2^{2+} ions (1.0×10^{-5} M). The decrease was about 15%. However, the interference by Ag^+ ion can be overcome by pretreating sample solution with chloride ions and thereby we avoided the interference from Ag^+ ion. This means that 2,2':6':2''-TPR modified GCE is a much higher affinity for Hg_2^{2+} ions than for other

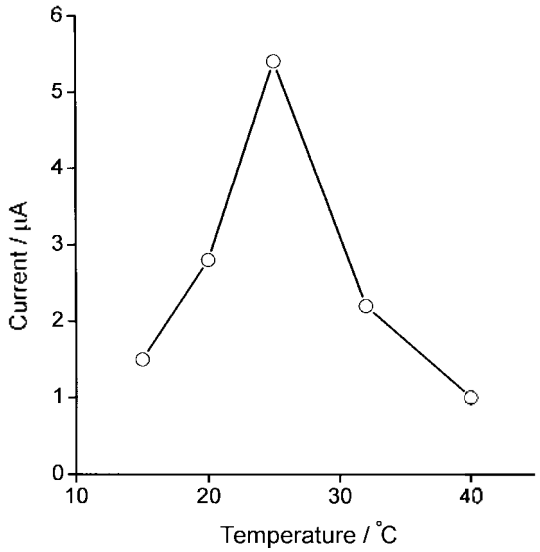

Figure 4. Effect of the preconcentration temperature on the stripping peak current of Hg_2^{2+} ions with the 2,2':6':2''-TPR modified GCE in a 0.1 M acetate buffer solution (pH 5.0) containing 1×10^{-4} M Hg_2^{2+} ions. (Preconcentration time: 25 min; and scan rate: 100 mV s^{-1}).

metal ions.

Figure 5 shows the calibration plot obtained from (a) LSV and (b) DPV with the 2,2':6':2''-TPR modified GCE in a 0.1 M acetate solution (pH 5.0). The calibration plot from LSV yields a linearity between 1.0×10^{-5} M and 3.0×10^{-6} M and the one from DPV between 1.0×10^{-6} M and 7.0×10^{-8} M. The least-square treatment of DPV data produced an equation of I [μA] = 0.031 + 0.005 C([Hg_2^{2+}] $\times 10^7$) with r = 0.980 (n = 5). The DPV measuring conditions were as follows: scan rate 5 mV s^{-1}; pulse height: 25 mV; pulse width: 50 ms. On the basis of a signal to background characteristics of the response (signal/noise = 3), a detection limit with linear sweep and differential pulse anodic voltammetry were 2.0×10^{-6} M and 8.0×10^{-8} M of Hg_2^{2+} ions, respectively.

Analytical Application for the Real Sample. To demonstrate the availability of the analysis method with the 2,2':6':2''-TPR modified GCE for a real sample, we examined the concentration dependence and a precision test. Prior to the preconcentration and voltammetric measurements, the decomposition of the urine sample was carried out in a concentrated nitric acid. Moreover, all the mercury ions were converted into Hg_2^{2+} ions through a treatment using five times the excess of hydroxylamine hydrochloride. The certified Hg concentration was 105 ppb (it was used after dilution to 84 ppb) and other metal ions presented in the 2-370 ppb range. Standard urine sample includes Ag, Al, As, Be, Cd, Ca, Cr, Cu, Au, Pb, Mg, Mn, Ni, Pt, K, Se, Na, and V. The determined concentration of Hg with the 2,2':6':2''-TPR modified GCE was 80 ppb (SD: 2.3 ppb) from five times differential pulse stripping voltammetric measurements. It shows that the experimental value sufficiently corresponds with the certified value of the urine sample without any big interference from the diverse metal ions.

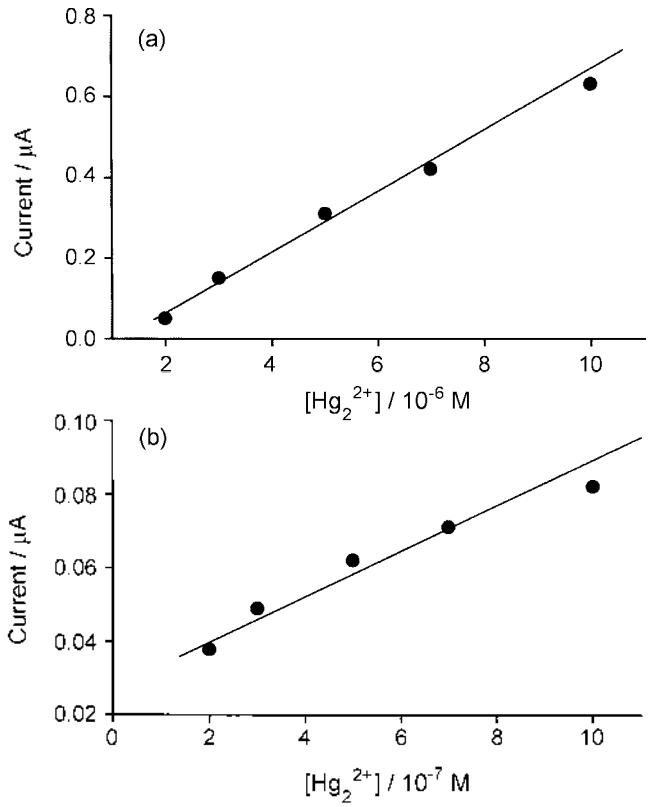

Figure 5. The calibration plot for the analysis of Hg_2^{2+} ions with the 2,2':6':2''-TPR modified GCE in a 0.1 M acetate buffer solution (pH 5.0) using the linear sweep voltammetry (curve-a) and differential pulse voltammetry (curve-b). (Preconcentration time: 25 min; scan rate: 5 mV s^{-1}; pulse height: 25 mV; and pulse width: 50 ms).

Conclusion

The 2,2':6':2''-TPR modified GCE shows highly specific interaction with Hg_2^{2+} than with other metal ions. In this study, optimum experimental conditions were as follows: the pH of the preconcentration solution was 5.0, the preconcentration time was 25 min, and the temperature of the preconcentration solution was 25°C. The detection limit of the Hg_2^{2+} ion was 2.0×10^{-6} M and 8.0×10^{-8} M, respectively. The electrode was successfully applied to determine trace amounts of Hg in a human urine sample without any interference from other metal ions.

Acknowledgment. This work was supported in part by the "Center for Integrated Molecular Systems" through The Korean Science and Engineering Foundation and the post-doctoral fellowship of Brain Korea 21 project.

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