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Electrodepositing of copper nanowires on layered double hydroxide film modified glassy carbon electrode for the determination of doxorubicin

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ABSTRACT

A novel doxorubicin sensor has been developed by electrodepositing of copper nanowires on Mg_2Al layered double hydroxides modified glassy carbon. The morphology of synthesized Cu nanowires/Mg_2 Al layered double hydroxides was characterized by field-emission scanning electron microscopy and energy dispersive X-ray spectroscopy. The modified electrode has a catalytic effect on the oxidation current of doxorubicin. The oxidation mechanism was studied using cyclic voltammetry, chronoamperometry and electrochemical impedance spectroscopy. Under optimized chemical and instrumental condition, the differential pulse voltammetry response of modified electrode toward doxorubicin shows a linear concentration range of 10.0–2110.0 nmol/L with the detection limit of 0.02 nmol/L at the signal to noise of 3. Satisfactory results have been achieved for the determination of doxorubicin with a doxorubicin injection solution and biological samples.

1. Introduction

Doxorubicin (also called adriamycin), is an anthracycline drug widely used in the treatment of a wide variety of cancer types [1,2]. It produces regression in disseminated neoplastic conditions such as leukemia, neuoblastoma, sarcomas and carcinoma [3]. Its clinical use is limited by some cytotoxic effects on neoplastic cells through the accumulation of reactive oxygen produced by scavenger enzyme activities. Therefore, development of highly sensitive analytical methods to determine trace amounts of doxorubicin is of great significance in clinical treatment. Several analytical techniques including electrochemical sensor [4,5], electrophoresis [6,7], liquid chromatography [8,9], and fluorimetry [10] were developed for the determination of doxorubicin. Among these techniques, electrochemical sensors have attracted great attention due to electroactivity of quinone and hydroxiquinone groups of doxorubicin. However, doxorubicin exhibits slow direct electron transfer at unmodified electrode. In recent years, modified electrodes such as silver-amalgam film electrode [11], nano-titania/nafion composite film modified electrode [12] and cyclodextrin-graphene hybrid nanosheets modified glassy carbon [13] have been developed to improve electrochemical sensing of doxorubicin at unmodified electrode. However, these methods suffer from serious problems such as complexity, expensive chemical materials for electrode modification, and they are time consuming. Furthermore, they require large amounts of toxic materials such as 3-(trimethoxysi lyl)-1-propanethiol and 1,6-hexanedithiol.

Layered double hydroxides, also called anionic clays, have recently attracted much attention because of their potentials in the field of catalysis, adsorption and nanocomposite [14]. The layered double hydroxides composition can be expressed as $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2]$ $(A_{X.}nH_2O)$ formula, where M^{2+} and M^{3+} can be most divalent and trivalent metal ions and A^- is any type of anion. Due to the unique properties of layered double hydroxides such as their compositional flexibility, anion exchangeability and biocompatibility, they are good candidates as stable substrates for electrodeposition of nanoparticles. The morphology and size of layered double hydroxides can be controlled by applying different preparation conditions [15,16]. In this work, the Mg_2 Al layered double hydroxides were prepared by the most effective hydrothermal method. The constructed layered double hydroxides, which provide a stable matrix for the formation of Cu nanowires, were then selected as a matrix for the electrodeposition of Cu nanowires. The resulting Cu nanowires/Mg_2 Al layered double hydroxides modified glassy carbon electrode exhibits a considerable electrocatalytic activity for the detection of doxorubicin and is applied for the determination of doxorubicin in pharmaceutical and biological samples. Table 1 compares the analytical parameters in previously reported methods with our proposed method for the determination of doxorubicin [17–19].

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Table 1
Comparison of figures of merit of the proposed method with recently published voltammetric methods for the determination of doxorubicin.

Electrode	Limit of detection (nmol/L)	Linear dynamic Range (nmol/L)	Interference	RSD%	Ref.
Gold electrode	0.00017	4.6×10^{-3}–0.09	Not found	6.9	3
Silver amalgam film electrode	3.0	9.0–109.7	Not reported	6	11
Glassy carbon electrode	1.0	5.0–2000.0	Uric acid, ascorbic acid	4.7	12
Gold electrode	0.002	1.8×10^{-3}–0.290	Not found	5.59	17
Glassy carbon electrode	1.7	8.2–19.0	Not reported	7.6	18
Glassy carbon electrode	0.33	0.1–1.0	Not reported	–	19
Glassy carbon electrode	0.02	10–2110.0	Ascorbic acid	4.3	This work

2. Experimental

2.1. Material and apparatus

$MgCl_2.6H_2O$, $AlCl_3.6H_2O$, Na_2CO_3 and $CuCl_2$ were obtained from Sigma–Aldrich. All other reagents were of analytical grade and were used without further purification. Phosphate buffer solution (0.1 mol/L) was used as the supporting electrolyte. Doubly distilled water was used in the experiment. All voltammetric measurements were carried out using an electrochemical system comprising the Metrohm instrument (Herisau, Switzerland), Model 797 VA and a conventional three electrode cell assembly containing an Ag/AgCl electrode as reference electrode, a platinum wire as counter electrode and Cu nanowires /Mg_2 Al layered double hydroxides modified glassy carbon electrode (Cu nanowires/Mg_2 Al-LDHs/GCE) as working electrode. The pH of the solutions was controlled with a Corning pH meter (model 146), and the modified electrode was characterized by field-emission scanning electron microscopy (FESEM) (Philips, XL30). The transmission electron microscope (TEM) images were obtained on a Tecnai F30 at an acceleration voltage of 20 kV. For TEM imaging, the freshly collected layered double hydroxides (LDHs) were dispersed in alcohol with ultrasonication for 20 min, and then a droplet was dropped on a copper grid coated with a carbon film. The analysis of chemical composition of the modified electrode was performed using an energy dispersive spectrometer (EDX) attached to FESEM (Seron technology AIS2300C).

2.2. Synthesis of Mg_2 Al-Cl-LDHs

The LDHs were synthesized according to Xu et al. method [20]. Typically, the mixture containing 10 mL salt solution of $MgCl_2$ (2.0 mmol) and $AlCl_3$ (1.0 mmol) was quickly added within 5 s into 40.0 mL basic solution containing NaOH solution (0.15 mol/L) and Na_2CO_3 (0.013 mol/L) under vigorous stirring. After 20 min stirring, the mixture was centrifuged and the precipitate was collected. The resulting precipitate was then washed twice with deionized water, and then dispersed into 40 mL water. This aqueous suspension was transferred to a Teflon – lined autoclave at the temperature of 100 °C for 16 h. After air cooling, stable and homogenous LDHs suspension was obtained with a concentration of 0.4 wt%.

2.3. Fabrication of modified Mg_2 Al-LDHs electrode

Prior to modification, the bare GC electrode was polished with alumina powder (5 μm), and then it was cleaned ultrasonically in ethanol and distilled water. Typically, 10 μL of LDHs suspension was dropped on the surface of the pretreated GC and allowed to dry in the air. Then, the electrode was immersed in 0.1 mol/L NaCl solution containing 2 mmol/L of $CuCl_2$ solution, and it was subjected to scanning from –0.6 V to 0.6 V at 50 mV/s for 20 cycles [21].

2.4. Preparation of real samples

Human blood samples were collected from patients suffering from cancer hospitalized at Seyedo Shohada Medical Center affiliated to Isfahan University of Medical Sciences. After 2 h, heparinized plasma samples were obtained by centrifugation and deproteination according to Aly et al. [22]. To each tube containing 100 μL of plasma, acetonitrile (200 μL) was added followed by vortex-mixing and centrifugation (2000 rpm, 5 min). The supernatant was transferred to a small conical flask and evaporated to dryness under the stream of nitrogen. The dry residue was diluted appropriately so that the concentrations of doxorubicin were in the dynamic range of its calibration curve. A blank solution was prepared by treating the drug-free plasma of healthy volunteers in the same way. The absolute recovery was determined by comparing the representative oxidation current of doxorubicin in the plasma sample and the standard drug at identical concentrations.

The urine sample was obtained from Isfahan University of Technology Health Center and was centrifuged (10 min at 1200 rpm). After filtering, it was diluted 10 times with doubly distilled water and was used without any additional pretreatment. 1.0 mL of each sample was used in each experiment and the pH was adjusted by 9.0 mL of 0.1 M PBS solution with pH 7.0. The standard addition method was used to determine doxorubicin in the spiked samples.

A 1.0 mg portion of finely ground and homogenized sample was dissolved with ultrasonication in 100 mL of water in order to measure doxorubicin in doxorubicin hydrochloride powder for injection (labeled 50 mg, Korea, United Pharm). After that, 60 μL of the solution plus 5 mL of the buffer (pH 7.0) was diluted with water in a 10-mL volume flask, and the resulting solution was used for analysis. The diluted sample solutions were then placed in an electrochemical cell to determine their concentrations using the DPV method.

3. Result and discussion

3.1. Characterization of LDHs nanowires

TEM is a strong technique used extensively to determine particle size distribution and morphology. As can be seen in Fig. 1A, the LDH nano flakes (80–100 nm lateral size) have a hexagonal platelet-like structure. The morphology of the prepared Mg_2 Al–LDHs on the surface of GCE is characterized by FESEM (Fig. 1B). It can be clearly seen that Mg/Al-LDHs have dispersed homogeneously on the surface of GCE. Fig. 1C illustrates the FESEM image of Cu nanowires coated Mg_2 Al-LDHs with an average length of 10 μm. In comparison, the size of Cu nanowires, which form on GCE in the same way, is about 25 μm. This proved that LDHs substrate induces the formation of Cu nanowires with smaller size leading to a higher surface area (Fig. 1D). EDX pattern of Cu nanowires/LDHs at modified electrode indicates that Cu, C, Mg and Al elements is the major components on the surface of the electrode (Fig. 1).

The CV responses of $[Fe(CN)_6]^{3-/4-}$ at the bare GCE and different modified electrodes were studied as shown in Fig. 2A. It can be