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Electro Analytical Method for Detection of Bacteria Using Amoxicillin Modified Carbon Paste Electrode: Analytical Application in Milk

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Abstract

A carbon paste electrode modified with amoxicillin antibiotic was employed for the quantification of bacteria from aqueous solutions. The new electrode (AMX-CPE) revealed interesting electroanalytical detection of *Staphylococcus aureus* based on the adsorption of bacteria onto amoxicillin under open circuit conditions. The influence of variables such as the accumulation time, pH solution and optical density (OD) was tested by square wave voltammetry (SWV) and cyclic voltammetry (CV). The response depends on the optical density of bacteria in the bulk solution as well as the parameters involved in the preconcentration, pH and the measurement steps. The elaborate electrode was used for the detection of *S. aureus* in milk and showed a very good limit of detection (3.00×10^{-6}) in milk.

Keywords: Modified electrodes; SWV; CV; Bacteria; Biosensor; *Staphylococcus aureus*

Introduction

The *Staphylococcus aureus* is a coccobactery positive Gram, positive catalase belonging to the family of *Staphylococcaceae* [1,2]. It has a diameter from approximately 0.5-1.5 µm, motionless, optionally anaerobic, usually laid out in bunches and fact part of the human flora and is especially present in the nose and on the skin [3]. One also finds it at several places at the human ones: groin, armpits, area perineal (men), mucous membranes, stops, glands mammals, hair, intestinal tract, genital-urinary apparatus and higher respiratory tracts [2,4,5]. Many animals are used as tanks, in particular the cows of which worse are infected [6]. It is the cause of multiple infections, which go from the cutaneous lesion (furuncle, whitlow, impetigo, etc.), with endocarditis, acute pneumonia, osteomyelitis or septicemia. This bacterium is one of the principal causes of food toxinfections, resulting from the food consumption contaminated by enterotoxins [4]. In our previous article, we proposed some methods to determine *S. aureus* in aqueous solution using amoxicillin modified carbon paste electrode (AMX-CPE) [7]. The aim of this work is to use this electrode in conjunction with square wave voltammetry (SWV) method for the determination of bacteria in milk.

Materials and Methods

Reagents

All chemicals were of analytical grade and were as received without any further purification. All solutions were prepared in double distilled water. 1 M NaCl solution is used as supporting electrolyte for the determination of *Staphylococcus aureus*. Amoxicillin and all reagents were purchased from Sigma. Carbon paste was supplied from (Carbone, Larraine, Ref 9900, French).

Instrument

All electrochemical experiments were performed by a potentiostat (model PGSTAT 100) interfaced to a personal computer and controlled by voltalab Master 4 software. A three electrode design consisting of a platinum counter electrode, SCE reference electrode and a modified carbon paste working electrode. Prior to any electrochemical measurements, the solutions were thoroughly degassed with nitrogen (BOC Gases), whilst a continuous flow of the gas was maintained through the electrochemical cell during the experiments. The pH meter (Radiometer Copenhagen, PHM210, Tacussel, French) was used for adjusting pH values.

Electrodes synthesis

AMX-modified carbon-paste electrode was prepared according the following procedure [8]. The modified carbon-paste (CP) electrode was prepared by mixing the graphite powder with the amoxicillin (AMX) to give an appropriate ratio AMX/CP. The mixture was grinding in a mortar agate and then a portion of the resulting composite material was housed in PTFE cylinder. The geometric surface area of the working electrode was 0.1256 cm^2. A bare of carbon vitreous inserted into carbon paste provided the electrical contact.

Analytical procedure

The modified carbon paste electrode was immersed in a cell containing bacteria sample to get a chemical accumulation. Meanwhile, the solution was rotated about 600 rpm at open circuit. After a desired contact time, the electrode was removed from the preconcentration cell, rinsed with DW and placed in the measurement cell containing the supporting electrolyte (1.0 mol L^{-1} NaCl). The square wave voltammograms were recorded in different bacteria concentrations using 5 mV of the pulse amplitude, step potential 50 mV and the duration time is 2 s at scan rate 100 mVs^{-1}.

Result and Discussion

Figure 1 shows a cyclic voltammograms (CV) performed between -2 to 2 V recorded for amoxicillin modified carbon paste electrode at 100 mVs^{-1}, in electrolytic solution, after exposure the AMX-CPE to different solution containing various concentrations of bacteria while 15 min. No significant peak is observed in the absence of bacteria in electrode surface. Contrary, when the prepared electrode is pre-concentrated in the solutions containing bacteria at different optical density, we see the emergence of two genuine redox peaks, the first one, at about -0.2 V in the cathodic direction of potential scan, the second one at 0.15 V in the anodic direction.

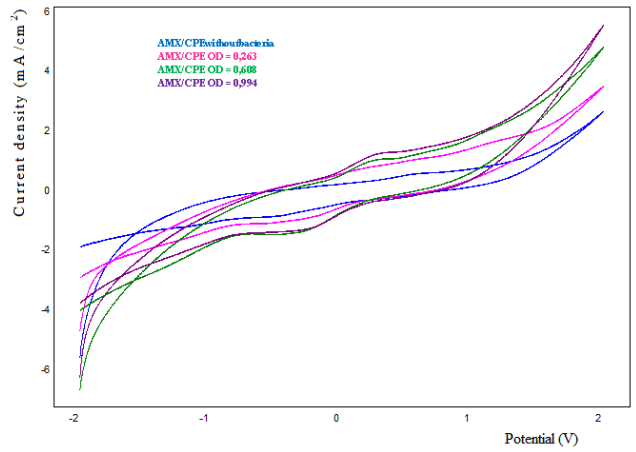

Figure 1: Cyclic voltammograms recorded in electrolytic solution, at 100 mV/s, at AMX-CPE, in 1 M NaCl, after exposure electrode to different solution containing different concentrations of bacteria.

The ability of the AMX-CPE to accumulate bacteria was studied. Figure 2 shows the square wave voltammograms obtained in 1.0 mol L^{-1} NaCl for prepared electrode after accumulation in the solutions containing bacteria (pH 7.4) for 15 min. the recorded SWV's give rise to a broad peak, for which the current density increases with increasing optical density (OD).

The dependence of peak current on the bacteria optical density solution was also investigated (Figure 3). The peak current increases with the increasing in the OD values, which can be expressed according to the following equation:

di = 135.39 OD + 111.93

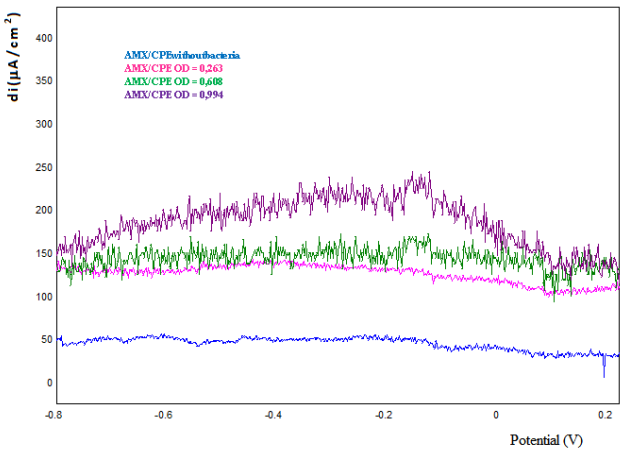

Figure 2: Square wave voltammograms recorded at AMX-CPE, in 1 M NaCl solution, the preconcentration time was 15 min, pH 7.4.

The current density of the peak moves from 52.07 $\mu A/cm^2$, in the absence of the bacteria in electrode surface, to 236.2 $\mu A/cm^2$ when the OD value reaches 0.994 (Table 1).

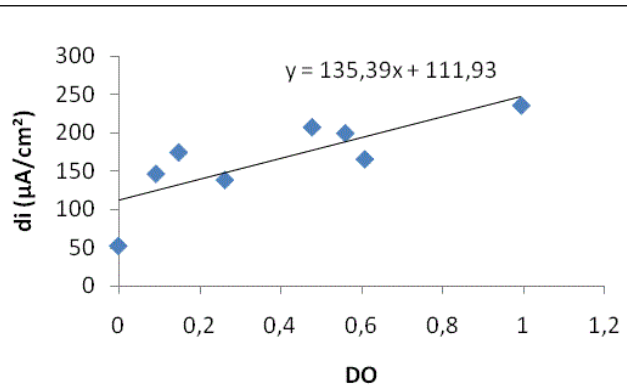

Figure 3: Density of current according to OD of the bacteria.

DO	0	0.093	0.149	0.263	0.478	0.560	0.608	0.994
di ($\mu A/cm^2$)	52.07	146.6	174.7	138.5	207.7	199.8	165.7	236.2

Table 1: Evolution of the current density peak, with the OD solution value.

A detection limit of 2.954×10^{-5} mol L^{-1} was determined using a 3σ/slope and the limit of quantification is 9.846×10^{-5} mol L^{-1}, where σ is the standard deviation of the mean value for height voltammograms of the blank, calculated according to Miller and Miller [9].

This biosensor was electrochemically characterized by the cyclic voltammetry in order to determine its duration of detection. It arises that after 50 cycles, that is to say one duration 66 min in the presence of the bacteria; the electrode posts the same pace while increasing in electro activity (Figure 4).

In a second step, the effect of pH on electrode response was investigated. Figure 5 shows the influence of the pH solution on the electrochemical response of bacteria presence at AMC-CPE. The current of the peak depend on the solution pH. The peak current density increases with pH in the range from 4.0 up to 9 and in pH 4.22

the peak current density gives a maximum peak. A decrease in the current is observed when the solution pH is higher than 7.0.

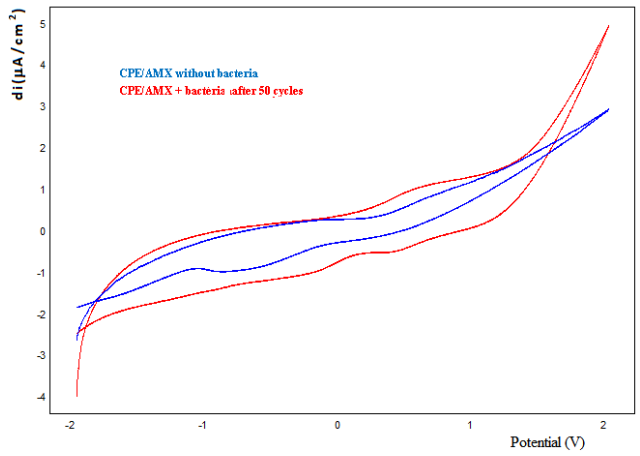

Figure 4: Comparison of the Cv's recorded at AMS-CPE, in electrolytic solution and after exposure electrode to bacteria solution (pH 7.4), scan rate: 100 mV/s.

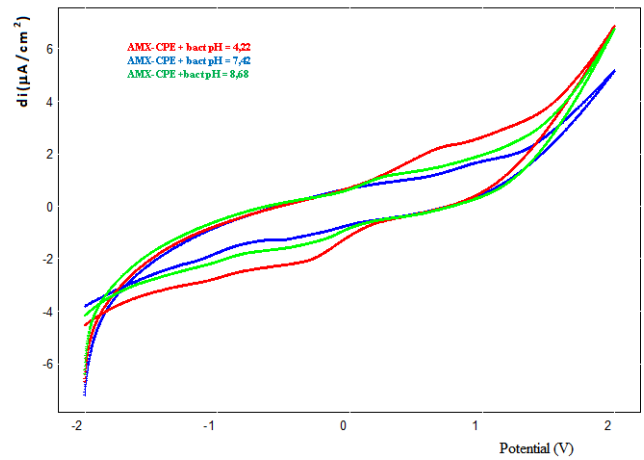

Figure 5: pH dependence of the peak current on CV response.

Practical Application

Under the optimized conditions, biosensor (CPE-AMX) was used for the detection of *Staphylococcus aureus* in the milk sample without a preliminary treatment. The bacteria were added to various optical densities in the samples of milk and then these samples were studied by the technique of the cyclic voltammetry and square wave voltammetry with the electrode. These results are presented in Figure 6.

The density of current increases with the evolution of the optical density of the bacteria, measured using a spectrophotometer. Figure 7 shows a typical linear answer, which can be expressed according to the following equation:

di = 66.765 OD + 166.45

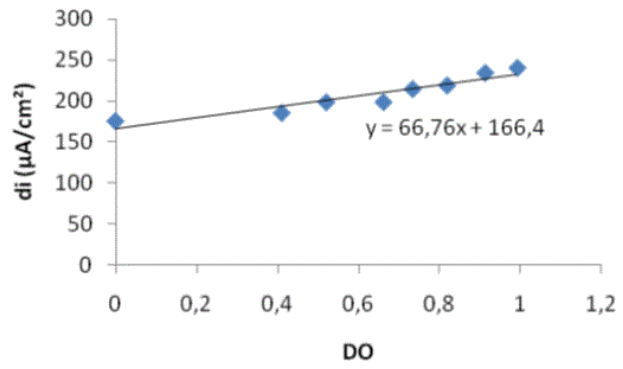

Figure 6: Density of current according to the optical density.

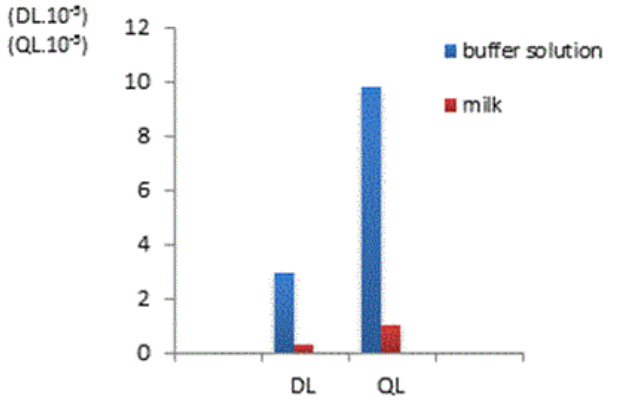

Figure 7: Comparison of DL and QL calculated respectively, in buffer solution and in milk solution.

Taking into consideration these result, it arises that the biosensor shows a better DL and QL in milk; that could be due to the fact that this medium is favourable to the bacteria. This medium thus supporting its growth, the bacterium becomes increasingly sensitive to the amoxicillin. Because indeed, the amoxicillin, like other penicillins, has a conformation which are connected in D-alanyl-D-alanine of the precursor of the peptidoglycan (component essential of the cellular wall) [7,10]. This similarity confers to him an affinity for the active site of the PLP (Proteins Binding Penicillin)

Conclusion

Biosensor (AMX-CPE) is extremely sensitive to the bacteria. The pH has an influence on the electro activity of this electrode and the acid medium seems more favourable. Also, its duration of detection is satisfactory. The analytical study in a milk sample showed good results.

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