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Amperometric Detection of Creatinine and Uric Acid at the Screen-Printed Electrode Modified By Gold Nanoparticles in Flow-Injection Analysis.

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ABSTRACT

A screen-printed carbon electrode (SPE) modified by gold nanoparticles (AuNPs/SPE) was suggested for the simultaneous amperometric determination of uric acid (UA) and creatinine (CR) in flow-injection analysis (FIA). Well-defined oxidation peaks of UA and CR were observed at 0.30 and 1.00 V on the modified electrode. The electrode's AuNPs/SPE response show high electrocatalytic activity for the oxidation of UA and CR since it greatly enhances the oxidation peak current of UA and CR as well as lowers their oxidation overpotential. Based on this, a very sensitive and simple amperometric method of the detection of UA and CR was developed in flow system. Using modified SPE with two working electrodes in FIA provides simultaneous detection of CR and UA at the same time. A sensitive linear dependence of amperometric response of the modified electrode from concentration of UA was obtained in the range of $5.0\times10^{-10} \div 5\times10^{-3}$ mol·L^{-1} and of CR in the range of $5.0\times10^{-7} \div 5\times10^{-3}$ mol·L^{-1}. Good stability and reproducibility were obtained for simultaneous determination of UA and CR.

Keywords: chemically modified electrodes, gold nanoparticles, amperometric detection of creatinine and uric acid, flow- injection analysis

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INTRODUCTION

Uric acid (UA) is a product of the metabolic breakdown of purines. After primary filtration by the kidney, the metabolic UA is reabsorbed into the blood-vascular system or secreted into the urine [1]. Abnormal concentration levels of UA in biological liquids are associated with various diseases, such as gout, renal disease and hyperuricemia [1-4]. In addition, creatinine (CR) is a fairly reliable indicator of kidney function. Elevated CR level signifies impaired kidney function or kidney disease [5]. Non-enzymatic dehydration and loss of phosphate from phosphocreatine leads to creatinine appearance. Therefore, the feasibility study of simultaneous determination of UA and CR for diagnosis of gouty arthritis has been of importance. Traditionally, UA is often quantified colorimetrically [6]. Generally accepted method of CR determination is the Jaffe reaction involving the use of picric acid. [7]. Though these methods are time-consuming, include unstable reactants and suffer from interferences of organic compounds that exist in human biological fluids.

Different techniques have been developed to determine these biologically active compounds including high performance liquid chromatography (HPLC) [8–10], capillary electrophoresis (CE) [11-13] and electrochemical methods [14–17]. Determination by HPLC and CE methods is associated with the necessity of sample preparation, the use of expensive equipment, highly skilled specialists. To solve these problems fringe methods were developed particularly electrochemical, which possess a number of advantages: low cost of equipment, high sensitivity, low detection limit, fast responce. In recent decades, voltammetry with chemically modified electrodes surface have become reliable electrochemical methods that is widely used for the biologically active compounds determination in human fluids and pharmaceutical preparations . Using chemically modified electrodes provides good selectivity, reproducibility, high sensitivity and low detection limit of analysis [18]. However, the methods of simultaneous determination of UA and CR using chemically modified electrodes are very bounded.

A promising area of modern analytical chemistry is the automation of the analysis, that provides high accuracy and performance of analysis. These automated systems make it possible to solve the problem of expanding quest for rapid analysis of a greate quantities of samples. To this end, flow-injection analysis (FIA) received particular attention. The use of amperometric detection at chemically modified electrodes in combination with FIA appears very promising , due to the fact to allow us to get a set of benefits as high performance sampling, analysis of samples in real time, universal equipment, low cost, lower limit of detection, higher selectivity and low reagents consumption. This automated analysis methods are becoming the major technique in the area of automatic analysis with large number of samples [19, 20]. Screen printed electrodes (SPE) have recently received increased interest and can be improved by chemical modification [21].

In the present work a sensitive and simple method for the fast simultaneously determination of CR and UA was developed. This goal was achieved by combining the FIA with amperometric detection at SPE modified by gold nanoparticles.

EXPERIMENTAL

Reagents and Solutions

All the reagents were of analytical grade and used without further purification. All solutions were prepared using distilled water. Creatinine, uric acid, $HAuCl_4$ and H_2SO_4 were purchased from Sigma Aldrich.

Apparatus

SPE with one or two carbon working electrodes, were purchased from Dropsens (Spain). They had a common three-electrode configuration printed on ceramic supports (3.4 cm×1.0 cm). Both working and counter electrodes were made of inks of carbon, while the pseudo reference electrode and electric contacts were made of silver. Electrochemical measurements were carried out with a bipotentiostat / galvanostat μstat 400 (DropSens, Spain). The data were obtained with the Dropview software (DropSens, Spain).

The flow injection system included a peristaltic pump «Perimax» (Germany), manual sample injection valve with a 1.0 mL sample loop, wall-jet flow cell for SPE with one and two working electrodes joined to a

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potentiostat. All of the flow injection system tubing was polyvinylchloride. All measurements were performed at room temperature.

Preparation of Gold Nanoparticles-Modified electrode

Modification of working surface of SPE with AuNPs was performed by immersion of the electrode into the 0.005 M $HAuCl_4$ solution and applying a potential of -0.3 V during 40 s. The prepared modified SPE was washed with distilled water and dried at room temperature.

Electrochemical measurement

For the CR and UA determination, the bare or electrode modified by AuNPs was dipped in 0.1 M H_2SO_4 containing different concentration of CR and UA. The cyclic voltammograms (CVs) were performed at a scan rate of 20 mV·s^{-1}.

RESULTS AND DISCUSSION

Electrocatalytic oxidation of creatinine and uric acid on modified SPE

CR did not oxidize at unmodified SPE in the 0.1 M H_2SO_4 solution; its CV in the potential range from 0.00 to 1.30 V does not differ from that of supporting electrolyte (fig.1a, curve 1).

In order to explore the effect of modification on organic compounds oxidation, CVs for CR or UA were obtained at the electrodes modified with AuNPs. Measurements were performed in the 0.1 M H_2SO_4 solution at pH 1.0 in the potential range -0.2 – 1.3 V.

The peak current of oxidation of AuNPs electrodeposited on SPE increases in the presence of UA at *E* 0.30 V and in the presence CR at *E* 1.00 V (fig.1, curve 3). Multiple increase of peaks current, decrease in the overpotential of the oxidation process, a linear relation of a current of peak from concentration of organic compounds, allows to attribute electrochemical processes to catalytic.

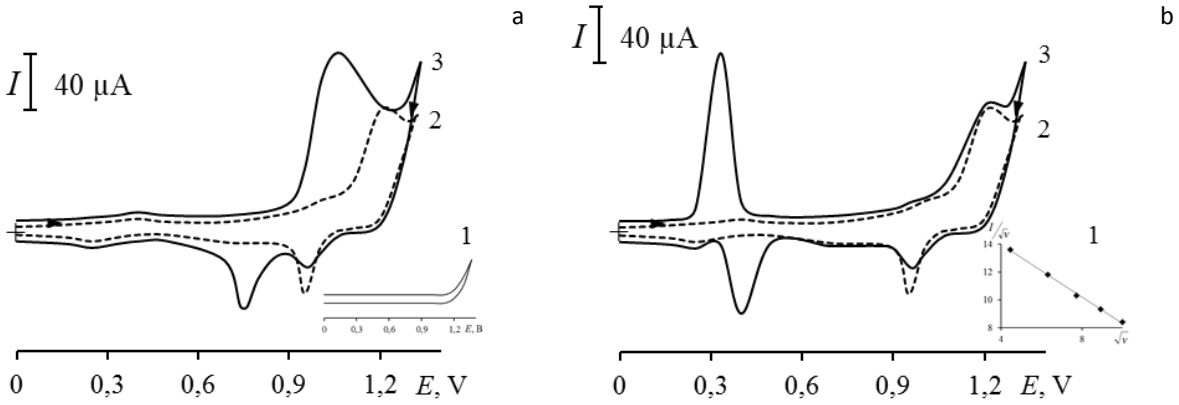

Fig. (1). – Cyclic voltammograms recorded at unmodified SPE for creatinine (a, curve 1), at AuNPs/SPEs for creatinine (a, curve 3) and uric acid (b, curve 3) in 0.1 M 0.1 M H_2SO_4 at a scan rate of 20 mV s^{-1}, the dotted line - background (a, b curve 2). The dependence of $I/\sqrt{v}$ from $\sqrt{v}$ for the oxidation of uric acid at AuNPs/SPE (1b).

The electrooxidation processes of CR and UA are usually demonstrated by the following scheme [23, 24]:

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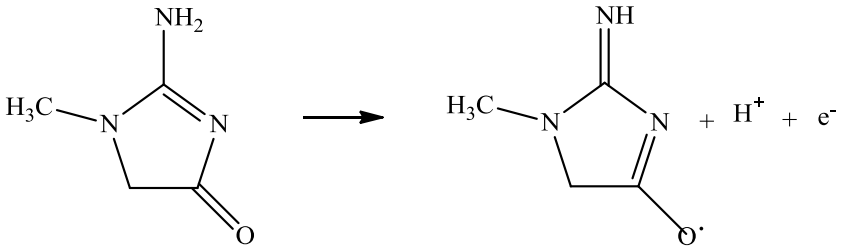
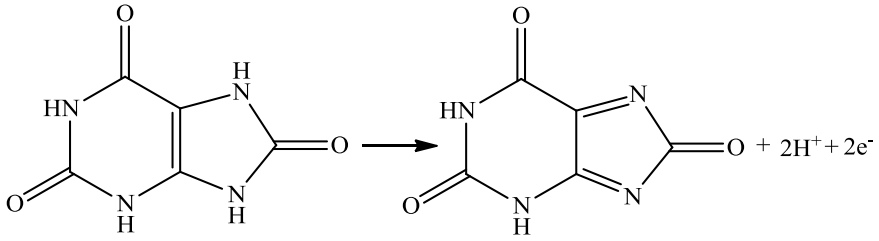
Creatinine



(1)

Uric acid



(2)

Scan rate can influence on the current responses of CR and UA and relevant electrochemical characteristics could be concluded from the relationship between the scan rate of potential sweep and current responses of organic compound oxidation. Scan rate studies were investigated to evaluate whether the oxidation processes on AuNPs/SPE were controlled by adsorption, diffusion or kinetics. In the studies of the dependence of the oxidation peak current from the scan rate, it was found that the slope of the linear – graph was negative (Fig. 1b), which pointed to the predominantly kinetic character of the current.

Thus, the modification of the electrode by AuNPs provided an improvement of the electrochemical characteristics of the electrode and a good surface for fast electron transfer. The response of gold nanoparticles electrodeposited on the SPE increased. The modified electrode exhibited excellent electrocatalytic activity toward CR and UA. The result obtained indicates that this modified electrode will be a good sensor for their determination.

Simultaneous determination of creatinine and uric acid

The proposed voltammetric method was employed for the simultaneous determination of CR and UA. As it could be seen from Fig.2a, the mixture of CR and UA exhibited two anodic peaks current at 0.30 and 1.00 V, corresponding to the oxidation of CR and UA, respectively. The potential differences is 0.70 V. These peaks were well separated and their simultaneous determination could be achieved.

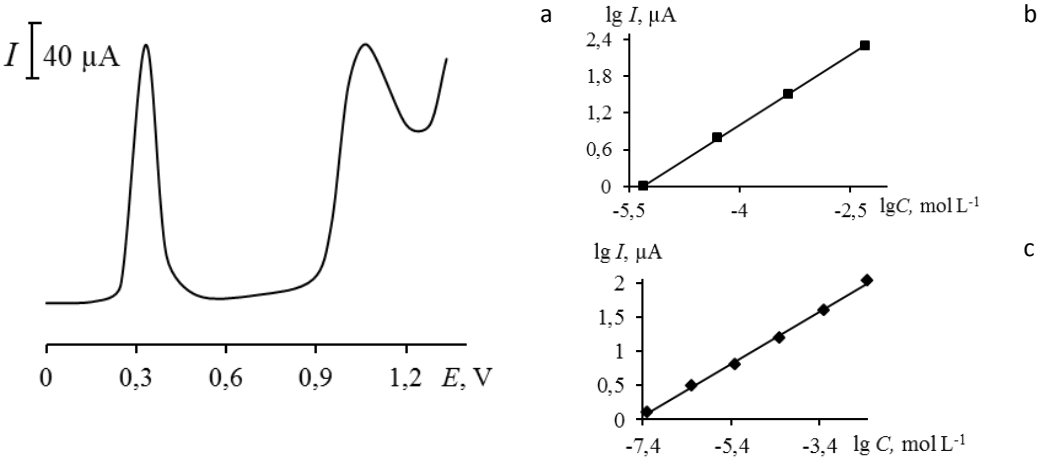


Fig. (2). – Anodic voltammogram of a mixture of 5×10^{-3} mol·L^{-1} creatinine and 5×10^{-3} mol·L^{-1} uric acid recorded at AuNPs/SPE in 0.1 M H_2SO_4 at a scan rate of 20 mV·s^{-1} (a). Logarithmic graphs of peak currents of uric acid (b) and creatinine (c) oxidation at AuNPs/SPE from organic compounds concentration.

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Thus, it could be concluded that, by employing the proposed voltammetric method, the simultaneous determination of these compounds was as efficient as their individual determinations.

Under the working conditions, the oxidation peak currents of various concentrations of CR and UA at the AuNPs/SPE were recorded. The peak current (I_p) was proportional to the concentration of CR in the range 5.0-5000 μmol·L^{-1} (Fig.2b) and of UA in the range 0.05-5000 μmol·L^{-1} (Fig.2c). The linear regression equations were lgI_p (μA) = 0.76 lgC (mol·L^{-1}) + 4.06 (r^2=0.9970) and lgI_p (μA) = 0.38 lgC (mol·L^{-1}) + 2.88 (r^2=0.9980) for CR and UA correspondently. A good sensitivity can be due to the efficiency of the electron transfer between the modified SPE and CR or UA.

Flow injection analysis

FIA system contained a pump system, manual sample injection valve with a 1.0 mL sample loop, wall-jet flow cell with SPE joined to a potentiostat. The carrier solution, 0.1 M sulfuric acid, was transported by the pump into the system. The samples were injected into the valve using a 1.0 mL syringe.

In order to find the most suitable conditions for the quantitative determination of CR and UA were studied some parameters in FIA. FIA-signal were recorded in the potentiometric mode. Therefore, the dependence of the current on the overlay potential were investigated. Accordingly, the working electrode potential was chosen *E*=1.2 V for CR and *E*=0.6 V for UA detection (Fig. 3a).

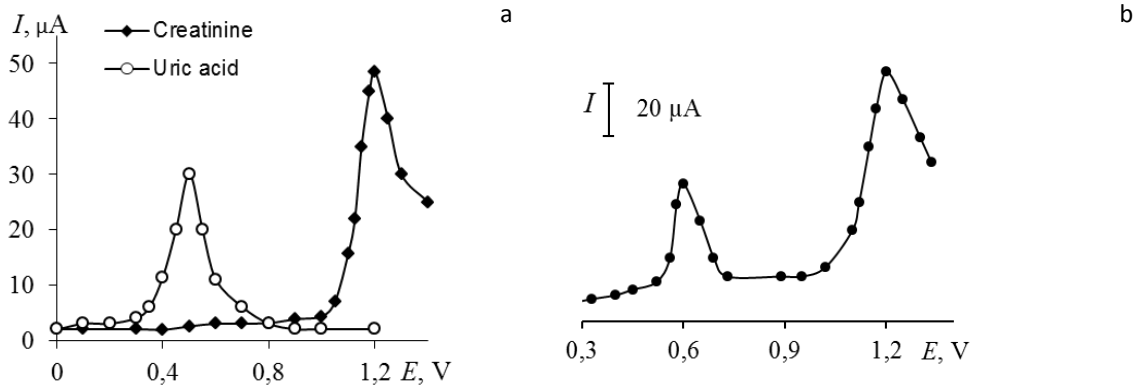

Fig. (3). – FIA amperometric response obtained at AuNPs/SPE for a 5×10^{-3} mol·L^{-1} creatinine and uric acid solutions (a) and mixture of creatinine and uric acid (b) as a function of the potential

The maximum response of the AuNPs/SPE was observed at a volume of the sample injected (*V*) of 400 μL (Fig. 4a) and at a flow rate (*u*) of 3.09 mL·min^{-1} (Fig. 4b) for both compounds.

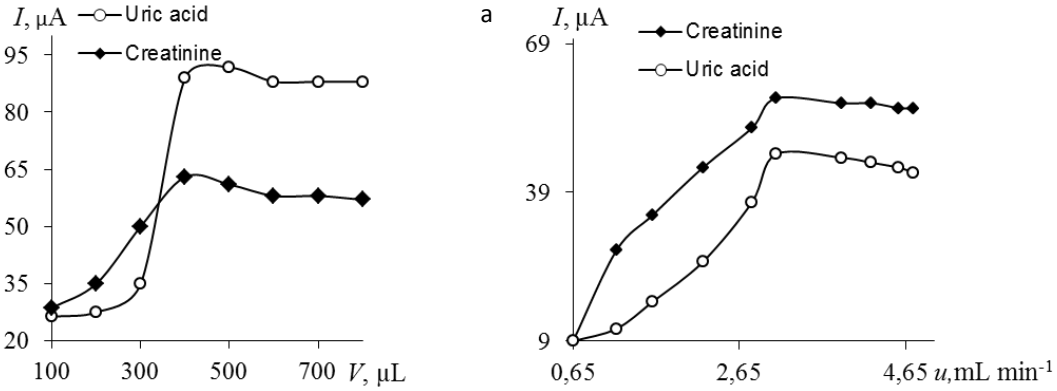

Fig. (4). – FIA amperometric response obtained at AuNPs/SPE for a 5×10^{-3} mol·L^{-1} creatinine and uric acid solutions as a function of the injection volume (a) and the flow rate (b)

The effect of electrode potential on the value of current at AuNPs/SPE in the solution contained mixture of CR and UA in FIA was studied. Fig. 3b displays the two well-defined anodic peaks that can be referred to the oxidation of UA and CR.

The method of simultaneous determination of CR and UA on the SPE with two working electrodes modified by AuNPs in the FIA conditions proposed. The parallel arrangement of two working electrodes on the surface of SPE allows determining of two compounds at the same time by registration of FIA amperometric responses of modified electrodes at different potentials.

The calibration plot of analyt determination is leaner in the range from 5×10^{-7} to 1×10^{-3} mol·L^{-1} for CR (linear regression giving a correlation coefficient of 0.9991) and 5×10^{-10} to 1×10^{-3} mol·L^{-1} for UA (linear regression giving a correlation coefficient of 0.9987).

The reproducibility of analytical signal height is quite satisfactory. The relative standard deflection did not exceed 2 %. Using of the electrocatalytic response of the AuNPs/SPE under FIA conditions can achieve a theoretical performance up to 180 samples/h with the electrode response time of 20 s.

Thus, SPE modified by AuNPs can be used for amperometric detection of these compounds in biological fluids for the clinical diagnosis of various diseases.

CONCLUSION

AuNPs/SPE shows electrocatalytic activity to the oxidation of UA and CR. It is exhibited in decreasing of overvoltage of organic compounds oxidation and in increasing of oxidation peak current of modifier. A very sensitive and simple amperometric method was developed for the detection of UA and CR on this modified electrode in FIA conditions. Using modified SPE with two working electrodes in FIA provides simultaneous detection of CR and UA at the same time. A sensitive linear dependence of amperometric response of modified electrode from concentration of UA was obtained in the concentration range of $5.0\times10^{-10} \div 5\times10^{-3}$ mol·L^{-1} and of CR in the range of $5.0\times10^{-7} \div 5\times10^{-3}$ mol·L^{-1}. Good stability and reproducibility were obtained for simultaneous determination of UA and CR. The advantage of this method is its high sensitivity, selectivity, rapidity, low cost and easy of operation in comparison with other methods.

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