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Characterization of cysteamine self assembled on gold functionalized with nitrilotriacetic acid and evaluation of copper(II) binding capacity with adsorption transfer stripping voltammetry

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ABSTRACT

A self assembled monolayer of cysteamine was prepared on the surface of gold disc electrode and further modified with nitrilotriacetic acid. Modified electrode was characterized with cyclic voltammetry and electrochemical impedance spectroscopy in presence of potassium hexacyanoferrate(II)/(III). Thus prepared electrode was tested for determination of Cu^{2+} with adsorption transfer stripping voltammetry. The binding of Cu^{2+} onto the modified electrode was successfully performed for a wide range of tested concentrations. Electrode response ($\log I_p$) was linearly proportional to $-\log c(Cu^{2+})$ with correlation coefficient $R^2 = 0.9759$. The detection limit was $1 \cdot 10^{-8}$ M. The influence of interfering substances (Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+} and Pb^{2+}) was tested and it was found that Mg^{2+} and Pb^{2+} do not interfere while Mn^{2+}, Co^{2+} and Ni^{2+} show slight interference. Real-world testing of copper content in urine samples confirmed practical application of prepared sensor.

1. Introduction

Significant amounts of heavy metal ions are found in the soil and water due to increased anthropogenic activity. They are circulating, and eventually accumulating, throughout the food chain, thus posing a threat to the ecosystem. Monitoring their level is imperative, considering their toxicity and longevity [1]. Several analytical techniques are used for copper detection. Atomic emission spectrometry [2], inductively coupled plasma mass spectrometry [3] and neutron activation analysis [4] are some of them. These techniques require time consuming manipulation steps and sophisticated instrumentation, so there is a need for fast, reliable and cheap method which can be used in the field. Electrochemical methods, in addition with modified electrodes, meet the above mentioned criteria.

Surface alteration, based on self assembled monolayers (SAMs), has been widely studied over the last 20 years [5,6]. The approach that is used, when changing electrode surface, depends on the type of the analyte. Modification of metal electrodes, with SAMs, can provide interface with required performance [7] i.e. SAMs can be used as a platform for detecting biologically important molecules such as epinephrine [8], glucose [9,10], dopamine [11,12], uric acid [13], proteins [14], cholesterol [15], vitamins [16], antibodies and antigens [17], DNA [18] and pesticides [19]. Possibility of tailoring SAMs properties, with different end groups, enables sensor construction for determination of metal ions and molecules like Zr^{4+}, Mg^{2+}, Ca^{2+}, Sr^{2+}, La^{3+} and Cd^{2+} [20–23]. Rubinstein et al. [24] were one of the first to modify electrode surface, with mixed SAM that selectively recognized Cu^{2+} ions. After that, a number of electrochemical techniques for copper measurement, based on SAM modified electrodes were published, such as cyclic voltammetry [25] and adsorptive stripping voltammetry [26] on gold electrode modified with L-cysteine, voltammetric determination on 3-mercaptopropionic SAM [27], differential pulse [28] and anodic stripping voltammetry [29] on *meso*-2,3-dimercaptosuccinic acid, anodic stripping voltammetry on 2,5-dimercapto-1,3,4-thiadiazol [30], differential pulse voltammetry on cysteamine functionalized with L-lysine [31] and linear sweep anodic stripping voltammetry on 2-mercaptoethanesulfonate [32].

Electrochemical impedance spectroscopy (EIS) is nondestructive, powerful tool used to study and characterize the phenomenon of corrosion, fuel cells and batteries, coatings, and conductive polymers, chemically modified electrodes and adsorption of thin films, modification of electrodes based on SAMs, electron transfer kinetics and the analytical determination of inorganic and organic substances [33]. The basic advantage of EIS is use of an electric model, i.e. equivalent circuit for description of any electrochemical

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system. In this way we obtain a large number of parameters and gain better understanding of investigated system.

Varieties of electrochemical methods are used in order to determine the analyte and to examine phenomena that occur on the electrode – solution interface. Commonly used techniques, for low concentration analyte measurement, are stripping methods.

Adsorption transfer stripping voltammetry (AdTSV) is analogous to the anodic and cathodic stripping voltammetry apart from accumulation step, which is not electrochemically controlled, but achieved through a process of adsorption. Possible adsorption mechanisms are either physical adsorption on the surface or chemical adsorption by complexation with modified electrode. Adsorption and measurement steps are conducted in different solutions [34]. This represents a comparative advantage over other methods because accumulation could be carried in the field, requiring only modified electrode, and measurement performed later in the laboratory.

In this paper we used nitrilotriacetic acid (NTA) as electrode modification substance. Carboxylic group is known as excellent metal ion chelating agent. NTA has three carboxylic groups and it forms stable complexes with transition metals [35]. Therefore, gold electrode modified with NTA, provides an assembly that allows investigation of complex formation and has potential for Cu^{2+} sensor construction.

The main purpose of this research was to characterize cysteamine (CA) self assembled layer on gold electrode (Au/CA) further functionalized with nitrilotriacetic acid (Au/CA/NTA) and to investigate possibility of thus prepared electrode for Cu^{2+} binding. Au/CA and Au/CA/NTA modifications were investigated with cyclic voltammetry (CV) and EIS using $[Fe(CN)_6]^{3-/4-}$ redox pair. Complexation of NTA and Cu^{2+} was tested and optimized with AdTSV.

2. Experimental

2.1. Chemicals and solutions

Cysteamine was obtained from Fluka (Germany), *N* hydroxysuccinimide (NHS), 1 ethyl 3 (3 dimethylaminopropyl)carbodiimide hydrochloride (EDC) and nitrilotriacetic acid disodium salt were from Sigma Aldrich (Germany). Disodium hydrogen phosphate dihydrate, sodium dihydrogen phosphate hydrate, acetic acid, sodium acetate, sodium perchlorate, ammonium chloride, 5 M hydrochloric acid and 5 M sodium hydroxide for adjusting buffer pH values, ethanol for CA preparation, potassium hexacyanoferrate(II) and EDTA for sensor recovery was from Kemika (Croatia). Copper(II) nitrate was from Alkaloid (Macedonia) and Ammonia from T.T.T. (Croatia). Perchloric acid, for electrochemical polishing and sensor recovery, and potassium hexacyanoferrate(III) were from Merck (Germany). Buffers, 0.1 M phosphate, PBS (disodium phosphate + monosodium phosphate) pH = 9.0, 0.1 M acetate, ACB (sodium acetate + acetic acid) pH = 3.0 and 0.1 M ammonia buffer, AMB (ammonium + ammonium chloride), all containing 0.1 M sodium perchlorate were used. All the chemicals were of analytical grade and all solutions were prepared with water from Millipore MilliQ system (USA).

2.2. Preparation of Au/CA/NTA electrode

A gold electrode of 1 mm diameter was polished on a flat pad to obtain mirror like surface with SiC powder of different mesh (240, 800 and 1200) and finally by 1 and 0.25 μm Al_2O_3 powder. After polishing, electrode was ultrasonically cleaned in ultra pure water for in the duration of 5 min followed by immersion in Piranha solution ($V(H_2O_2, 30\%)$:$V(H_2SO_4$, conc.) = 1:3) for 5 min and sonication in ethanol. Subsequently, the gold electrode was electrochemically polished in 0.1 M $HClO_4$ between 0 and 1.5 V until the stable voltammogram was obtained (data not shown). The real electrode surface was calculated from the integration of reduction peak at 0.9 V vs. reference electrode assuming charge density of 482 $\mu C\,cm^{-2}$ for reduction of AuO monolayer [36]. A roughness factor was calculated as the ratio of real and geometric surface and was 1.4. Modification of a gold electrode was performed in 18 mM CA in ethanol for approximately 48 h at the room temperature in darkness (part 1 in Fig. 1). After removal from CA solution, the electrode was thoroughly rinsed with ethanol to eliminate physically adsorbed species. EDC + NHS + NTA solution was prepared by mixing 2.9 mg NHS, 1.9 mg EDC and 11.8 mg NTA in 0.1 M PBS, pH = 5.0 and held in darkness for 3 h to activate carboxyl group of NTA (part 2 in Fig. 1). After preparation, Au/CA electrode was immersed in prepared EDC + NHS + NTA solution for 24 h to form Au/CA/NTA electrode (part 3 in Fig. 1). Fig. 1 shows one possible way of coupling NTA to Au/CA surface.

Accumulation of Cu^{2+} on the modified surface was performed at open circuit potential by immersion Au/CA/NTA electrode for 10 min in 10 mL of a stirred solution. Initial potential of the scan was +0.6 V.

2.2.1. Urine sample preparation

The samples of urine were collected and diluted 1:1 with 0.1 M ammonia buffer, pH = 7.0 and spiked with known concentration of $Cu(NO_3)_2$. The initial concentration of Cu^{2+} in the 1:1 diluted urine sample was measured with sensor. Afterward diluted urine samples were spiked with known concentration of Cu^{2+} and signal was quantified. The concentration of Cu^{2+} was determined as the difference between the signal value with and without the addition of Cu^{2+}.

2.3. Instrumentation

CV measurements were carried out on Potentiostat 264 A (Princeton Applied Research, USA) connected to a computer for data collecting and analysis using EG&G PowerSuite software. All electrochemical measurements were carried out in a three electrode cell. Gold disc electrode was used as working electrode, platinum wire and $Hg|Hg_2Cl_2|$ KCl saturated (SCE) electrode were used as counter and reference electrode, respectively. The applied potential scan rate in CV was 50 $mV\,s^{-1}$. CV was carried out on Potentiostat/Galvanostat 263A (Princeton Applied Research, USA) combined with Frequency Response Detector 5210 (EG&G Instruments, PAR, USA) connected to a computer for data acquisition and analysis using EG&G PowerSuite software for EIS measurements. The frequency range for impedance measurements was between 100 mHz and 100 kHz. Small sinusoidal AC signal of ±5 mV was applied to potential of +200 mV vs. SCE. Equivalent circuit parameters were calculated by fitting the EIS data using appropriate circuit by *ZsimpWin* software provided by EG&G. AdTSV measurements were carried out on a polarographic analyzer/stripping voltmeter 264A (Princeton Applied Research, USA) connected to a computer for data acquisition with E-Corder 410 using “Chart & Scope software”. All solutions were deaerated with nitrogen gas for 10 min before measurements. All measurements were performed at room temperature.

3. Results and discussion

3.1. Characterization of Au/CA/NTA electrode

3.1.1. Cyclic voltammetry

The behavior of reversible redox couple can be used to investigate structure of the monolayer [37]. In general, formation of SAM