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Electrochemical Sensing of Phenicol Antibiotics at Gold

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Phenicols are an effective and a broad spectrum class of antibiotics which has lost favour due to their side effects on human health. A rapid and sensitive electrochemical detection system is developed for the simultaneous detection of chloramphenicol (CAP), thiamphenicol (TAP) and florfenicol (FF). The electrochemical behaviour of CAP in the presence of its derivatives was investigated by cyclic voltammetry (CV) and square wave voltammetry (SWV). At a gold electrode, CAP gives rise to a sensitive cathodic peak at −0.68V (versus SCE) in a tris buffer solution (pH 7.6). This behavior gives us the opportunity to introduce a method for sensing CAP electrochemically in the presence of its derivatives. Calibration graphs were linear in the 2.5-7.4 μmol L^{-1} concentration range. Deviations from linearity were observed for higher concentrations and this was interpreted to be due to kinetic limitation caused by the saturation of CAP and its reduction products onto the gold electrode surface. A limit of detection of 1 μmol L^{-1} was found.

Keywords: electrochemical detection; antibiotics; chloramphenicol; florfenicol; thiamphenicol

1. INTRODUCTION

Veterinary antimicrobial drugs such as chloramphenicol (CAP), florfenicol (FF), tiamulin and tilmicosin are used in food-producing animals to prevent diseases caused by infections and for improving food efficiency [1]. The administration of antibiotics to animals results in entering bactericide and bacteriostatic components into animal tissues and their products such as milk and honey. This phenomenon has been resulting in antibiotic-resistant strains of bacteria with potential human health problems. Therefore, residue of these drugs may cause a health risk to consumers and monitoring the residues level is an important and critical part of quality control programs [1,2].

The phenicol class of antibiotics consists of CAP, FF and thiamphenicol (TAP). These are rather small molecules and all are very lipid-soluble in physiological pH [2-4]. The main member of

the group, CAP (Fig. 1), is a neutral nitrobenzene derivative and is effective as antibiotic (wide spectrum). In 1947, Ehrlich and coworkers reported the purification of CAP from *Streptomyces venezuelae* [2]. It has been used for more than 60 years but due to its side effects in humans, the drug is no longer recommended for the treatment of minor diseases [3-6].

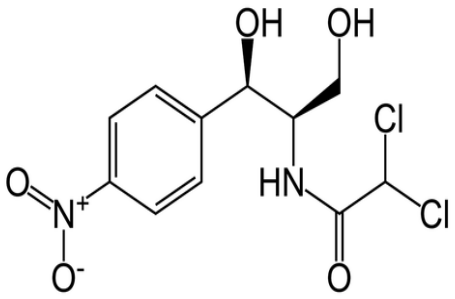


Figure 1. Chemical structure of chloramphenicol (CAP).

CAP has two kind of side effects on humans. The most serious effect is an irreversible aplastic anaemia. The second form involves reversible bone marrow suppression [4,6]. Subsequently, it has been banned from use in food-producing animals in the EU, US and Canada [2]. Minimum required performance limit (MRPL) has been published in the Commission Decision 2002/657/EC and is described as "minimum content of an analyte in a sample, which at least has to be detected". This value is used as the reference value for evaluating the food quality. Until now, the MRPL reported for CAP is 0.3 μg kg^{-1} [2,8,9]. A rapid, accurate and inexpensive analytical method is essential for detecting this limit. Simultaneous detection of analogous components is also critical in this area.

TAP (Fig. 2 (a)) is an analogue of CAP in which the p-nitro group at the benzene ring is changed by a methylsulphonyl group. This compound shows lesser antibacterial activity. Although it is an effective antibiotics, it can show hematological toxicity [7]. FF (Fig.2 (b)) is a fluorine derivative of CAP, having a fluorine atom instead of a hydroxyl group. In spite of being a structural analogue of TAP, FF has a superior spectrum of activity and greater potency. It can easily enters most body tissues but to a lesser extent than CAP, therefore, it is strongly banned from use in humans [6-8].

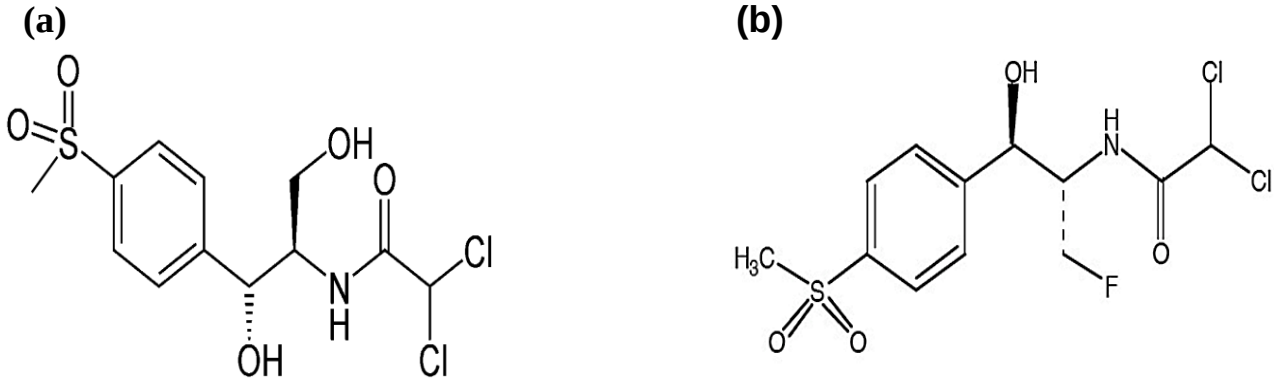


Figure 2. Chemical structure of (a) thiamphenicol (TAP) (b) florfenicol (FF).

Different strategies are used for the determination of CAP and its derivatives. Gas chromatography (GC) [10-13] and high performance liquid chromatography (HPLC) [14] are among the commonly used methods. These methods are time consuming, expensive and suffer from lack of selectivity. Moreover, they need several cleanup steps and a very selective detection system.

An alternative is the use of electrochemical sensors which are widely used in pharmaceutical applications. These analytical devices consist of an electrochemical transducer that converts a biological or chemical recognition process into an electrical signal which is related to the recognition process and thus proportional to the analyte concentration [15-19]. This technique offers some advantages such as easy to operate, fast and low cost. The sensitivity of electrochemical methods is often greater than the classical methods such as chromatography. Electrochemical sensors have potential for miniaturization and construction for portable equipment applications. Moreover, they make a fast analysis and a continuous quality control during the food processing possible [1,18].

Voltammetric determination of CAP at electrochemically activated carbon fibre microelectrodes [20], at multiwall carbon nanotube-modified electrodes [21] and at a pretreated glassy carbon have been reported [22]. However, these methods suffer from non-selectivity and the electrochemical behaviour of CAP derivatives isn't included.

This article aims a profound electrochemical study of the electrochemical behaviour of the phenicol class of antibiotics at a gold electrode. The strength of this article is the detection of the compounds in a matrix of all phenicol compounds. This article gains fundamental knowledge for the electrochemical detection of antibiotics in a broader context of biosensor development. We recently developed an aptamer modified electrode for the detection of chloramphenicol. This article is a key element in understanding the electrochemical behavior of chloramphenicol at an aptasensor.

2. EXPERIMENTAL

2.1. Chemicals

CAP, FF, TAP (Sigma, Belgium) and Tris buffer (100 mmol L^{-1} NaCl, 20 mmol L^{-1} Tris HCl, 2 mmol L^{-1} $MgCl_2$, 5 mmol L^{-1} KCl , 1 mmol L^{-1} $CaCl_2$, 0.02 mmol L^{-1} Tween 20, PH=7.6) (VWR, Belgium) were used.

Stock solutions of CAP were prepared by means of deionized water and ethanol. The working solutions for the voltammetric investigations were prepared by dilution of the stock solution with aqueous buffer solutions. Stock solutions of CAP were prevented from decomposition by keeping in 8°C. The solutions were thoroughly deoxygenated by flowing nitrogen through the cell solution for 30 min and a nitrogen atmosphere was maintained over the solution during the experiment. All measurements were performed at room temperature.

2.2. Electrochemical measurements

All electrochemical measurements were performed by using a PGSTAT20 potentiostat controlled by GPES 4.9 005 software package running (ECO Chemie, The Netherlands).

Electrochemical experiments were performed in a three electrode cell configuration using a reference electrode (SCE) and a platinum counter electrode. All potentials in the text are referred to the SCE. The working electrodes were gold in laid disk electrodes (d=1.2 mm) which were mechanically polished with alumina polishing pads.

Cyclic voltammetry (CV) and square wave voltammetry (SWV) were used for electrochemical characterization.

3. RESULTS AND DISCUSSION

3.1. Electrochemical detection of CAP

Fig. 3 (dashed line) presents the voltammetric response of a bare gold electrode in a Tris buffer solution (pH 7.6) in a potential window from −0.8 versus +0.8 V with a scan rate of 50 mV s^{-1}. In this potential range, an oxidation wave can be observed between +0.2 and +0.8 V that is related to gold oxidation with formation of gold oxides [24-27].

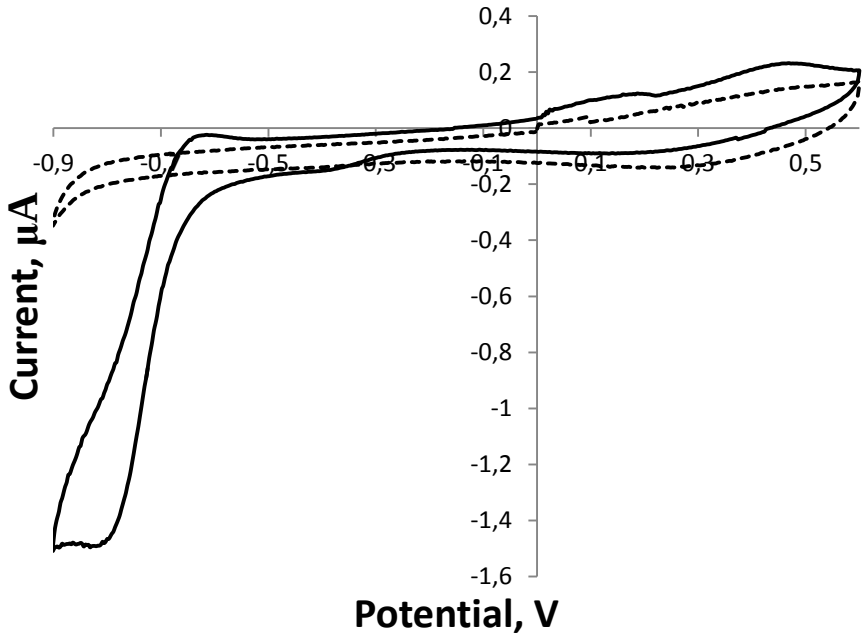
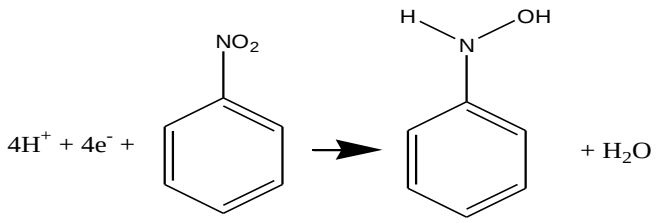


Figure3. Cyclic voltammograms of a gold electrode in a blank Tris buffer solution (dashed line) and in the presence of 7.4 µmol L^{-1} CAP (solid line). Scan rate is 50 mVs^{-1}.

When 1 mmol L^{-1} CAP is added to the electrolyte solution, the corresponding current potential behavior is shown as the solid line. An oxidation wave and two reduction peaks are observed that shows the presence of intermediates in redox reactions of chloramphenicol. A considerable reduction peak (A) is observed with a well-defined reductive peak potential of −0.68 V. This process can be linked to the irreversible reduction of the nitro group (NO_2) present in the CAP molecule with the

formation of hydroxylamine group (NHOH) (Scheme 1) [21,23]. Because a much higher peak current value was found, the first cathodic peak was chosen to be used in the electrochemical detection of CAP.

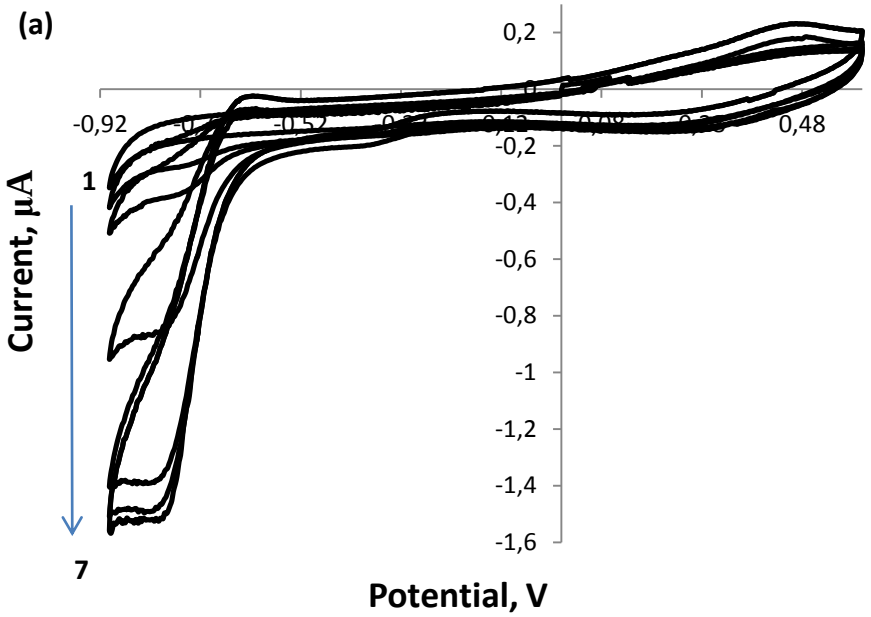


Scheme1. Redox reaction of chloramphenicol.

3.2. Influence of the CAP concentration

The mechanism of the electrochemical process is revealed by varying the CAP concentration. Fig. 4a shows the cyclic voltammetric behavior of a gold electrode in the buffer solution containing different CAP concentrations. The same reduction peak (A) as described in Fig. 3 is observed for all concentrations. When the CAP concentration increases, the reduction current increases.

As can be observed in Fig. 4b, the peak current is only linearly proportional to the bulk concentration of the analyte species for the lowest CAP concentrations. When the signal to noise ratio is 3, the detection limit is 1 μmol L^{-1}. Deviations from linearity were observed for higher concentrations. When the CAP concentration exceeds the value of 6.2 μmol L^{-1}, there is no further increase of the reduction current.



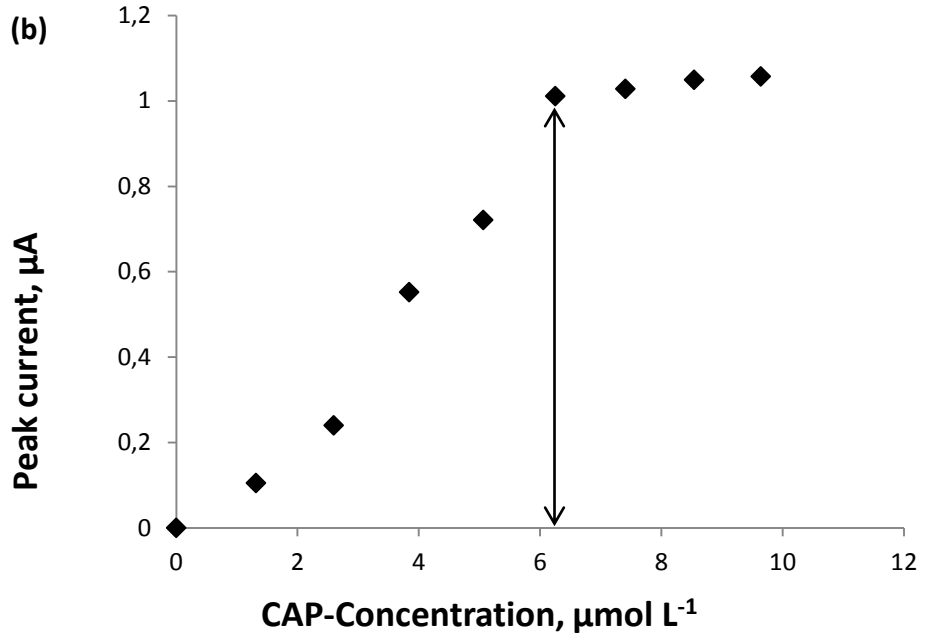


Figure 4. Cyclic voltammograms of a gold electrode in a Tris buffer solution with different CAP concentrations: 0 (1), 1.3 (2), 2.5 (3), 3.5 (4), 5 (5), 6.2 (6) and 7.2 (7) µmol L^{-1} (a). Calibration plot (b).

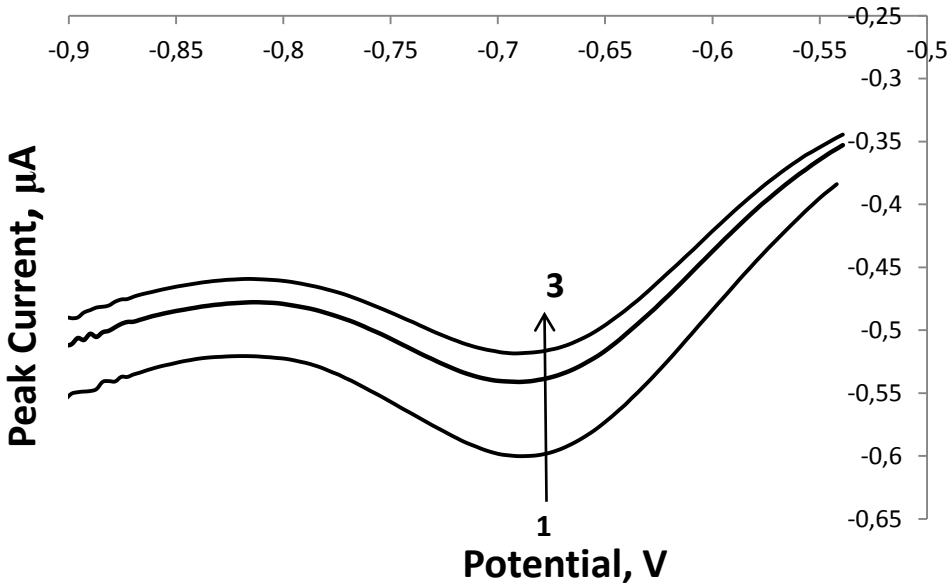


Figure 5. Successive cyclic voltammograms recorded at a gold electrode in a 3.8 µmol L^{-1} CAP solution (pH 7.6): scan # 1 (1), scan # 2 (2) and scan # 3 (3).

This phenomenon can be explained by the fact that the electrode surface is blocked by NHOH formation during CAP reduction, making the interface unavailable for further reduction of CAP molecules [23]. The blocking effect can also be proven by recording successive cyclic voltammograms. The cyclic voltammograms shown in Fig. 5 exhibits an obvious signal decrease

during successive recordings. Again, this phenomenon results from the adsorption of reduction products blocking the gold electrode surface. The schematic representation of the redox reaction of chloramphenicol is shown in scheme 1.

3.3. Influence of the scan rate

The dependence of the scan rate on the peak current I_p was investigated to determine the nature of the process. Fig. 6 shows the reduction of 1 mmol L^{-1} CAP at a gold electrode in a Tris buffer solution over a wide range of scan rates.

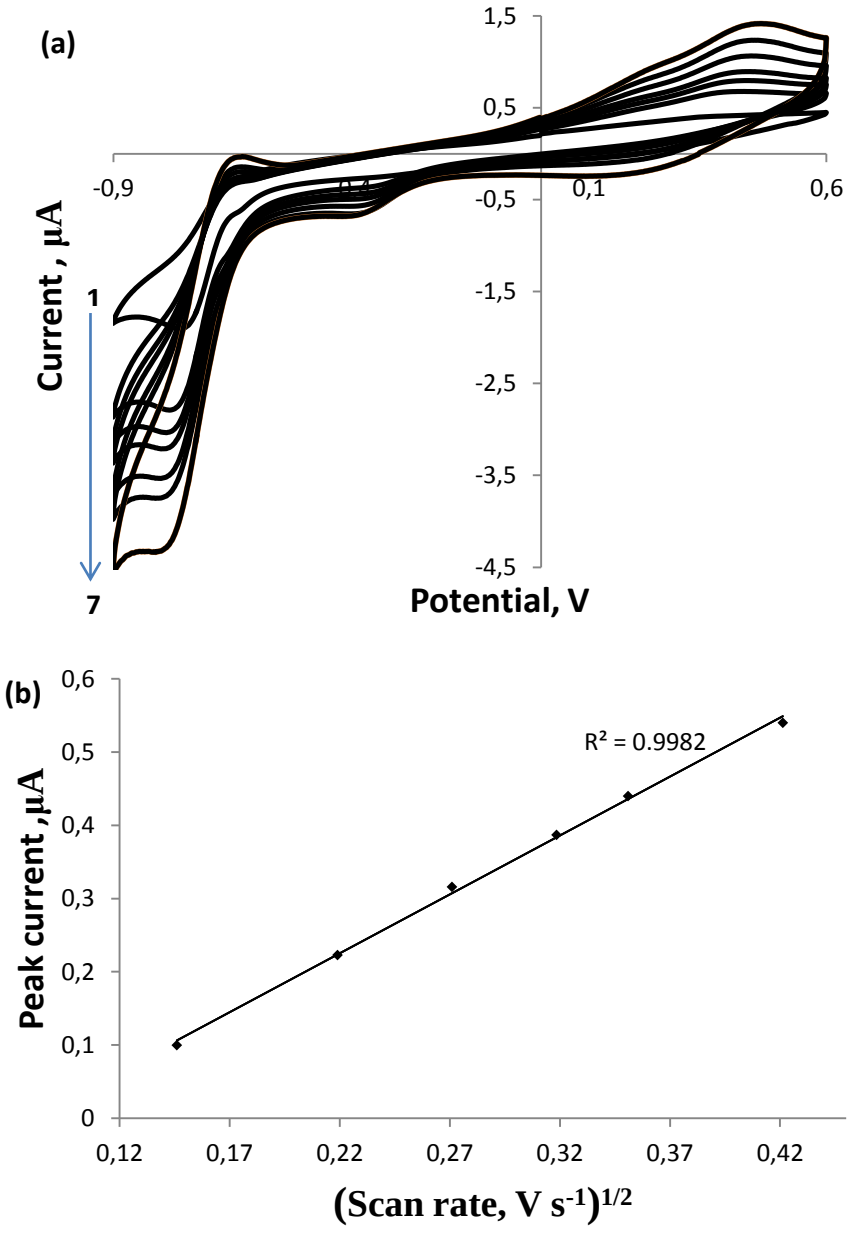


Figure 6. Cyclic voltammograms of a gold electrode in a 1 mmol L^{-1} CAP solution at different scan rates: 0.01 (1), 0.05 (2), 0.075 (3) 0.1 (4) 0.15 (5) 0.2 (6) and 0.3 (7) Vs^{-1}. (b) Relationship between I_p and $v^{1/2}$.

The influence of scan rate is explained in terms of the diffusion layer thickness. The size of the diffusion layer close to the electrode surface will depend on the voltage scan rate. In lower scan rates the diffusion layer will grow more than a fast scan. Consequently, the flux to the electrode surface is considerably smaller at slow scan rates than it is at faster rates. Since the current is proportional to the electron flux towards the electrode the amount of the current will be lower at slow scan rates and higher at high scan rates [18,21,29].

The net cathodic peak current has a linear relationship with the square root of the scan rate ($\nu^{1/2}$) with a correlation coefficient (R^2) of 0.9982. The results suggest that the electrochemical behaviour of chloramphenicol is a diffusion controlled process.

3.4. Square wave voltammetry

Square wave voltammograms were recorded for further investigation of the electrochemical CAP response.

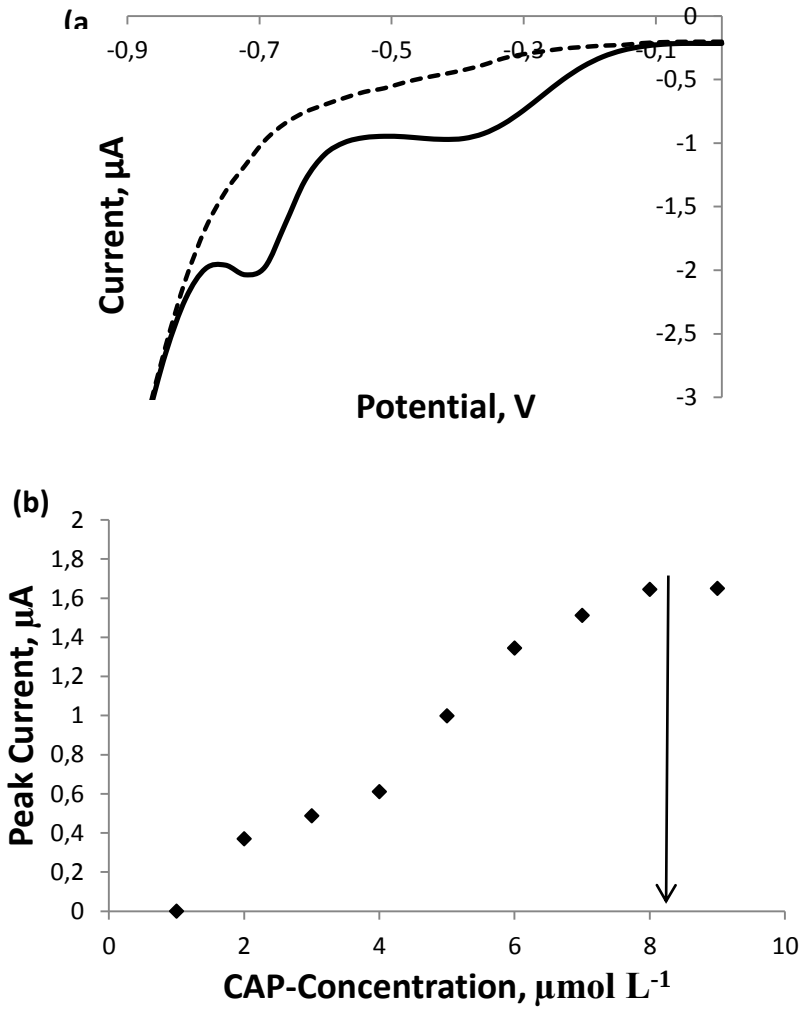


Figure 7. (a) Square wave voltammogram of a gold electrode in a Tris pH 7.6 buffer solution containing 7.4 µmol.L^{-1} CAP. (b) Calibration plot.

The curve shown in Fig. 7 belongs to the net current-potential curves of the square wave voltammograms. The scan consists of pulses changing in forward and reverse direction. The faradaic current is sampled at the end of each half cycle to reduce the influence of the capacitance current. Therefore, the current is sampled twice during each square wave cycle. The net current is the difference between the forward current and the reverse current. Subsequently, it is expected that the linear range is broader and the detection limit become lower in this technique.

The SWV behaviour of the sensing interface in the presence and absence of 7.4 μmol L^{-1} CAP is investigated, and the results are shown in Fig.7.

A cathodic peak at ca. -0.7 V, with a high net current appeared upon adding the 7.4 μmol L^{-1} CAP to the solution. This peak is related to the irreversible reduction of the nitro group in CAP molecules with the formation of hydroxylamine [9,18]. A calibration plot could be obtained for the concentrations varying between 1 and 8 μmol L^{-1} CAP (Fig. 7(b)). For concentrations above this value, a similar blocking effect is observed as for CV. Due to the fact that SWV is a more sensitive technique, the blocking effect starts at higher concentrations. Thus, when performing SWV, the linear relationship between peak current and concentration is valid over a broader range.

3.5. Electrochemical detection of CAP in the presence of FF and TAP

The major problem in detecting the residue of phenicols in foods is the different (electro) chemical property of the analogue structures which makes their simultaneous determination more complex.

Electrochemical response of a bare gold electrode in a blank buffer solution is shown in Fig. 8 (dashed line).

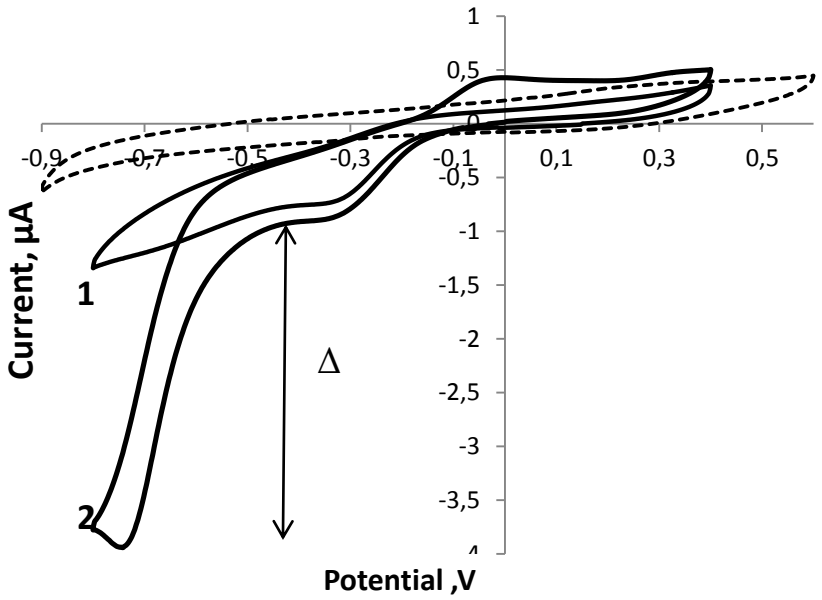


Figure 8. Cyclic voltammograms of a gold electrode in a blank Tris buffer solution (dashed line), containing 1 mmol L^{-1} FF (solid line-1) and 1mmol L^{-1} FF + 1 mmol L^{-1} CAP (solid line-2).

Upon the addition of 1 mmol L^{-1} FF, curve 1 is obtained. The reduction wave appeared at -0.35 V could be linked to reduction of sulfonyl group attached to the benzene present in FF molecules [30]. When 1 mmol L^{-1} CAP is then added to this FF solution, curve 2 is obtained. The NO_2 originated reduction peak of CAP is observed around -0.7V. The net current (ΔI) corresponds to the amount of CAP present in the buffer solution, indicating that this device at this potential and in the presence of FF shows selectivity towards CAP. The reduction wave at ca. -0.3 V occurs for both CAP and FF. Therefore, this wave can be explained as a reduction process of the groups which attached to ring structure [16,18,23].

A similar approach was used to reveal the influence of TAP on the electrochemical behavior of CAP. Fig. 9 shows the electrochemical behavior of a bare gold electrode in a blank buffer solution (dashed line).

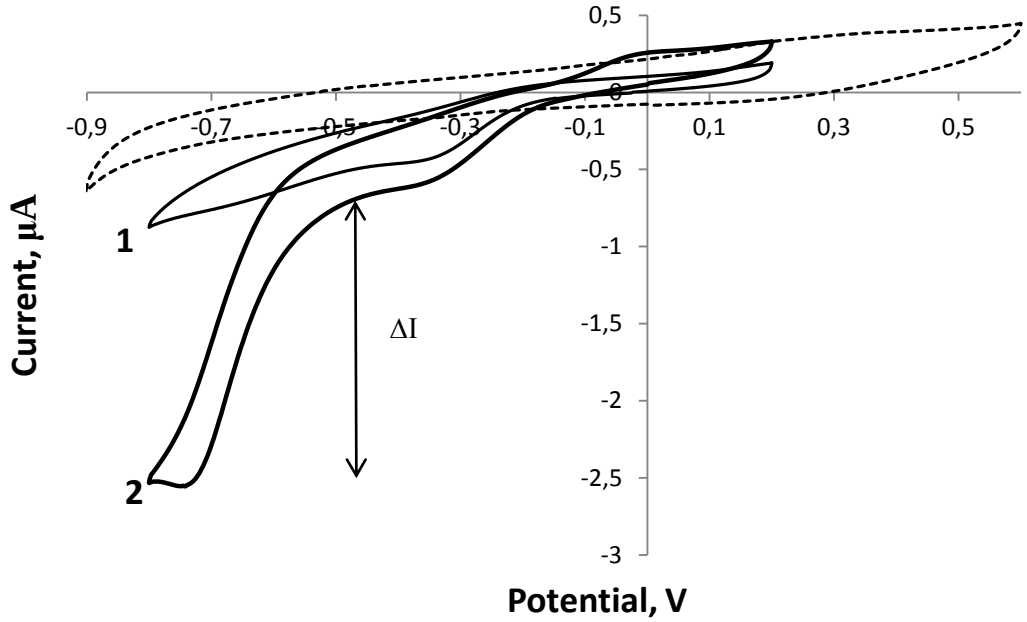


Figure 9. Cyclic voltammograms of a gold electrode in a blank Tris buffer solution (dashed line) containing 1 mmol L^{-1} TAP (solid line-1) and 1 mmol L^{-1} TAP + 1 mmol L^{-1} CAP (solid line-2).

Once adding 1 mmol L^{-1} TAP, a reduction wave at ca. -0.3 V is observed in curve 1 that could be caused from reduction of sulfonyl group attached to benzene ring presents in TAP molecule [29,30]. Curve 2 in Fig. 9 represents the electrochemical response of a gold bare electrode in the mixture of 1 mmol L^{-1} TAP and 1 mmol L^{-1} CAP solution. The peak related to the reduction of the nitro group in CAP molecules is illustrated. Additionaly, the net current (ΔI) corresponds to the amount of CAP present in the buffer solution, indicating that this device at this potential and in the presence of TAP shows selectivity towards CAP. The reduction wave at ca. -0.3 V occurs for both CAP and TAP. Therefore, this wave can be explained as a reduction process of the groups which attached to ring structure [23-25].

Fig.10 shows the difference in electrochemical response of a gold bare electrode in a blank buffer solution (dashed line) and in a solution containing 1 mmol L^{-1} CAP, 1 mmol L^{-1} TAP and 1 mmol L^{-1} FF (solid line).

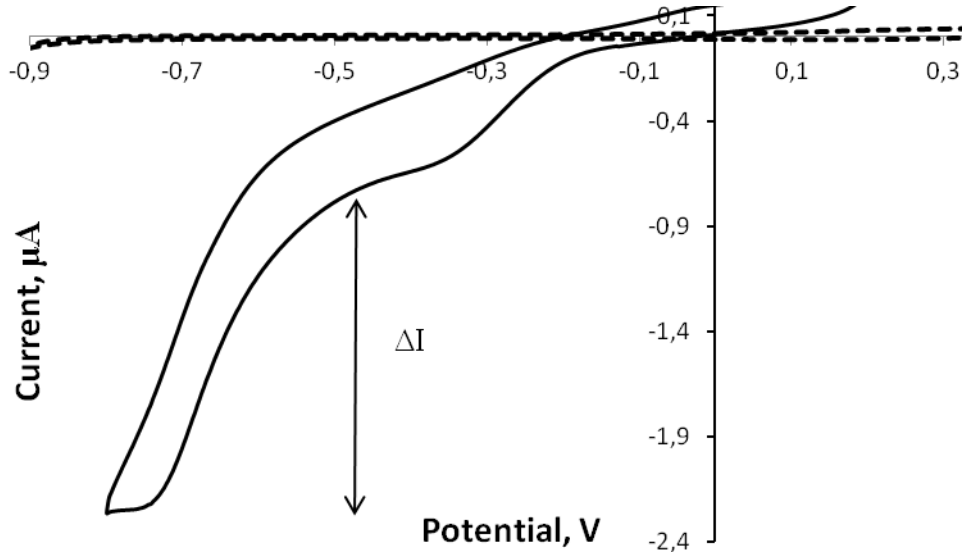


Figure 10. Cyclic voltammograms of a gold electrode in a blank Tris buffer solution (dashed line) containing 1 mmol L^{-1} FF + 1 mmol L^{-1} TAP + 1 mmol L^{-1} CAP (solid line).

The characteristic reduction peak of CAP (reduction of NO_2 group) and the sulfonyl reduction of FF and TAP appear at two different potentials. This makes a quantitative (ΔI) and selective detection of CAP in the presence of its derivates possible.

4. CONCLUSION

In this work, a profound study of the current potential behavior of the phenicol class of antibiotics was done. The reduction peak current related to the presence of CAP increases with concentration with a detection limit of 1.0 µmol L^{-1} with high selectivity towards chloramphenicol in the presence of other CAP derivatives. Moreover, it is fast in replying and easy to construct. The detection of chloramphenicol occurs in a selective way. This article is of fundamental importancy in a broader context of biosensor development. This article is a key element in understanding the electrochemical behavior of chloramphenicol at biosensors.

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