Molecularly imprinted polymer-modified electrochemical sensor for simultaneous determination of copper and zinc

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ABSTRACT

A procedure for the simultaneous determination of copper and zinc using molecularly imprinted polymer-modified sensor is developed. The linear range of copper and zinc was observed in the concentration window 0.098-23.80 µg L^{-1}, with detection limits (S/N=3) as low as 0.0275 µg L^{-1} (zinc) and 0.0159 µg L^{-1} (copper). Copyright © 2011 VBRI press.

Keywords: Molecularly imprinted polymer; double imprinting, differential pulse anodic stripping voltammetry; copper and zinc.

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contribution in analytical chemistry. He also went to Ranbaxy Ltd., India for about one-and-half year and elaborated a protocol for pharmaceutical analysis, interfaced with several sophisticated instruments. His research interests include environmental chemistry, chromatography, electroanalysis, and detection principle for chemical analysis and development of biomimetic chemical sensor using molecularly imprinted polymers for clinical, pharmaceutical and biological analyses.

Introduction

The formation of polymer materials in the presence of a template is a method for creating polymers that have a bias toward rebinding of the template molecule versus other compounds. Most often referred to as molecularly imprinted polymers (MIPs), these materials are useful in the fields of separations, sensors, assays, and catalysis [**1-3**]. In molecular imprinting, specific recognition for an artificial receptor can be created either by a single-template approach [**4**] or a mixture-template approach [**5**]. In single-template approach, a MIP with pre-defined single activity is produced, and this activity can be shown with the template molecule or with other compounds having similar structures. However, with a mixture of such compounds, it is not possible for a single-template MIP to recognize them all, because it is not feasible to produce an MIP with such a broad range of activity. Likewise, the use of a mixture of single template MIPs is labor-intensive, since several MIPs have to be synthesized and their recognition abilities standardized. By contrast, advantages of multiple-template imprinting are that several different classes of compounds can be extracted, separated, assayed, detected, or otherwise

analyzed at one time. Multi imprinting has been done widely in case of organic substrates [**6**]. Whereas, inorganic imprinting where the template used is an inorganic ion like Copper, Zinc, Iron etc. so far has not been reported. As we know, zinc and copper are essential elements for our body and for our diet too. However, these elements can also be toxic at high concentrations. Zinc is among the most important of the trace elements in human nutrition. It is vital for the immune system, the expression of genes and the transfer of nervous signals [**7**]. Whereas, copper is required for iron utilization, and as a cofactor for enzymes involved in glucose metabolism and the synthesis of hemoglobin, connective tissue and phospholipids [**8**]. Both metals are chemically active and can easily make alloys with other metals. This is why it is used so often in industry. The widespread use of zinc and copper has led to increased monitoring of their levels in the environment to safeguard human life [**9**]. The low-cost and the high sensitivity of electrochemical methods make them very appealing for the detection and quantization of heavy metals. Disposable pencil graphite electrodes (PGEs) have been used by virtue of their high electrochemical reactivity, good mechanical rigidity, low-cost, low-technology, low-background current, wide-potential window, chemical inertness and ease of modification, renewal, and miniaturization. The PGE, when combined with a highly sensitive and accurate voltammetric technique such as differential pulse voltammetry, becomes an attractive tool for trace analysis.

Experimental

Reagents

Copper sulphate ($CuSO_4.5H_2O$), zinc sulphate ($ZnSO_4.7H_2O$), multiwalled carbon naotubes (MWCNTs), Bis- (2-acryloylamino-ethyl)-phosphinic acid (BAAP), azoisobutyronitrile (AIBN), ethylene glycol dimethacrylate (EGDMA), and other solvents were purchased from Sigma Aldrich Chemie (Steinheim, Germany). Pencil rods (0.5mm x 5cm) were purchased from Hi Par, Camlin, Mumbai. The stock solutions of Cu (II) and Zn (II) of 500 mg L^{-1} were prepared by dissolving the appropriate amounts of copper and zinc sulphate in triple-distilled water.

Apparatus

All voltammetric measurements were performed on EG & G Princeton Applied Research 264A potentiostat model 303A electrode assembly and RE0089 X-Y chart recorder. The three electrode cell consisted of modified pencil graphite, a saturated Ag/AgCl and a platinum wire as working, reference, and auxiliary electrodes, respectively. ^{1}H NMR spectra were recorded on JEOL AL 300 FT/NMR (Japan) spectrometer. Morphological images of modified PGEs were recorded using scanning electron microscope (SEM; JEOL, JSM, model 840 A, Netherlands).

MIP-modified pencil graphite electrode (PGE) fabrication

A pretreated (6M HNO_3 for 15 min) pencil rod (2B) was inserted into a Teflon tube where the tip of the pencil rod at one end was gently rubbed with an emery paper (No. 400) to level the pencil surface along the tube orifice. Electrical

contact was obtained by soldering a metallic wire to the exposed reverse side of the pencil rod. For preparation of MIP-modified PGE, monomer (BAAP, 0.8 mmol, 1.5mL DMSO) and templates (both $CuSO_4.5H_2O$ and $ZnSO_4.7H_2O$ in 1.5mL DMSO) solutions were mixed together, followed by the addition of EGDMA (2 mmol, 350 μL) as a cross-linker and AIBN (0.1 mmol, 600μL DMSO) as an initiator. To this mixture, an optimized amount (60 mg) of MWCNTs were added and stirred for 10 min and degassed gently with a N_2 stream for 10 min. Herein, the purpose of using MWCNTs to make non-conducting MIP film on PGE surface, conducting. MWCNTs possess high surface area, high electroconductivity, good stability, and unique electronic and nano-structural properties to stimulate electrodics better than many carbon electrodes. Finally, 2 μL of this mixture was spin coated (for 30s at 3000 rpm) on the pretreated PGE surface and the coated PGE was cured at 60 [0]C for 4h. The non-imprinted polymer (NIP)-modified PGE was also prepared in the similar manner. For template extraction, modified PGEs were immersed in 0.1 mmol EDTA solution for 1h. The complete template removal from PGE surface was ensured till no voltammetric transduction response was noticed. Binding interactions between MIP and templates are shown in **Scheme 1**.
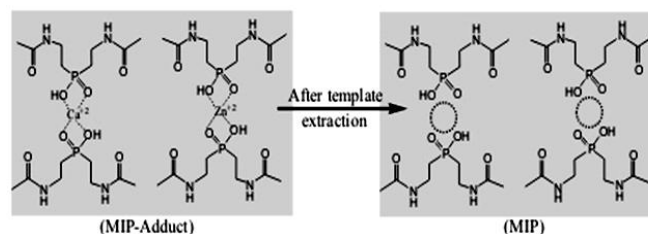
Scheme 1. Binding interactions between MIP and templates.

Voltammetric procedure

For the electrochemical measurement, the metal ions were first accumulated as uncharged metal (after cathodic reduction) with stirring for 90 s at -1.5 V. After 15s equilibration, differential pulse anodic stripping voltammetric (DPASV) runs were scanned from -1.5 to -0.3 V at scan rate 10 mVs^{-1}. All DPASV runs for each concentration of test analyte were quantified using the method of standard addition. The limit of detection (*LOD*) was calculated as three times the standard deviation of the blank measurement (in absence of analyte) divided by the slope of calibration plot between analyte concentration and DPASV current [**10**].

Results and discussion

Spectral characterization

The proton NMR spectra of MIP-adduct and MIP suggest the binding interactions between MIP and template (as shown in **scheme 1**), as follows: monomer peaks due to −OH (2.0 ppm), -NH- (8.0 ppm), and −CH_2 (1.6 and 3.0 ppm) were shifted downfield to 2.4, 8.6, 1.9, and 3.4 ppm, respectively, after binding with template in MIP-adduct and later on reinstated at their original positions after the template extraction.

Morphological characterization

Fig. 1 represents SEM images of the surfaces MIP- (a) and NIP- (b) modified PGEs, respectively. It can be clearly visualized that the carbon nanotubes are dispersed as well as embedded throughout the polymer matrix (either MIP or NIP) to from an interconnected electro-conductive texture. MIP-modified PGE surface (a) is rather porous as compared to NIP-modified PGE surface (b), due to the presence of cavities in the MIP matrix.
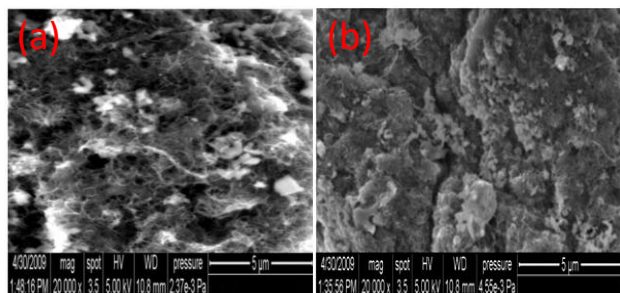
Fig. 1. SEM images of MIP (a) and NIP (b) modified PGE surfaces.
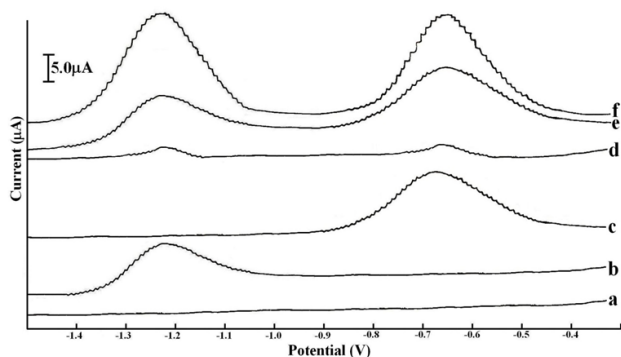
Fig. 2. Voltammograms of Zn and Cu at bare (d) and modified PGE in the absence (a) and presence of either single (b and c) or mixture of analytes (e and f).

Simultaneous determination of Cu (II) and Zn (II) ions

Fig. 2 shows the anodic stripping of Cu and Zn (reduced forms) at the surface of a bare PGE and MIP-modified PGE. The results showed that both metals were oxidized with well-defined and distinguishable sharp peak potentials. On the other hand, the indistinguishable and broad peaks at a bare PGE indicate a slow electron-transfer kinetic (curve d). In order to check the intermolecular effects between Cu (II) and Zn (II) ions, two different experiments were carried out under optimum conditions at a pH 10.0. In each experiment, the concentration of one of the two compounds was changed while keeping the concentration of the other constant. The oxidative stripping peak currents for Cu and Zn increased linearly with increases in their respective concentrations without considerable effects on the other peak currents (**Fig. 2** curves e and f). The oxidation peak potentials of Cu and Zn on the modified electrode are separated completely into two well-defined peaks at -0.75 and -1.2 V vs. Ag/AgCl, respectively.

DPASV currents were recorded for various concentrations of both analytes ranging from 0.098 to 23.80 $\mu g\ L^{-1}$). The calibration equations between peak current (i_p, μA) and concentration (C, $\mu g\ L^{-1}$) along with respective recoveries and *LOD*s in aqueous sample, are given below:

a) For Zn (II): $i_p=(1.963\pm1.004) + (10.907\pm3.914)C$, $r^2= 0.997$, recovery range=94.0-99.2%, *LOD*=0.0275 $\mu g\ L^{-1}$
b) For Cu (II): $i_p=(2.575\pm1.891) + (19.368\pm7.828)C$, $r^2= 0.996$, recovery range=95.0-99.8%, *LOD*=0.0159 $\mu g\ L^{-1}$

These results confirm that the oxidation processes of both metals at MIP-modified PGE are independent from each other, and as such they help in the simultaneous determination of two compounds, without any interference.

Interference study

In order to evaluate the selectivity of the method for the simultaneous determination of the Cu (II) and Zn (II), the influence of potentially interfering substances on the determination of these compounds was investigated. The tolerance limit was defined as the maximum concentration of foreign substances, with a relative error of less than 1.5%. Interference studies were conducted by exposing the MIP-modified PGE in a solution containing test analyte in the presence of interfering substance like Fe^{+3}, Ca^{2+}, Mg^{2+}, nitrate, urea, ascorbic acid, and dopamine. It was found that both MIP and NIP-modified PGEs were not responsive for any interferents studied.

Stability and reproducibility of the electrode

The most attractive feature with the use of MIP-modified PGE for the determination of metal ions is the easy renewal of surface for the next use by the method of template extraction. Any single electrode could be used for as many as 55 times with quantitative recoveries. To examine the intraday precision, 10 repetitive measurements were carried out which showed a *RSD* of 0.87%. To ascertain the reproducibility of results, three electrodes were prepared in different batches, which responded equal current for the same concentration of analyte with *RSD* 0.8%. Thus, the modified electrode possesses good reproducibility and ruggedness toward the quantitative assay of metal ions.

Conclusion

We have presented a novel and an easy method for the simultaneous detection of metal ions by MIP-modified PGE based on multi-imprinting technology. The modified electrode separated the anodic oxidation peak potentials of Zn and Cu with respective well-defined peaks in the presence of each of the other compounds to determine metal ions simultaneously, without any intermolecular effects.

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