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A novel cysteine sensor based on modification of carbon paste electrode by Fe(II)-exchanged zeolite X nanoparticles

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ABSTRACT

An electrochemical sensor based on carbon paste electrode (CPE) modified with iron(II) doped into a synthesized nano-particles of zeolite X (Fe(II)-NX/ZCME) was constructed, which is highly sensitive for detection of cysteine (Cys). The modified electrode showed an excellent electro-activity for oxidation of Cys in phosphate buffer at pH 7.4. It has been found that anodic peak potential of Cys oxidation, compared with the unmodified CPE (UCPE), was shifted towards negative values at the surface of the modified electrode under the optimum condition. The peak current increased linearly with the Cys concentration in the wide range of 5.0×10^{-9}–3.0×10^{-3} mol L^{-1}. The very low detection limit was obtained to be 1.5×10^{-10} mol L^{-1}. Finally, the modified electrode was used as a selective, simple and precise new electrochemical sensor for the determination of Cys in the real samples, such as pharmaceutical and biological fluids.

1. Introduction

Cysteine, a thiol compound containing a sulfhydryl [SH] group, is a precursor of taurine in organisms and is normally obtained from the diet and by a transsulfuration pathway from methionine. This compound is an essential biological molecule that is necessary for detoxifying function, immunological competence, growth and delay senility of cells, and tissues in living systems. Lack of cysteine can bring about health problems including retarded growth, hair depigmentation, and liver damage [2]. Therefore, selective and sensitive detection of the intracellular Cys has attracted increasing attention [3,4]. The determination of Cys in biological samples such as blood and urine has been addressed and emphasized by a number of researches [5–7]. Several methods reported for its determination confirmed that electrochemical techniques have the inherent advantages of simplicity, ease of miniaturization, high sensitivity, and relatively low cost [8] toward other methods. An important theme in electrochemical studies is the modification of electrode surfaces to improve their selectivity or sensitivity.

The interest of zeolites for the electrochemists is due to the individual properties of the materials including: stable at high temperatures, insoluble in most organic solvents, and offer better resistance to extreme experimental conditions than numerous organic polymers commonly employed to modify electrode surfaces. Due to their rigid, three-dimensional and negatively charged lattice, zeolites offer size, shape and charge selectivities [9]. These attractive features were caused to using of zeolites for the modification of electrode surfaces by designing zeolite-modified electrodes (ZMEs) with wide applications including molecular recognition, mediated electrocatalysis and electroanalysis fields [10–13]. In general, most uses of ZMEs include: voltammetric analysis after accumulation at open circuit, direct electrocatalytic detection, indirect amperometric detection of non-electroactive cations, some biosensing applications and zeolite-based composite membranes in potentiometric measurements of different cationic and anionic species [14–20]. Both the zeolite nature and the membrane preparation procedure strongly affect the potentiometric responses [21]. Under equilibrium conditions, only nonsize-excluded cations and neutral species are able to diffuse freely into the zeolite framework. High ion exchange capacity caused to using of zeolites as pre-concentration media for various inorganic, organic and organometallic cations [9]. This cause to promote the selective pre-concentration of analytes at an electrochemical interface, to confine electron transfer mediators in a micro heterogeneous environment or to synthesize size-excluded entities inside the large cavities of the zeolite, by binding, for example, corresponding counter-ions. Incorporation of a zeolite in an electrode surface creates the electrochemical interface in a micro-structured environment dominated by the zeolite three-dimensional framework. Also, numerous electron transfer reactions occur in zeolites present in the ZMEs. Ion exchange properties of zeolites have been successfully coupled to the redox

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properties of the exchanged cations, especially to demonstrate the existence of transient species, to perform selective redox reactions, to prepare micro- and ultra-microstructured conductor or semiconductor clusters and to create electron transfer chains inside electrically insulating microporous systems [9].

As we know, cation exchange capacity (CEC) of zeolites significantly depends to their Si/Al ratio, so with decreasing this value the CEC of the corresponding zeolite significantly increases. This parameter significantly affects the voltammetric behavior of the corresponding ZME, because higher CEC of the used zeolite causes to higher voltammetric current of the corresponding ZME. Although, using of natural zeolites is economically important but their lower CECs may limit their use in ZMEs. Based on our preliminary knowledge about the Si/Al ratio [11] of the common synthetic and natural zeolites (zeolite Y: 2.5–4.1; zeolite A: 1.2–1.7; zeolite X: 1.2; zeolite β: 12.5; clinoptilolite: 4.8–5; mordenite: 5) and the simplicity of the used procedure for synthesis of the zeolite X nano-particles which no needs to high temperature, pressure or especial equipments (see Section 2.2), this zeolite was used in this work. Nanomaterials have improved physical, chemical and biological properties because of their small dimension and large external surface areas [1]. In addition, due to high dispersibility of nanozeolites, they were identified at an early stage as promising candidates for the modification of electrodes.

Modified electrodes containing iron cations as a modifier have been used in voltammetric determination of different organic and pharmaceutical compounds [22,23]. In this work, as synthesized nanozeolite X was ion exchanged in Fe(II) solution and used for the modification of a carbon paste electrode (Fe(II)-NX/ZCME). The modified electrode was then used for the voltammetric determination of Cys. After optimization of several experimental parameters influencing the voltammetric behavior of the electrode in the presence of Cys, the modified Fe(II)-NX/ZCME electrode was used for the electrochemical determination of Cys in some real samples including: N-acetyl cysteine (ACC) tablets, powdered poultry feed (PPE) and biological fluids (human blood serum and urine).

2. Experimental section

2.1. Chemicals and apparatus

Materials used to synthesize of nano-zeolite X include tetraethyl orthosilicate (TEOS), iron(II) chloride, sodium hydroxide, aluminum hydroxide, and sodium hydroxide pellets (99%). For preparation of acetate buffer, sufficient amount of dilute sodium hydroxide solution was added to a 0.4 mol L^{-1} standardized acetic acid solution and variation of pH was followed using a pH-meter to reach pH 4.7 (in this case, pH = pKa = 4.7). Similar method was used for preparation of phosphate buffer with pH 7.4 using a 0.4 mol L^{-1} phosphoric acid solution and sodium hydroxide solution (pH ≈ pKa2 = 7.2). The pH of the solutions was appropriately adjusted with 1 mol L^{-1} hydrochloric acid and sodium hydroxide solutions (where buffer not require). Aqueous stock solutions of Cys were prepared by dilution of the respective standard 1 mmol L^{-1} solution. All reagents were of analytical-reagent grade from Merck Company and used without further purification. All solutions were prepared in de-ionized double distilled water.

Powder X-ray diffraction pattern of the sample was recorded using a Bruker D8ADVANCE diffractometer by means of Cu-Ka radiation. The particles size of the synthesized zeolite (S) was estimated by the following Scherrer's equation:

$$S = (K\lambda)/[\beta\cos\theta] \qquad (1)$$

where S is the mean crystallite dimension, K the crystallite shape constant (0.89), θ the Bragg angle, λ the X-ray wavelength, and β the full width at half maximum (FWHM) of the peak in radians [24]. The surface morphology of the samples was obtained using a Hitachi Model S-3500 N scanning electron microscope (SEM). The TEM images of sample were recorded using Transmission Electron Microscope S-3500N with Absorbed Electron Detector S-6542 (Hitachi Science System Ltd). FT-IR spectrum was recorded on Nicolet 400D Impact spectrophotometer in KBr pellets.

2.2. Zeolite synthesis and preparation of the electrode

The zeolite nanoparticles were synthesized by hydrothermal crystallization in a temperature-controlled condition [24]. For this goal, an aluminosilicate gel containing 5.34 g of NaOH, 2.42 g of $NaAlO_2$, 3.43 g of SiO_2, and 50.0 g of H_2O was prepared in a closed polyethylene bottle and the solution was stirred into an ice-water bath (1 h). Then, TEOS (12.6 mL TEOS ≈ 11.89 g TEOS ≈ 3.43 g SiO_2) was added and stirring continued at 0 °C for 6 h and then at room temperature for another 24 h. Hydrothermal crystallization was conducted at 60 °C (4 days) in a shaker with a rotation rate of 250 rpm. The suspension was finally centrifuged and the solid material was re-suspended in water to reach $pH < 8$. The final suspension was centrifuged and the filtrate air dried for 24 h.

To prepare the Fe(II)-exchanged nano-zeolite X (Fe(II)-NX), 2.2 g of iron chloride ($FeCl_2$) was dissolved in 50 mL of acidified (HCl) water (containing slight amount of hydroxyl amine hydrochloride to prevent Fe(II) oxidation) and then 4 g of NX was added under continuous shaking for 24 h. After centrifuging (with 12,000 rpm), the obtained powder was carefully re-suspended in dilute HCl solution (pH 2.0) and then in water in order to remove occluded materials and surface adherent salts. Then, the Fe(II)-NX was dried at 100 °C for 1 h. The amount of the loaded Fe(II) into the NX was measured by atomic absorption spectroscopy (Perkin Elmer AAnalyst 300) and it turned out to be 12.5% (w/w).

The working electrodes were constructed in the following way: A graphite-Fe/NX (2–15 wt.% with respect to carbon paste) mixture was added to the Nujol oil and thoroughly mixed. A portion of the composite mixture was packed into the end of a Teflon tube. A copper wire was used as an electrical contact and it was made by forcing positioned into glass tubing, down the Teflon tube and into back of a paste. Unmodified carbon paste electrode (UCPE) was prepared in a similar way with the exception of omitting the zeolite addition step. The electrode surface was polished using a soft paper and then rinsed with ethanol and water, respectively.

2.3. Voltammetric measurements

Electrochemical measurements were recorded with a potentiostat/galvanostat (PerkinElmer, EG&G 273 A) with a three-electrode system consisting of a Fe(II)-NX/ZCME working electrode, a platinum counter electrode and Ag/AgCl reference electrode. The system was run by a PC through Power Suite software. The pH of solutions was adjusted using a digital pH meter (Jenway 370). The reaction solutions were prepared immediately before use and cyclic voltammetric experiments were performed under nitrogen atmosphere to prevent the oxidation of Cys to cysteine (CySSCy oxidized form of Cys) by atmospheric oxygen, especially in basic solutions [25]. The potential was scanned at scan range of −0.4 V to +0.6 V for CV and −1.0 to +1.1 V for SqWV (with 100 mV s^{-1} scan rate). The electrodes were kept in open air when not in use.

2.4. Preparation of real samples

The modified electrode was used for the determination of N-acetylcysteine (ACC) tablets, powdered poultry feed (PPE), urine and biological fluids. Tablets of ACC and PPF as the pharmaceutical samples were purchased respectively from Fluimucil Company of Swiss and Razak Company of Iran. Also, patient blood serum and urine as the clinical samples were taken from healthy volunteers. For preparing a