

## Simultaneous determination of lead and copper by anodic stripping voltammetry using a poly(L-glutamic acid) modified electrode

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Received 25 September 2016; revised and accepted 27 January 2017

A new anodic stripping voltammetric method using a poly(L-glutamic acid) modified electrode has been developed for the separation and simultaneous determination of trace amounts of lead(II) and copper(II). The stripping peak currents are well proportional to the concentration of lead and copper over the range of  $5.00 \times 10^{-8}$ – $1.00 \times 10^{-4}$  mol L<sup>-1</sup>, with detection limits of  $5.0 \times 10^{-9}$  mol L<sup>-1</sup> and  $1.0 \times 10^{-8}$  mol L<sup>-1</sup>, respectively. The method has been successfully applied to the analysis of Pb<sup>2+</sup> and Cu<sup>2+</sup> in the waste water samples with good precision and accuracy.

**Keywords:** Electroanalysis, Anodic stripping voltammetry, L-Glutamic acid, Modified electrodes, Lead, Copper

Lead is known to be a toxic heavy metal that can damage nervous system, immune system and genital system due to its slow metabolic process in the human body<sup>1-4</sup>. Evidence suggests that lead, even in very low concentrations, exhibits deleterious effects on humans and animals<sup>5</sup>. Also, exposure to lead is responsible for 0.6% of global diseases according to World Health Organization<sup>6</sup>. Furthermore, copper is an essential microelement for plants, animals and humans, but excess of copper can cause various serious adverse health effects<sup>7-11</sup>. The extensive utilization of lead and copper leading to extensive environmental pollution and deleterious effects on humans, makes it necessary to develop sensitive, simple and inexpensive methods for trace determination of these two metal ions. Besides, different metal ions often coexist in the same sample and being able to simultaneously determine these ions is important<sup>12</sup>.

Various techniques have been applied to the simultaneous trace metal analyses, including atomic absorption spectrophotometry (AAS)<sup>13-15</sup>, inductively coupled plasma atomic emission spectrometry

(ICP-AES)<sup>16,17</sup> and inductively coupled plasma mass spectrometry (ICP-MS)<sup>18,19</sup>. Although these techniques are highly selective, sensitive and offer a wide range of linearity, they are expensive and require careful sample preparation. Chemometrics methods based on professional analysis software such as second-derivative spectrophotometry method<sup>20</sup>, neural network method<sup>21</sup> and least-squares method<sup>21</sup> also have been widely used for the simultaneous determination of metal ions and have shown good results. However, multiple analytical procedures are required in these methods. An alternative powerful tool is the electrochemical technique of anodic stripping voltammetry (ASV), which is inexpensive, remarkably sensitive, simple to operate, compact instrumentation and convenient approach to metals analysis<sup>12</sup>.

In electrochemistry, chemosensors and chemically modified electrodes have been proven to be important and reliable methods for the simultaneous determination of trace metal ions. Some chemosensors applied to the simultaneous analysis of metal ions have shown good selectivity and sensitivity<sup>23</sup>, however, long preparation time is needed. Chemically modified electrodes obtained by mechanical or electrochemical deposition of the modifier have proved to be more sensitive, selective and effective than bare electrodes in the determination of metals by electrochemical method<sup>24</sup>. In order to obtain better detection, a variety of polymers have been applied to modify electrode surfaces. Currently, modifiers used for the simultaneous determination of metals include 2-aminothiazole-silica-gel<sup>25</sup>, pyruvaldehyde bis(N,N-dibutyl thiosemicarbazone)<sup>26</sup>, and carbon-ink screen-printed polymer<sup>12</sup>. Compared with these polymers, poly(amino acid) as a biocompatible polymer is very promising. Amino acids with amine and carboxylic acid functional groups possess specific properties such as stability and good biocompatibility. Moreover, amino acids are easily available materials and can be easily deposited on bare electrode surfaces<sup>27</sup>. The poly(amino acids) modified glassy carbon electrodes have been widely used in many fields, especially for the determination of medicine, biological material and small organic molecule<sup>28-33</sup>. To the best of our knowledge, the simultaneous determination of Pb<sup>2+</sup> and Cu<sup>2+</sup> using a poly(L-glutamic acid) modified electrode has not been studied.

Herein, we aim to develop a convenient and selective electrochemical method for simultaneous determination of lead(II) and Cu(II) by ASV using a poly(L-glutamic acid) modified glassy carbon electrode (PLA/GCE).

### Experimental

Electrochemical measurements were obtained using a BAS 100B/W electrochemical work station (BAS group, USA). A conventional three-electrode system was used for all electrochemical experiments, including an Ag/AgCl (saturated KCl) as the reference electrode, a platinum wire as the auxiliary electrode, and the bare glassy carbon electrode (3 mm in radius) or poly(L-glutamic acid)-modified glassy carbon electrode as the working electrode. The pH values were determined with a PHS-3C digital pH meter (Shanghai Leici Device Works, China). Flame atomic absorption spectrometry analysis was carried out with a Shimidzu AA-6701f atomic absorption spectrophotometer (Shimidzu Corporation, Japan).

L-Glutamic acid,  $\text{Cu}(\text{NO}_3)_2$ , and  $\text{Pb}(\text{NO}_3)_2$  were purchased from Sigma-Aldrich. All the other chemicals used were of analytical grade purity. The pH of buffer solution was adjusted to 6.0 by addition of  $0.1 \text{ mol L}^{-1}$  HAc and  $0.1 \text{ mol L}^{-1}$  NaAc. All the solutions were prepared with doubly distilled deionized water.

For the preparation of the poly(L-glutamic acid) modified electrode, the bare GCE was polished with  $0.05 \mu\text{m}$   $\text{Al}_2\text{O}_3$  powder, up to a mirror finish and then it was successively rinsed with 1:1  $\text{HNO}_3$  solution, ethanol and doubly distilled water. After that, the GCE was placed in an ultrasonic bath for 10 minutes. After dry in air, L-glutamic acid was electrochemically deposited on the bare GCE by cyclic sweeping from  $-1.0$  to  $2.1 \text{ V}$  at the scan rate of  $120 \text{ mV s}^{-1}$  for 6 cycles in  $0.1 \text{ mol L}^{-1}$  NaAc-HAc buffer solution (pH 3.0) containing  $2.25 \times 10^{-3} \text{ mol L}^{-1}$  L-glutamic acid. After polymerization, the poly(L-glutamic acid)-modified electrode was washed in doubly distilled water and dried at room temperature.

$\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  were deposited on PLA/GCE by dipping the electrode into  $0.1 \text{ mol L}^{-1}$  NaAc-HAc buffer solution (pH 3.0) containing the heavy metal ions at an applied potential of  $-0.8 \text{ V}$  for 5 minutes. After a quiet time of 30 s, the linear sweep anodic stripping voltammograms were recorded in the potential range of  $-0.8$  to  $0.4 \text{ V}$  at a scan rate of  $2 \text{ mV s}^{-1}$ . All experiments were performed at room temperature.

### Results and discussion

Electrochemical impedance spectra (EIS) is a powerful method to study diffusion effects at modified electrodes<sup>34</sup> and a  $[\text{Fe}(\text{CN})_6]^{4-/3-}$  redox couple is an important electro active probe. Fig. 1 depicts the EIS of the bare GCE (curve 1) and the PLA/GCE (curve 2) in  $5.0 \times 10^{-3} \text{ mol L}^{-1}$   $\text{K}_3[\text{Fe}(\text{CN})_6]$  and  $1.0 \text{ mol L}^{-1}$  KCl. From Fig. 1, it can be seen that the semicircle diameter in higher frequency region of the PLA/GCE is smaller than that of bare GCE, indicating fast electron transfer kinetics at the PLA/GCE. This indicates great electron transfer resistance of PLA/GCE due to electron transfer being facilitated by the modification of L-glutamic acid on the bare GCE. Meanwhile, the linear part of the spectrum represents the diffusional limited electron transfer process.

The dependence of the anodic peak currents of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  on the scan rates at the PLA/GCE was studied individually. Figure 2 displays the cyclic voltammograms of  $1.00 \times 10^{-5} \text{ mol L}^{-1}$   $\text{Pb}^{2+}$  (Fig. 2(a)) and  $\text{Cu}^{2+}$  (Fig. 2(b)) at different scan rates using the PLA/GCE. The anodic peak currents of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  were linearly proportional to the square root of scan rate. The regression equations are:  $I_{pa}(\text{Pb}^{2+}) = -1.252 \times 10^{-7} + 1.343 \times 10^{-6} \sqrt{v}$ ,  $R = 0.9989$ ;  $I_{pa}(\text{Cu}^{2+}) = 2.425 \times 10^{-7} + 1.78 \times 10^{-7} \sqrt{v}$ ,  $R = 0.9946$ . This clearly reveals that the PLA/GCE reactions of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  are both diffusion-controlled processes.

For comparison, the ASV of bare GCE and PLA/GCE for the simultaneous determination of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  in  $0.1 \text{ mol L}^{-1}$  NaAc-HAc buffer solution at pH 6.0 were also recorded. As is shown in Fig. 3, with the bare GCE (curve 1) or PLA/GCE (curve 2), the anodic peak currents of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  obtained on PLA/GCE are much larger and the stripping peaks are

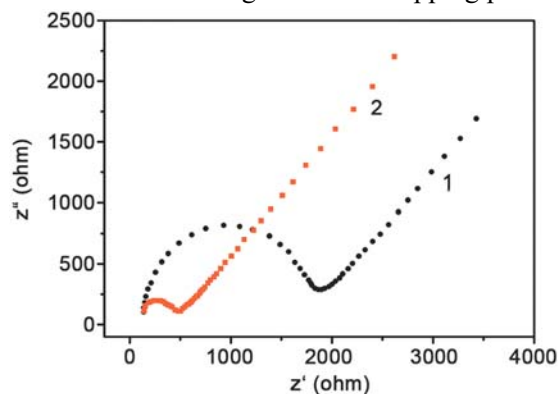


Fig. 1—Electrochemical impedance spectra of bare GCE (1) and poly(L-glutamic acid) modified glassy carbon electrode (2) in the solution of  $\text{K}_3[\text{Fe}(\text{CN})_6]$  and KCl.

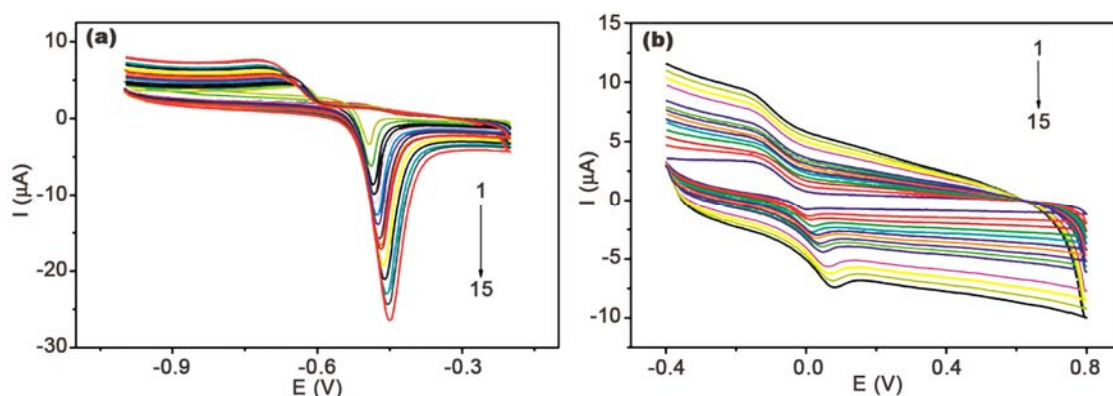


Fig. 2—Cyclic voltammograms of  $1.00 \times 10^{-5} \text{ mol L}^{-1} \text{ Pb}^{2+}$  (a) and  $\text{Cu}^{2+}$  (b) at various scan rates. [1-15: 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 240, 280, 320, 360,  $400 \text{ mV s}^{-1}$ ].

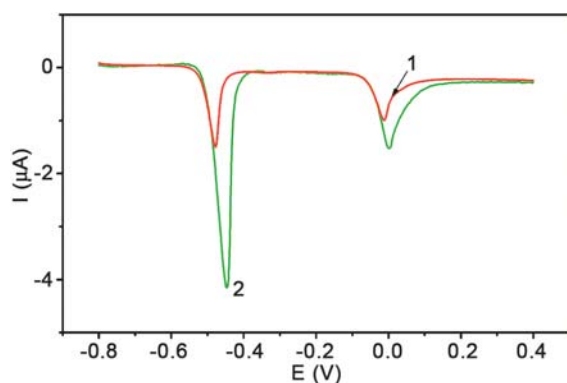


Fig. 3—Stripping voltammograms of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  at bare GCE (1) and poly(L-glutamic acid) modified glassy carbon electrode (2).

much sharper, which may be due to the increased electrochemical activity of L-glutamic acid with amidogen and carboxyl. In addition, it can also be seen from Fig. 3 that the potential scan between  $-0.8 \text{ V}$  and  $0.4 \text{ V}$  can be used for the simultaneous determination of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$ .

To investigate the pH effect, the electrochemical responses to  $1.00 \times 10^{-5} \text{ mol L}^{-1} \text{ Pb}^{2+}$  and  $\text{Cu}^{2+}$  at PLA/GCE were recorded in the pH range of 3.0–7.0 (Supplementary data, Fig. S1). It was observed that the maximum peak currents of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  appeared at 3.0 with good peak separation. Therefore, pH 3.0 was selected as the best buffer solution value for simultaneous determination of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$ .

To further achieve good sensitivity, the influence of scan rates was also investigated in the range of  $2\text{--}20 \text{ mV s}^{-1}$ . The results showed that  $2 \text{ mV s}^{-1}$  was optimum for simultaneous determination condition for  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$ , based on the largest peak currents. At the lower scan rates, the slower oxidation rate resulted in the increase in the amount of unoxidized metal which can transfer electrons faster.

In order to obtain the appropriate deposition potential value, the stripping voltammetry experiments with solution containing  $1.00 \times 10^{-5} \text{ mol L}^{-1} \text{ Pb}^{2+}$  and  $\text{Cu}^{2+}$  were recorded in the deposition potential range of  $-0.7$  to  $-1.2 \text{ V}$ . The results indicated that  $-0.8 \text{ V}$ , and hence  $-0.8 \text{ V}$  was considered as the optimum deposition potential value corresponded to the maximum current value.

To validate the effect of deposition time on the simultaneous determination of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$ , the stripping voltammetric signals were studied on the modified electrode from 1–8 min under fixed pH 3.0 and deposition potential  $-0.8 \text{ V}$ . The peak currents increased obviously as the deposition time increased from 1 to 4 min, whereas it increased slightly when the deposition time increased 4 to 8 min. Considering a high signal and a reasonable detection period, deposition time of 5 min was used in further experiments.

Under the above optimum conditions, the stripping performance for the simultaneous determination of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  on the PLA/GCE by ASV was recorded. Well resolved peaks were recorded with increasing concentrations of the two metal ions (Fig. 4a) and the stripping signals increased linearly with concentration of the two metal ions (Fig. 4b). The analytical parameters for the individual and simultaneous determination of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  are listed in Table 1.

The effect of many common interferences was tested on the simultaneous determination of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  by the proposed ASV method. A relative error of less than  $\pm 5\%$  was considered as insignificant interference. It was observed that the signals of  $1.00 \times 10^{-5} \text{ mol L}^{-1} \text{ Pb}^{2+}$  and  $\text{Cu}^{2+}$  were not affected by 1000 folds of threonine, histidine, ascorbic acid, formaldehyde, ethyl alcohol, phenol,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{SO}_4^{2-}$ ,  $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$  and  $\text{Cl}^-$ .

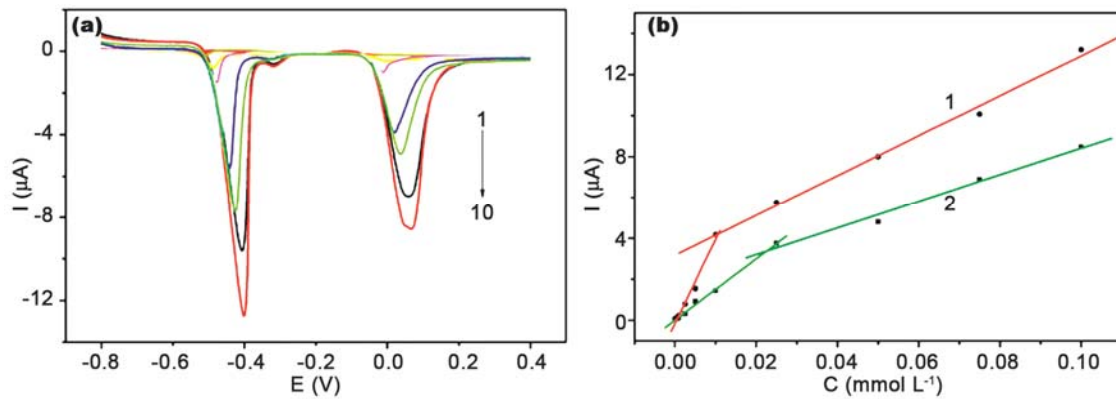


Fig. 4—(a) Stripping voltammograms of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  at various concentrations (1-10:  $5 \times 10^{-8}$ ;  $5 \times 10^{-7}$ ;  $1 \times 10^{-6}$ ;  $2.5 \times 10^{-6}$ ;  $5 \times 10^{-6}$ ;  $1 \times 10^{-5}$ ;  $2.5 \times 10^{-5}$ ;  $5 \times 10^{-5}$ ;  $7.5 \times 10^{-5}$ ;  $1 \times 10^{-4}$  mol  $\text{L}^{-1}$ , and, (b) relationship curves between the peak current and varying concentrations of  $\text{Pb}^{2+}$  (1) and  $\text{Cu}^{2+}$  (2).

Table 1 — Analytical parameters for the individual and simultaneous determination of  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  at poly(L-glutamic acid) modified electrode

Method	Analyte	Linear range (mol $\text{L}^{-1}$ )	Linear regression eq. <sup>a</sup>	Corr. coeff.	Detection limit (mol $\text{L}^{-1}$ )
Individual	$\text{Pb}^{2+}$	$5.00 \times 10^{-8} - 1.00 \times 10^{-5}$	$I_{pa} = 9.930 \times 10^{-8} + 0.3872c$	0.9925	$5.00 \times 10^{-9}$
		$1.00 \times 10^{-5} - 1.00 \times 10^{-4}$	$I_{pa} = -3.766 \times 10^{-8} + 0.2778c$	0.9950	
	$\text{Cu}^{2+}$	$1.00 \times 10^{-7} - 2.50 \times 10^{-5}$	$I_{pa} = 7.760 \times 10^{-7} + 0.1606c$	0.9988	
		$2.50 \times 10^{-5} - 1.00 \times 10^{-4}$	$I_{pa} = 2.746 \times 10^{-6} + 0.07005c$	0.9900	
Simultaneous	$\text{Pb}^{2+}$	$5.00 \times 10^{-8} - 1.00 \times 10^{-5}$	$I_{pa} = 1.539 \times 10^{-7} + 0.4123c$	0.9903	$5.00 \times 10^{-9}$
		$1.00 \times 10^{-5} - 1.00 \times 10^{-4}$	$I_{pa} = 3.182 \times 10^{-6} + 0.09726c$	0.9972	
	$\text{Cu}^{2+}$	$5.00 \times 10^{-8} - 2.50 \times 10^{-5}$	$I_{pa} = 1.251 \times 10^{-8} + 0.1492c$	0.9983	
		$2.50 \times 10^{-5} - 1.00 \times 10^{-4}$	$I_{pa} = 1.916 \times 10^{-6} + 0.06498c$	0.9930	

<sup>a</sup> $I$  in (A);  $c$  in (mol  $\text{L}^{-1}$ )

Table 2—Determination of Cu(II) and Pb(II) in waste water by the proposed ASV ( $n_1=6$ ) and FAAS ( $n_2=6$ ) methods

Analyte	Method	Mean value ( $10^{-6}$ mol $\text{L}^{-1}$ )	RSD (%)	Recovery (%)	$t^t$ (Student's t-test)	$t_{0.05,10}$
$\text{Pb}^{2+}$	ASV	7.285	1.6%	104	1.07	2.23
	FAAS	7.198	2.3%	—		
$\text{Cu}^{2+}$	ASV	3.595	3.0%	98	1.59	
	FAAS	3.499	3.5%	—		

$$t = \frac{|\bar{X}_1 - \bar{X}_2|}{s} \sqrt{\frac{n_1 - n_2}{n_1 + n_2}}; s = \sqrt{\frac{\sum(x_{1i} - \bar{x}_1)^2 + \sum(x_{2i} - \bar{x}_2)^2}{(n_1 - 1) + (n_2 - 1)}}$$

The results clearly proved the proposed method is highly selective towards lead and copper.

The reproducibility of the modified electrode was evaluated under optimal conditions for 15 repetitive measurements in the presence of  $1.00 \times 10^{-5}$  mol  $\text{L}^{-1}$   $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$ . The relative standard deviations (RSD) were below 2.8% and 4.1% for  $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$  respectively. The results show good reproducibility of the present PLA-modified electrode. The stability of the PLA/GCE was also investigated by measuring the peak currents of  $1.00 \times 10^{-5}$  mol  $\text{L}^{-1}$   $\text{Pb}^{2+}$  and  $\text{Cu}^{2+}$

acetaminophen for 15 days. The current response of the modified electrode showed almost no decline compared to its original response, indicating good stability of the modified electrode.

The proposed method was employed for the determination of the two metal ions in waste water collected from the contaminated river in our region to assess its practical applicability ability. From the results shown in Table 2, both the RSD and the recovery values were satisfactory. As a comparison, the standard method FAAS was also applied to

determinate the Pb(II) and Cu(II) in the same waste water (Table 2). Based on the student's t-test ( $t < t_{0.05,10}$ ), there was no significant difference between the results of the two methods, which affirmed the reliability and accuracy of the proposed ASV method for simultaneous determination of Pb(II) and Cu(II) in real samples.

In conclusion, a poly(L-glutamic acid) modified electrode was prepared by a simple and fast stripping voltammetry method for simultaneous determination of  $Pb^{2+}$  and  $Cu^{2+}$ . The modified electrode exhibited higher sensitivity and better selectivity, as compared with the bare GCE. Using the modified electrode with the proposed stripping voltammetry method,  $Pb^{2+}$  and  $Cu^{2+}$  could be measured simultaneously in the waste water samples with satisfactory results. This method provides an alternative to other procedures and shows great potential for estimation of other non-volatile metals.

#### Supplementary data

Supplementary data associated with this article are available in the electronic form at [http://www.niscair.res.in/jinfo/ijca/IJCA\\_56A\(02\)238-242\\_SupplData.pdf](http://www.niscair.res.in/jinfo/ijca/IJCA_56A(02)238-242_SupplData.pdf).

#### Acknowledgement

This work was supported by the Key Project of Science and Technology of Anhui Educational Bureau (KJ2016A642, Information College, Huaibei Normal University, Huaibei, 235000, China).

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