Sensors and Actuators B 176 (2013) 307–314

Contents lists available at SciVerse ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Conducting polymer film based electrochemical sensor for the determination of amoxicillin in micellar media

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ARTICLE INFO

Article history:
Received 15 April 2012
Received in revised form 3 September 2012
Accepted 4 September 2012
Available online 15 September 2012

Keywords:
Polyaniline film modified CPE
Amoxicillin
Pharmaceuticals
Adsorptive stripping voltammetry
SWAdSV
DPAdSV

ABSTRACT

The objective of the present work is to develop a sensitive and selective voltammetric method for the determination of amoxicillin (AMX) based on the polyaniline film modified carbon past electrode (CPE) in micellar media. The adsorptive voltammetric behavior of amoxicillin at polyaniline film modified carbon past electrode was investigated and validated in pharmaceuticals and biological fluids by cyclic voltammetry (CV), differential pulse adsorptive stripping voltammetry (DPAdSV), and squarewave adsorptive stripping voltammetry (SWAdSV). The voltammograms of the AMX in 0.2 M acetate buffers of pH 2.0–6.5 exhibit a single well defined oxidation peak which may be due to the oxidation of —OH— group. The cyclic voltammetric studies indicate, the oxidation of amoxicillin at the electrode surface is a reversible step and diffusion-controlled. Various chemical and instrumental parameters affecting the monitored electroanalytical response were investigated and optimized for amoxicillin determination. The achieved limits of detection and quantification were 3.5×10^{-10} M and 1.5×10^{-9} M by SWAdSV and 7.3×10^{-10} M and 5.4×10^{-9} M by DPAdSV, respectively. The applicability of the proposed method was tested in biological samples and pharmaceutical preparations.

1. Introduction

Amoxicillin (AMX), D-α-amino-p-hydroxybenzylpenicillin trihydrate is one of the most frequently used lactam antibiotics in the world and it is employed to treat humans and animals. As other lactam antibiotics present a structure based on a lactam ring responsible for the antibacterial activity and variable side chains that account for the major differences in their chemical and pharmacological properties. Despite a high level of clinical success, a serious mechanism of resistance had emerged demanding high dose regimen and new pharmacokinetic combination. AMX is one of the more important antibiotics used in the treatment of bacterial infections and its determination [1–3].

Polymer films modified electrodes [4–16] have been receiving great attentions recently due to their wide applications in the fields of chemical sensors and biosensors. Such modified films can significantly improve the electrocatalytic properties of species, decrease the overpotential, increase the reaction rate and improve the stability and reproducibility of the electrode response in the field of electroanalysis. Up to now, different methodologies have been used to prepare polymeric films modified electrodes. Among them, electro polymerization has demonstrated to be a very convenient means for immobilizing polymers on various conductive substrates because the deposition can be controlled by adjusting the electrochemical parameters and the process is located at the electrode surfaces. Thus, the thickness, permeation and charge transport characteristics of the modified polymeric films can be well defined.

Electrochemical methods [17–25] such as square-wave voltammetry (SWV), stripping voltammetry (SV), differential pulse voltammetry (DPV) and differential pulse polarography (DPP) have been widely applied for the determination of pharmaceuticals. Electrochemical techniques are time saving, cost effective, provide qualitative and quantitative information.

Additions of surface-active agents have proven to play an effective role in the electroanalysis of biologically active compounds and drugs. The use of surfactants as drug carriers makes necessary the study of the interaction of drugs with micellar systems, implying the elucidation of the nature of these interactions. Solubilization of electroactive compounds in aqueous solutions containing surfactants provides a new medium for electrochemical studies. Surfactants influence the electrochemical processes of electroactive species and thus are widely used in electroanalytical chemistry to improve the sensitivity and selectivity [26–34].

Few analytical methods have been reported to quantify amoxicillin based on spectrophotometric [35–38], fluorimetric [39] flow injection analysis [40], HPLC [41] and polarography/voltammetry [1–3,42]. Furthermore there appears to be no adsorptive stripping voltammetric method for the determination of amoxicillin in micellar media at polyaniline film modified carbon past electrode (CPE).

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http://dx.doi.org/10.1016/j.snb.2012.09.007

The purpose of the present work is to prepare sensitive electrochemical sensor for the study of voltammetric behaviour of amoxicillin in micellar media and to establish the methodology for their trace determination using differential pulse adsorptive stripping voltammetry (DPAdSV) and square-wave adsorptive stripping voltammetry (SWAdSV) in bulk form, pharmaceutical formulations and biological fluids.

2. Experimental

2.1. Materials and methods

Amoxicillin (98% purity) was obtained from Sigma–Adrich Laboratories, Mumbai, India. Tablets containing amoxicillin [Amoxil] labeled 500 mg was obtained from commercial source. Blood was obtained from healthy volunteers. Blood was kept frozen until assay. After gentle thawing, the samples were spiked with appropriate concentrations of the drug. For the preparation of a standard stock solution of amoxicillin (0.001 M) 36.5 mg of amoxicillin was accurately weighed, dissolved in distilled water, and then adjusted to 100 ml to give the appropriate concentration. Standard working solutions were prepared by appropriate dilutions of the stock solution.

2.2. Instrumentation

The voltammetric studies were carried out in exploratory and determination mode on a software connected Ω Metrohm 797 VA Computrace (ion analyzer). The voltammetric cell consisted of a three electrode assembly and a stirrer with polyaniline film modified carbon past electrode (CPE) as a working electrode, a platinum wire as auxiliary electrode and Ag/AgCl electrode as reference electrode. Nitrogen gas was purged through the solution for 5 min. A systronics digital μpH meter model-361 was used for pH measurements.

2.3. Procedure

The general procedure adopted for obtaining differential pulse voltammograms was as follows: a 10 ml aliquot of acetate buffer at desired pH was pipette out in clean and dry voltammetric cell and the required standard solution of amoxicillin was added. The test solution was purged with nitrogen for 5 min initially, while the solution was stirred. The selected accumulation potential vs. Ag/AgCl was applied to a modified electrode while the solution was stirred for 90 s. Following the preconcentration period; the anodic scans were carried out over the range 0.0 to +1.0 V. All measurements were made at room temperature.

2.4. Operational conditions of electrochemical measurements

For CV, DPV and SWV measurements, a known volume of amoxicillin was pipetted into a 10 ml volume calibrated flask and then completed to the volume with acetate buffer. Then purified nitrogen was passed for 5 min to remove the dissolved oxygen under stirred conditions. The selected accumulation potential was applied at the working electrode for a selected time by keeping the constant pulse amplitude while the solution was stirred. At the end of the accumulation time period, the stirrer was stopped and 10 s was allowed for the solution to become quiescent. Then voltammograms were recorded by scanning the potential towards the positive direction over the range of 0.0 to +1.0 V vs. Ag/AgCl reference electrode by applying the square-wave waveform. All data were obtained at room temperature.

2.5. Construction of calibration curve

Aliquots of amoxicillin were transferred to 25 ml volumetric flask and were completed to the mark with acetate buffer. The solution was then transferred into a voltammetric cell, and pure N_2 gas was passed for 5 min. Cyclic, square-wave and differential pulse voltammograms were then recorded in the range of 0.0 to +1.0 V. The calibration graph was obtained by plotting a known concentration of amoxicillin with peak current. The recovery was calculated using the standard addition method.

2.6. Preparation of modified carbon paste electrode

Prior to the electrode modification, the carbon paste electrode was mechanically polished with alumina powder (Al_2O_3, 0.05 μm) to a mirror finish and ultrasonicated in distilled water for 5 min. Then CPE was electrochemically activated by using 10 times cyclic potential sweeps in the range of −0.5 to +2.0 V in 0.1 M nitric acid solution at a scan rate of 50 mV s^{-1}. Electrochemical modification of the pretreated CPE was performed using cyclic voltammetry in 0.5 mM polyaniline solution, the CPE electrode was cycled under the same conditions. After the electropolymerization, the modified electrode was rinsed thoroughly with distilled water and then dried in air for 30 min at room temperature. For comparison, CPE and modified CPE electrodes were used to investigate their electrochemical properties toward the analytes under investigation.

2.7. Application procedures

2.7.1. Tablet assay procedure

Amoxicillin determination was performed on the commercially available tablet dosage form amoxil. Each film-coated amoxil tablet contains 500 mg of AMX. Ten tablets were accurately weighed and thoroughly ground to a fine powder. A portion of the powder equivalent to the average weight of one tablet was transferred into a 100 ml volumetric flask. The mixture was sonicated for 15 min to provide complete dissolution and then centrifuged. The resulting suspension was allowed to settle, and an aliquot of the clear supernatant was transferred quantitatively into a calibrated flask and then completed to volume with the same solvent to get a final concentration. Aliquots of this solution were diluted to a final volume of 10 ml with selected supporting electrolyte and then transferred to a voltammetric cell. The square-wave and differential pulse voltammograms were then recorded.

2.7.2. Serum and plasma assay procedure

Drug-free human blood obtained from healthy volunteers was centrifuged at 5000 rpm for 30 min to separate serum and plasma at room temperature. Separated serum and plasma samples were stored froze until analysis. Then separated serum and plasma samples were treated with 1.0 ml of acetonitrile as protein denaturing and precipitating agent. After vortexing for 30 s, the serum and plasma samples were centrifuged for 10 min at 5000 rpm in order to eliminate serum and plasma protein residues. An aliquot of serum and plasma samples were fortified with AMX solution to achieve a final concentration of 2.5×10^{-3} M. Appropriate volumes of these spiked samples were transferred into the voltammetric cell containing acetate buffer of pH 3.5. Quantification was performed by means of calibration curve method.

3. Results and discussion

3.1. Voltammetric behavior of amoxicillin

Cyclic voltammograms obtained for electrochemical oxidation of 4.0×10^{-5} M amoxicillin in 0.2 M acetate buffer (pH 3.5) in 3 mM