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Electrochemical characterization of cefadroxil β-lactam antibiotic and Cu(II) complex formation

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Abstract

The electrochemical behaviour of cefadroxil, a first-generation β-lactam antibiotic, was studied at glassy carbon electrodes in aqueous media over a wide range of pH. The first oxidation process is of the phenol moiety and follows an ECE mechanism, generating catechol and resorcinol derivatives as sub-products, which are then reduced and oxidized in subsequent cycles. The sulphur heteroatom present in the cyclic structure close to the β-lactam moiety is oxidized in two steps generating sulphoxide and sulphone. This process was identified from direct comparison with amoxicillin, which has a similar molecular structure, although they belong to different classes of β-lactam antibiotics. For amoxicillin, oxidation of the sulphur heteroatom occurred at more positive potentials, most likely due to structural difficulties in stabilizing the charged oxidized species. Formation of a complex between copper (II) and each of the antibiotics was studied by cyclic voltammetry. Finally, determination of cefadroxil in commercial samples was successfully carried out.

Keywords: β-lactam antibiotic; cefadroxil; amoxicillin; Cu(II) complex; electrochemical characterization.

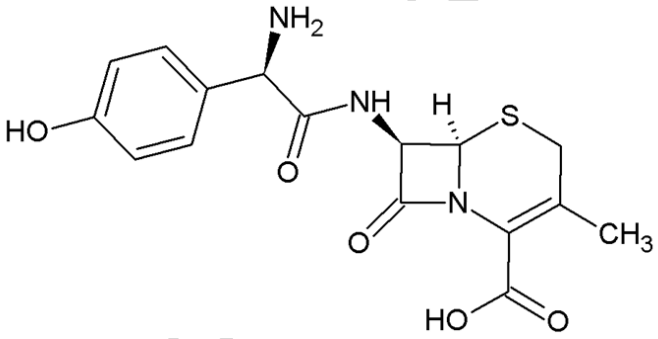
1. Introduction

β-lactam antibiotics contain a β-lactam ring in their molecular structure and are used as pharmaceuticals against several types of bacteria [1]. Their bactericidal action resides in the inhibition of the active site of penicillin-binding proteins (PBP), which are responsible for the last stage of transpeptidation in peptidoglycan biosynthesis, a component that confers rigidity to the cell wall of prokaryotes. The lack of pentaglycine chains in the protein strains, due to the inactivity of PBP, disrupts the cell wall and causes the bacteria's death [2]. The similarity between the molecular structure of the β-lactam ring and D-alanine residues (substrate of the enzyme present in these strains) is responsible for the inhibition of PBP, even though their activity is most likely associated with the conformational structures rather than their isosterism [3]. Additionally, the carboxyl and methyl groups anchored to the cyclic structure alongside the β-lactam moiety assist the interaction of the antibiotic with PBP. Nevertheless, beta-lactamases are present in resistant bacteria, are capable of disrupting the β-lactam ring and make its final conformation less suitable for anchoring in the active site of PBP [4]. The development of new pharmaceuticals that may be less susceptible to the action of beta-lactamases (resistant to cleavage) is still a focus of research. Their administration together with inhibitors such as clavulanic acid, sulbactam, or tazoctam, which inactivates the active site of beta-lactamases, is often used, preventing the cleavage of the β-lactam moiety present in these antibiotics [5].

Cephalosporins are a group of β-lactam antibiotics related to penicillins, with structural differences in the dihydrothiazine vs. thiazolidine ring close to the β-lactam moiety [6]. Cefadroxil belongs to the class of cephalosporins and is a first-generation semisynthetic broad-spectrum β-lactam antibiotic, effective against a variety of gram-positive and gram-negative organisms after oral administration, being adsorbed in the gastrointestinal tract, Figure 1A. It has been used in the treatment of mild to moderate infections most often caused

by *S. pneumoniae*, *S. aureus*, *S. pyogenes*, *E. coli* and *Proteus mirabilis*, and also of patients who have penicillin allergic reactions [7]. Amoxicillin, Figure 1B, is the corresponding penicillin, which resembles the cefadroxil molecule in its structure, and is also a first-generation broad-spectrum β-lactam antibiotic, often administered with clavulanic acid, a beta-lactamase inhibitor. It was the second aminopenicillin to be commercially available (after ampicillin) and is widely used due to its high efficiency against bacterial infections caused by *Streptococcus*, *Bacillus subtilis*, *Enterococcus*, *Haemophilus*, *Helicobacter* and *Moraxella*, *E. coli*, as well as its low cost and easy administration [8].

A



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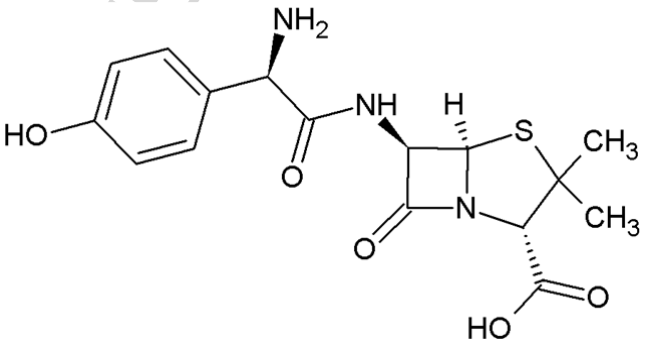


Figure 1 Molecular structures of (A) cefadroxil and (B) amoxicillin.

The determination of β-lactam antibiotics is extremely important for their therapeutic monitoring, in quality and residue control, to evaluate its bioavailability and bioequivalence, and to obtain its pharmacokinetic parameters. In the literature, there is a predominance of high-pressure liquid chromatography (HPLC) with UV absorption spectroscopy detection [9–

12]. The recent International Pharmacopoeia method prescribed for the assay of cefadroxil and amoxicillin employs this technique [13], which involves the use of toxic reagents.

The mechanism of action of pharmaceuticals often involves charge transfer processes which can be investigated *in vitro* using electrochemical techniques, since most of their products are also generated *in vivo* after enzymatic intermediation [14]. This electrochemical approach can detect reactive intermediates (usually radicals) and can be correlated with the compound's biological activity [15–17], using less toxic reagents, which are usually aqueous buffer solutions, with a shorter analysis time and lower instrumentation cost.

The study of β-lactam antibiotics at hanging mercury drop electrodes [18–20], boron doped diamond electrodes [21], unmodified carbon electrodes [22–25] and modified with metal nanoparticles [26–30], multi-walled carbon nanotubes [31] or even inorganic nanostructures [28] using electrochemical techniques such as voltammetry, amperometry and polarography has been described, but mainly focus on their quantification. Oxidation of the sulphur heteroatom present in the dihydrothiazine or thiazolidine ring of β-lactam antibiotics always occurs, but oxidation of side-chain substituents can also take place. Substituents in the side chains at the C^7 and C^3 positions, (cephalosporins), and C^6 positions (penicillins) [32] can be reduced [33], and such reductions are frequently related to the presence of a methoxyimino group. The electrochemical behaviour of cephalosporin complexes with cations such as Cu(II), Cd(II), Pd(II), Zn(II), which may accelerate the rates of chemical reactions, has also been studied using polarographic [34] and voltammetric techniques [35–40], the corresponding redox processes usually occurring at negative potentials.

The electrochemical processes of these molecules are not well elucidated, especially of the moieties which are close to the β-lactam ring. These products, which may be generated *in vivo*, change the final conformation of the initial compound, affect absorption of the drug through membranes and, therefore, hinder their efficiency [41,42].

This paper describes the electrochemical oxidation of cefadroxil and amoxicillin. The formation of a complex with Cu(II) ions, which interferes with the compound's biological activity, is investigated and a method for determination of cefadroxil in commercial samples is described.

2. Experimental

2.1 Reagents and materials

Acetic acid, phosphoric acid and boric acid were purchased from Fischer, and 0.040 M Britton-Robinson (BR) buffer solution was prepared over a wide pH range (2.0 – 12.0). HEPES ((4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) was purchased from Sigma-Aldrich and used to prepare 0.1 M HEPES buffer solution pH 7.0. The pH values of both buffer solutions used were adjusted with 6 M NaOH from Baker, Analyze. Cefadroxil and amoxicillin were purchased from Sigma-Aldrich. Each capsule of Duracef^{RM} from Zamora, Spain, was weighed, fully dissolved in water and then filtered. The precipitate on the filter was again washed with water at least 3 times and this water was added to the filtrate solution, in order to obtain 1 L of each capsule sample solution. Copper(II) nitrate trihydrate ($Cu(NO_3)_2 \cdot 3H_2O$) was purchased from Merck.

All solutions were prepared using Millipore Direct-Q ultrapure water with resistivity ≥ 18 MΩ cm and all reagents were of analytical grade. The experiments were performed at room temperature (25 ± 1ºC) without oxygen removal.

2.2 Instrumentation

Electrochemical measurements were performed using a computer-controlled μ-Autolab Type II potentiostat-galvanostat running with GPES (General Purpose Electrochemical System) for Windows version 4.9, software (EcoChemie, Utrecht, The Netherlands). Spectrophotometric

measurements were performed on a U-2810 Spectrophotometer Digilab® Hitachi from Tokyo, Japan, with UV Solutions Program.

Buffer solution pH measurements were done with a CRISON 2001 micro pH-meter (Crison, Spain).

2.3 Electrochemical measurements

All electrochemical measurements were conducted in a one-compartment 3 mL electrochemical cell containing a 3.0 mm diameter glassy carbon electrode (GCE) with a geometric area of 0.071 cm^2 as working electrode (BAS, USA), a platinum wire as auxiliary electrode and an Ag/AgCl (3 M KCl) reference electrode, purchased from ALS Co., Japan.

2.4 Spectrophotometric measurements

All UV-vis spectra were measured from $200 < \lambda < 400$ nm, at a scan speed of 100 nm/min, sampling interval of 0.2 nm and path length of 1 cm. The wavelength used for the construction of the calibration plot and commercial sample determination, was 229 nm, using the standard addition method, with 5µL additions from a 3 mM stock solution of cefadroxil.

2.5 Surface cleaning procedure

The bare glassy carbon electrode (GCE) surface was polished with diamond spray down to 1.0 µm particle size and rinsed thoroughly with deionized water. Cyclic voltammetry ($0.0\ V \leq E_{apl} \leq 1.0\ V$, 100 mV s^{-1}), was performed at a bare GCE in 0.04 M BR buffer solution pH 7.0 for 4 cycles in order to stabilize the baseline.

3. Results and Discussion

The electrochemical characterization of cefadroxil was performed by cyclic voltammetry (CV), differential pulse voltammetry (DPV) and square wave voltammetry (SWV) at bare

GCE in BR electrolyte with different pH values. Since amoxicillin has a molecular structure similar to cefadroxil, its electrochemical behaviour was also investigated, in order to clarify the oxidation processes that this molecule might share with the cephalosporin under study. The difference in reactivity of the thiazine/thiazolidine moieties regarding the oxidation of the sulphur heteroatom was also evaluated. Given that cefadroxil (and other cephalosporins and penicillins) has the ability to form complexes with metal cations, the conditions for the formation of Cu(II)-cefadroxil complexes were also investigated, as the biological activity of this class of pharmaceuticals might be affected since complex formation occurs close to the β-lactam ring. For voltammetric characterization, a pH value of 7 was used, except when studying the influence of pH, since it is close to the biological pH value, and it presents a high magnitude of current.

3.1 Voltammetric characterization

Cyclic voltammograms obtained in 0.04 M BR buffer pH 7.0 containing 100 μM cefadroxil, Figure 2A, show up to three oxidation processes at E_{p1a} = 0.721 V (1a), E_{p2a} = 1.102 V (2a) and E_{p3a} = 1.420 V (3a). In the reverse potential sweep, one reduction peak is observed at E_{p0c} = 0.049 V (0c), with an anodic component in the second scan at E_{p0a} = 0.097 V (0a). The processes (0c and 0a) are most likely from the same species, with ΔE_p = 48 mV and I_{pa}/I_{pc} = 0.85, and only occur after process 1a has taken place.

DP voltammograms on the second scan, Figure 2B (dotted line), restricting the potential window of the first scan until after peaks 1a (A), 2a (B) or 3a (C) show clearly that the product generated by the first oxidation process (1a) is electroactive, and responsible for the redox couple 0c/0a. A new oxidation process was also observed, 0a'. It is possible that the oxidation product of process 1a undergoes chemical coupled reactions, generating two different electroactive species, which are oxidized at different potentials.

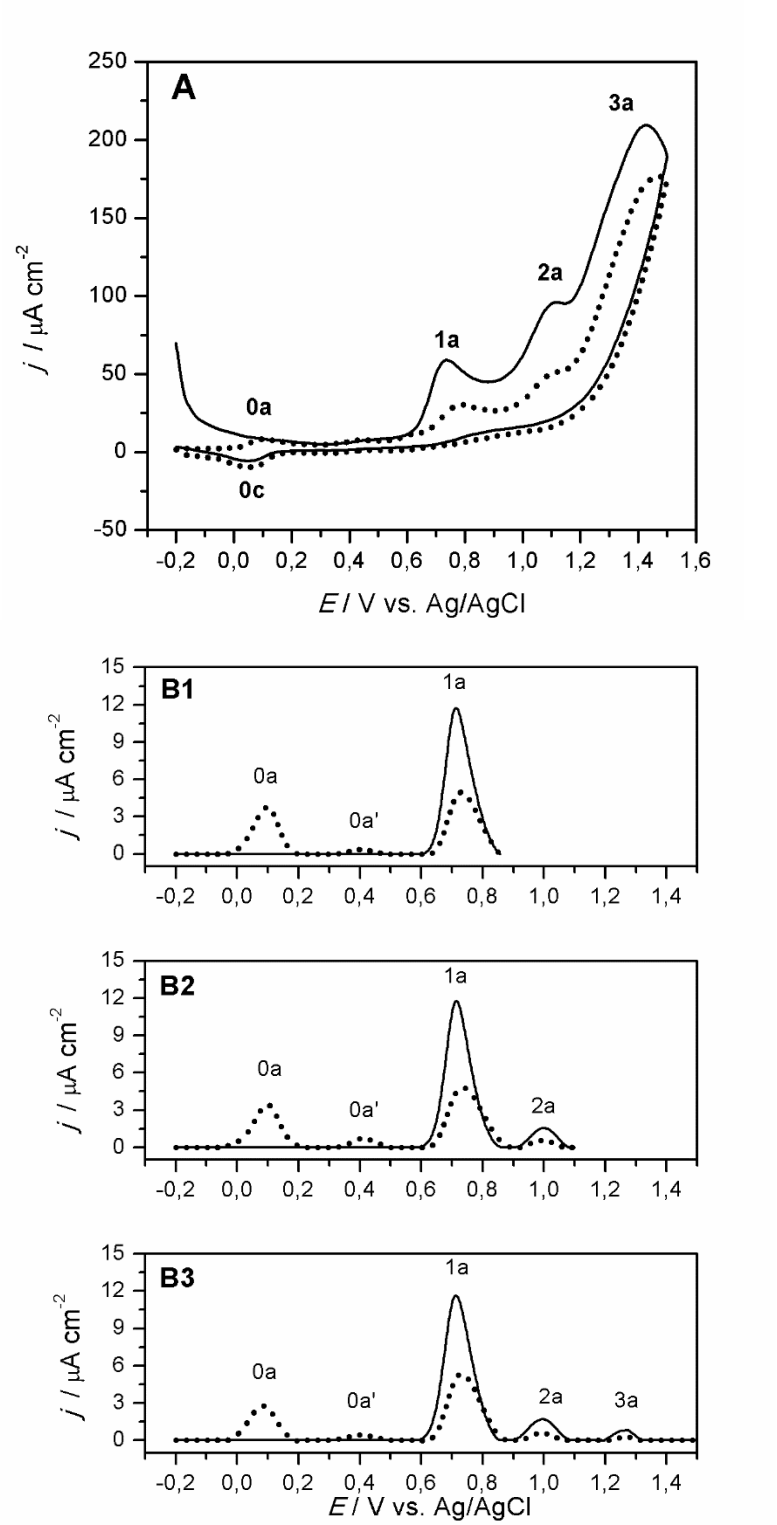


Figure 2 (A) CV (baseline-corrected) at bare GCE in BR buffer pH 7.0 containing 100 µM of cefadroxil. Experimental parameters: ν = 100 mV s^{-1}, ΔE_s = 2 mV.
(B) DP voltammograms, baseline corrected, at bare GCE in BR buffer pH 7.0 containing 100 µM of cefadroxil, E_i = -0.2 V and E_f = 0.9 V (B1), E_f = 1.1 V (B2), E_f = 1.5 V (B3). Pulse amplitude = 50 mV, step potential = 2.5 mV, ν = 5 mV s^{-1}. First scan (—), second scan (●●).

Table 1 - Peak width at half height ($W_{1/2}$) obtained from the DP voltammograms presented in Figure 2B.

$W_{1/2}$ (mV)	E_{p1a} / mV	E_{p2a} / mV	E_{p3a} / mV	E_{p0a} / mV	$E_{p0a'}$ / mV
1st scan	107	108	110	-	-
n. of e-	1	1	1	-	-
2nd scan	-	-	-	68	63
n. of e-	-	-	-	2	2

In the second scan, peaks 1a, 2a and 3a shift slightly to more positive potentials with a smaller current magnitude, indicating that one of the generated products may adsorb on the electrode surface and partially block it. In agreement with this, the currents on the third and subsequent scans show a progressive decrease in the currents. The value of $W_{1/2}$, obtained from DP voltammograms, allows an estimation of the number of electrons involved in each oxidation process, Table 1.

The oxidation processes 0a and 0a' can both be attributed to the oxidation of an adsorbed product generated after process 1a. According to the peak width at half height, $W_{1/2}$, see Table 1, with values less than 90 mV ($W_{1/2} \geq 90/n$ mV), each of these processes involves 2 electrons.

DP voltammograms recorded after the first scan and after transferring the electrode to electrolyte solution without cefadroxil show that the processes 0a and 0a' are still present (data not shown). This confirms that these species are adsorbed on the surface of the electrode.

The shift in peak potential observed in the second scan may be due to products being chemically generated after the first electrode process.

The reversibility of the oxidation processes was evaluated by square wave voltammetry at 100 mV s^{-1} effective scan rate (data not shown). It was expected that process 0a' would not be

present, due to lack of time for the chemical step to be significantly established. However, both processes (0a and 0a') were observed most probably due to the higher sensitivity of the technique compared to cyclic voltammetry. They presented a reversible profile (I_a/I_c = 1 for 0a and I_a/I_c = 1 for 0a'), with the same peak potentials as previously observed, whilst the irreversibility of processes 1a and 2a was confirmed. The last process, 3a, was not present in these measurements, probably owing to the shorter timescale being too fast for the oxidation signal to develop. According to CV measurements and the nature of the oxidation pathway, process 3a is also irreversible, and dependent on process 2a.

The effect of scan rate on the peak currents obtained at GCE in BR buffer pH 7.0 containing cefadroxil, was also investigated for the first oxidation process, 1a, by cyclic voltammetry. A linear response for I_p vs. $\nu^{1/2}$ was observed, which indicates diffusion control. The value of the diffusion coefficient for cefadroxil oxidation in 0.04 M BR buffer solution was estimated, since, for an irreversible process,

$$I_{p1a} / A = 2.69 \times 10^5 \, n \, (\alpha_a n')^{1/2} \, A \, D^{1/2} \, [O]_\infty \, \nu^{1/2} \quad (1)$$

where I_{p1a} is the current (A), A is the electroactive area of the bare GCE (cm^2), n is the number of electrons, $[O]_\infty$ is the concentration of cefadroxil in the electrochemical cell (mol cm^{-3}), v is the scan rate (V s^{-1}) and D is the diffusion coefficient. This led to the I_{p1a} vs. $\nu^{1/2}$ plot shown in Figure 3A, described by the equation $I_{p1a} / \mu A = 1.71 \times 10^{-7} + 5.99 \times 10^{-6} \, v^{1/2}$ / V s^{-2}, R^2 = 0.972 for scan rates in the range 10 mV s^{-1} to 1000 mV s^{-1}.

For an irreversible diffusion-controlled anodic process, $|E_p - E_{p/2}| = 47.7 / (\alpha_a n')$, where α is the charge transfer coefficient and n' is the number of electrons in the rate-determining step. The value calculated for α_a in oxidation process 1a was 0.59 considering n' = 1, and the electroactive area of the bare GCE of 0.0345 cm^2 was obtained from the slope of the I_p vs. $\nu^{1/2}$ plot in a 0.1 M KCl solution containing 1 mM of $K_4Fe(CN)_6$, with D = 6.3 x 10^{-6} cm^2 s^{-1} [43].

The diffusion coefficient for cefadroxil obtained was D = 4.2 x 10^{-5} cm^2 s^{-1} which is in agreement with the values obtained from similar cephalosporins in the literature [20].

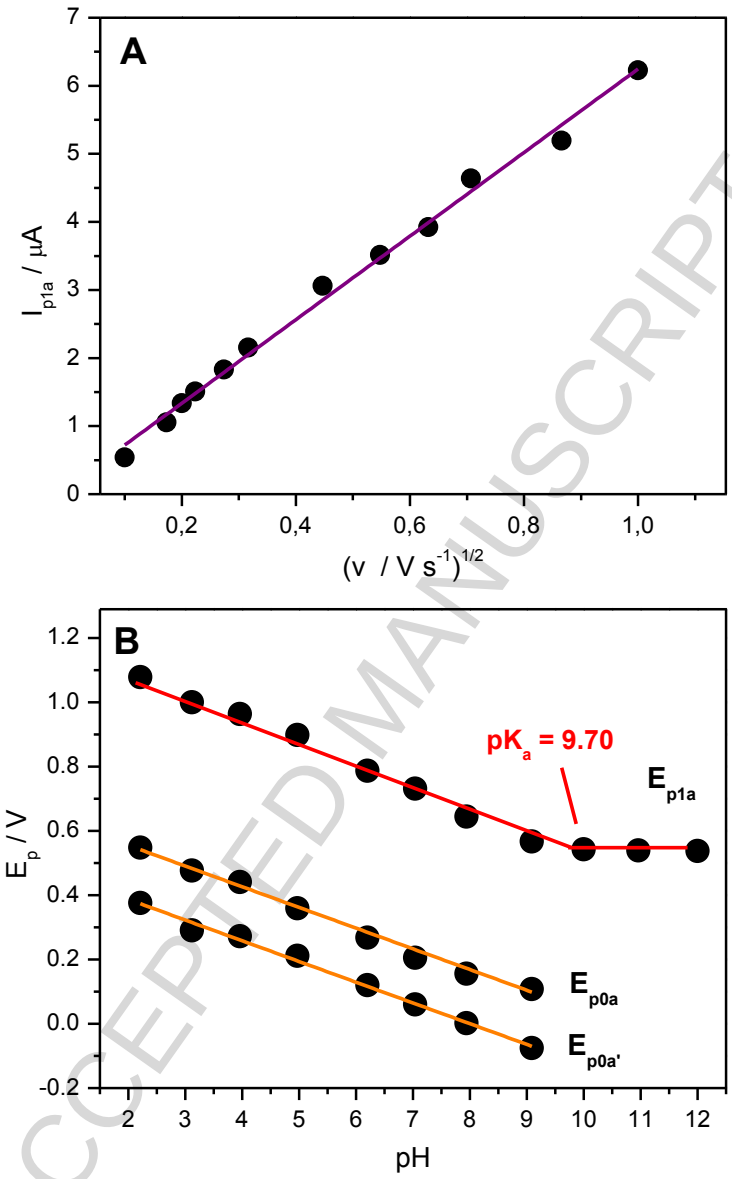


Figure 3 (A) I_{p1a} vs. $v^{1/2}$ plot obtained from CVs using bare GCE in 0.04 M BR buffer solution pH 7.0 containing 100µM cefadroxil, E_i = -0.2 V, E_f = 1.4 V, 1st cycle. (B) E_p vs. pH plot obtained from DP voltammograms at bare GCE in 0.04 M BR buffer solution containing 100 µM cefadroxil, (— E_{p0a}, — $E_{p0a'}$ and — E_{p1a}).

The influence of pH on the oxidation processes was also investigated, in the range 2 to 12. The resulting E_p vs. pH plots are shown in Figure 3B. The equations describing the sloping linear parts of the plots are:

E_{p0a} / V = 0.516 – 0.064 pH, R^2 = 0.998 (2)

$E_{p0a'}$ / V = 0.684 – 0.064 pH, R^2 = 0.993 (3)

E_{p1a} / V = 1.210 – 0.066 pH, R^2 = 0.980 (4)

The ratio of the number of protons to electrons can be estimated from the slopes of the plots, -64, -64 and -66 mV, respectively, to be one. The pK_a of the group involved in the first electrochemical oxidation process (1a) was also estimated, since E_p becomes invariant with pH at high pH values. The intersection between the sloping and horizontal parts of the plot leads to a value of pK_a of 9.70.

The second oxidation process (2a) does not present any significant change with pH, nor does process 3a. In order to determine in which moiety these oxidation processes take place, a comparative study was performed with amoxicillin, which resembles the cefadroxil molecule in structure, sharing its phenol moiety.

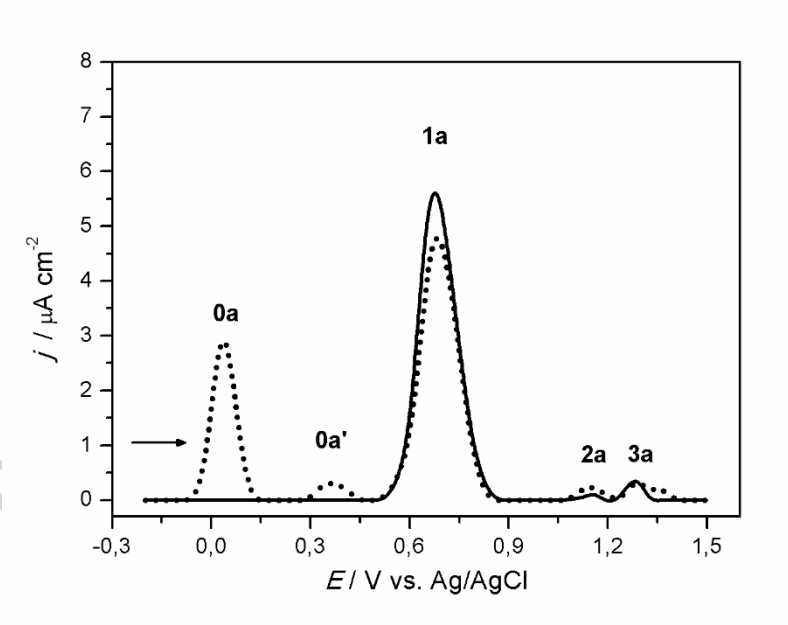


Figure 4 DP voltammograms obtained at bare GCE in 0.04M BR buffer pH 7.0 containing 100 µM of amoxicillin, E_i = -0.2 V, E_f = 1.5 V. Pulse amplitude = 50 mV, step potential = 2.5 mV, ν = 5 mV s^{-1} (▬) First scan and (●●) second scan.

DP voltammograms obtained in similar conditions as those previously described for cefadroxil, but with amoxicillin, Figure 4, presented the same oxidation processes 1a, 0a and 0a' as observed with cefadroxil, but different 2a and 3a. For amoxicillin, there are two ill-defined peaks at E_p = 1.15 V and E_p = 0.128 V. These give indications that these oxidation processes most likely take place at the sulphur atom present in the thiazine moiety of cefadroxil. This may be due to the easier stabilization of the radical intermediate species due to the hexacyclic ring in cefadroxil rather than the pentacyclic ring in amoxicillin.

3.2 Oxidation mechanism in aqueous media

An ECE mechanism can be attributed to the first oxidation processes, since the phenol moiety of the cefadroxil molecule is oxidized involving one electron and one proton (1a), Figure 5. The pK_a value obtained from the E_{p1a} vs. pH plot in Figure 3B is in agreement with the theoretical value for the phenolic group, pK_a = 9.48 [44]. This generates a phenoxyl radical, which then undergoes nucleophilic attack by water, generating *o*-hydroquinone (catechol) and *m*-hydroquinone (resorcinol). It is highly unlikely that the *p*-hydroquinone is formed, since the moiety present at that position of the molecule is a poor leaving group. Figure 5 shows the formation of the catechol moiety. These hydroquinone derivatives are immediately oxidized to quinones, reduced in the reverse sweep (peak 0c for catechol derivative, Figure 2A) and then reoxidized in the subsequent cycle (peaks 0a and 0a' for catechol and resorcinol derivatives, respectively, Figure 2B) involving two electrons and two protons [45]. This part of the molecule is the same as in amoxicillin, see Fig. 1, which follows an identical first oxidation step.

The oxidation mechanism proposed for processes 2a and 3a is more complicated, Figure 5, since it occurs at higher potential values and the charge transfer can be hindered by the presence of molecules derived from quinones, already adsorbed on the surface of the

electrode from immediate electro-oxidation of the hydroxylated product formed after the first oxidation process, 1a.

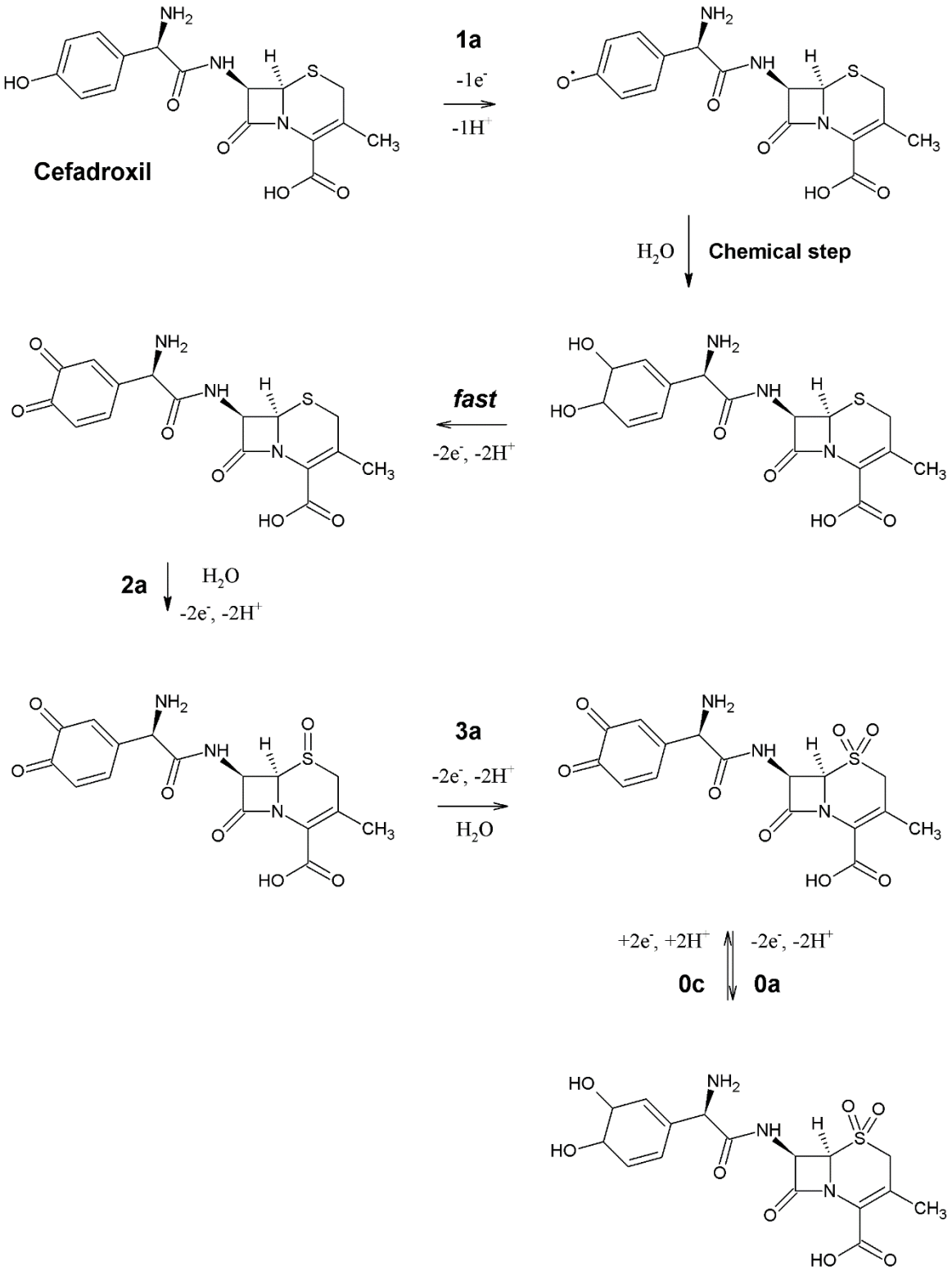


Figure 5 Proposed ECE mechanism for the first oxidation process (1a) of the phenol moiety of cefadroxil, generating a catechol moiety that can be reduced and reoxidized (processes 0c and 0a), and for oxidation of the sulphur heteroatom (processes 2a and 3a).

It is believed that the sulphur present in the hexacyclic ring near the β-lactam moiety is oxidized in processes 2a and 3a, each involving 2 electrons, 2 protons and a water molecule, first generating sulfoxide and then sulfone in the subsequent step.

This process is only observed for amoxicillin at higher potential values and with lower currents (compare Figures 2 and 4) since, according to the basic structure of penicillin and as indicated previously, the intermediate radical formed is less stable due to the pentacyclic, rather than hexacyclic, ring. Also, the process that corresponds to the generation of sulphoxide arises from the adsorption and oxidation of the intermediate species on GCE, contrary to the results reported in [16] with boron-doped diamond electrodes (BDDE) in which only the oxidation that leads directly to sulphone generation is present. The same mechanism is observed for methionine using BDDE [46].

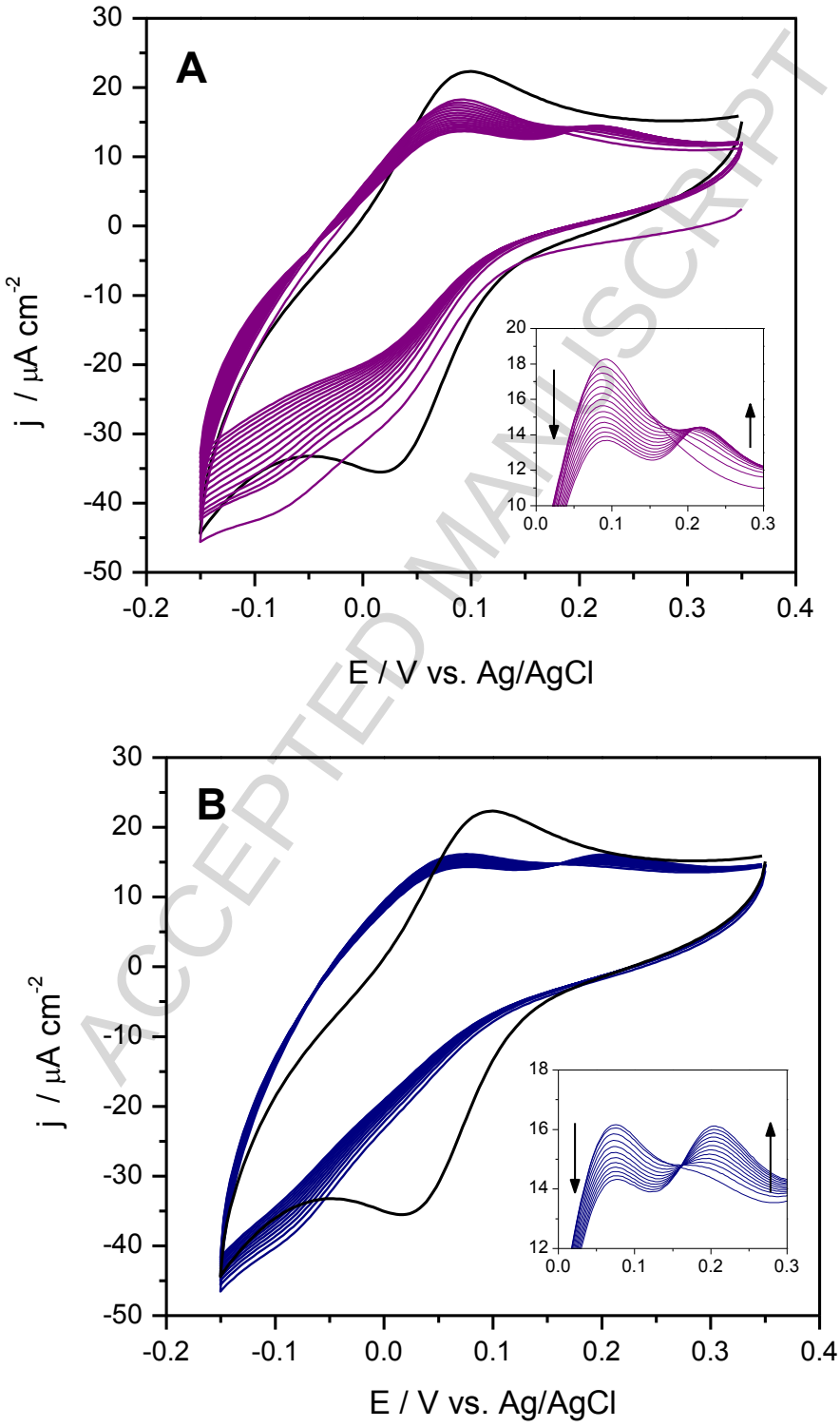
3.3 Formation of Cu(II) complexes

The electrochemical behaviour of Cu(II) complexes of a number of antibiotics with Cu(II) cations has been previously investigated [34,35]. To evaluate the interaction between copper (II) ions and cefadroxil, cyclic voltammograms were recorded in solutions containing 200 μM Cu^{2+} and concentrations of cefadroxil of 100, 200 and 400 μM in 0.1 M HEPES buffer pH 7.4.

This buffer solution was used in order to avoid interference of complexing agents such as phosphate and chloride ions present in other biological buffers. The redox process of the Cu(II) species changes when the antibiotic is added, Figure 6.

The peak potential for copper (II) reduction is shifted to more negative values and the magnitude of the current is drastically decreased. At the same time, the oxidation process shifts to less positive potentials, but the current decreases with the number of cycles as another oxidation process appears at more positive potentials, the corresponding current

increasing with each cycle. There is thus a progressive change in the CV profile as the amount of cefadroxil is increased from one similar to that of Cu^{2+} to one in which the Cu redox process is suppressed, demonstrating the formation of a complex.



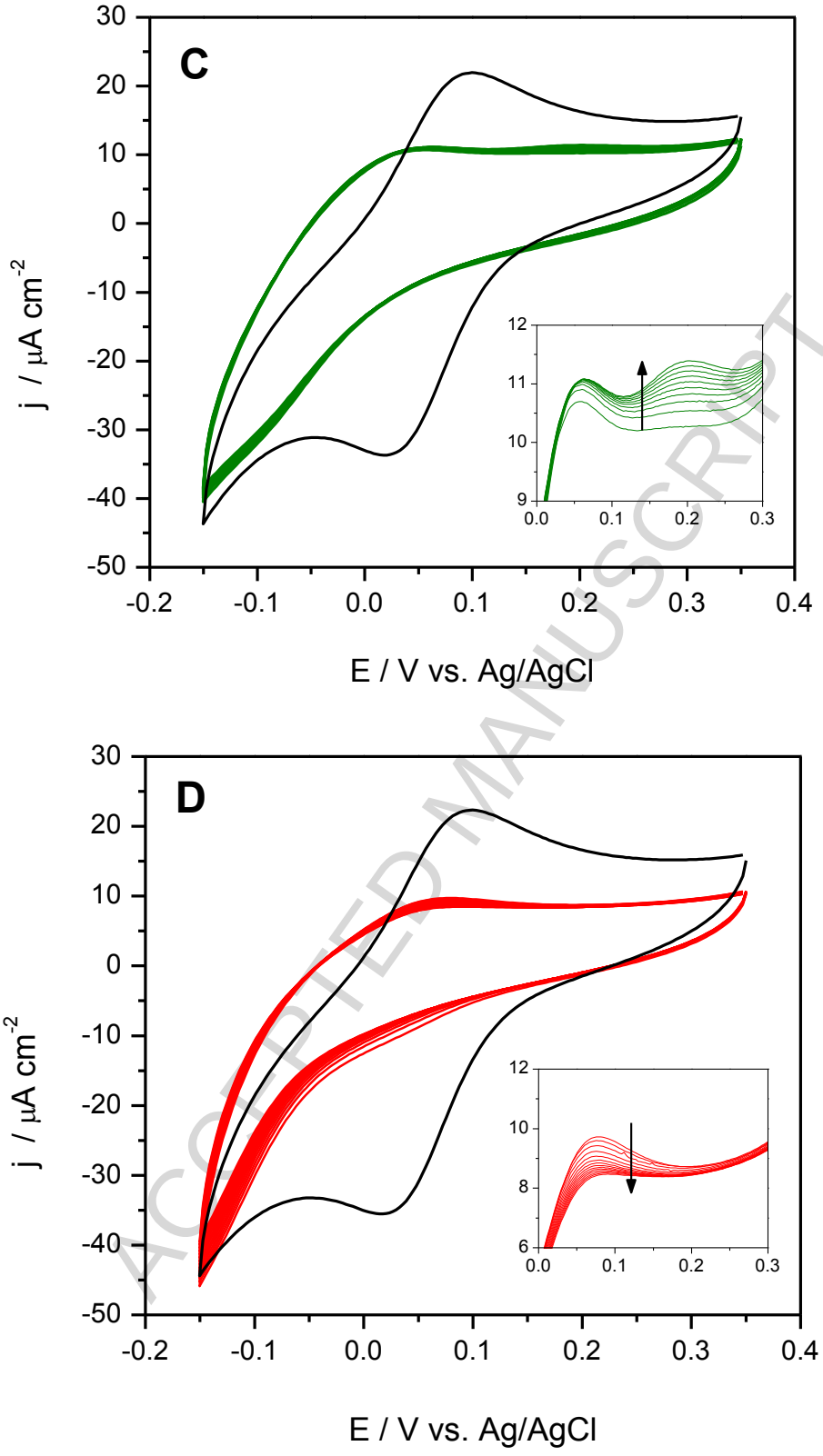


Figure 6 Cyclic voltammograms at bare GCE in 0.1 M HEPES buffer pH 7.4 containing 200 µM Cu^{2+} (—) and (A) (—) cefadroxil, ratio 2:1 (B) (—) cefadroxil, ratio 1:1 (C) (—) cefadroxil, ratio 1:2 (D) (—) amoxicillin, ratio 1:1. Scan rate = 100 mV s^{-1}.

For amoxicillin, Figure 6 (D), evidence that the complex is formed can also be deduced from the disappearance of the reduction process for copper (II), but no oxidation process appears in the positive going scan, even with the decreasing oxidation process already observed for copper (II) species. The complex may be more stable or it may oxidize at even more positive potentials, outside the potential window available in HEPES buffer solution.

3.4 Analytical determination

3.4.1 Calibration plot

A calibration curve for cefadroxil was constructed from the values of the peak current of the first oxidation process (1a) in DP voltammograms using 0.04 M BR buffer pH 7.0, with additions of 1 μM cefadroxil up to 5.0 μM (n=3), Figure 7. This value of pH was used since it is close to the biological pH value, and it presents a high magnitude of current. At lower values of pH the magnitude of current for this process is higher, but there is an influence from the second oxidation process, 2a.

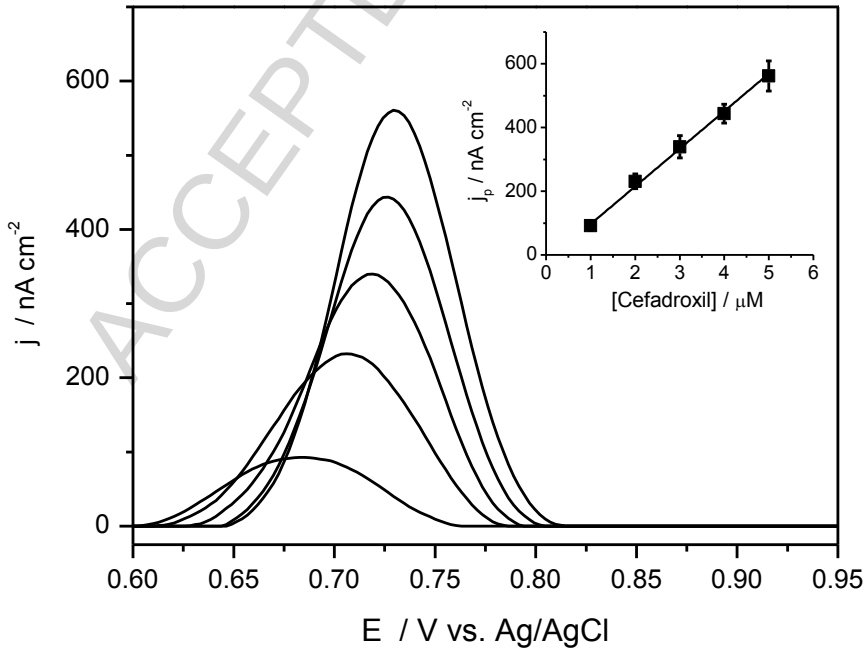


Figure 7 Differential pulse voltammograms at bare GCE in 0.04 M BR pH 7.0 containing increasing concentrations of cefadroxil, E_i = 0.55 V, E_f = 0.90 V. Pulse amplitude = 50 mV, step potential = 2.5 mV, ν = 5 mV s^{-1}. Inset: calibration plot of j_p vs. [Cefadroxil].

Process 1a is diffusion-controlled, presents the highest magnitude of current compared to the other processes and, since it is the first oxidation process, there is no influence from any adsorbed species generated. According to the calibration plot, j_p / nA cm^{-2} = 2.14 + 118 **[**Cefadroxil / μM**]**, R^2 = 0.994. The potential shifts to more positive values with increasing concentration of cefadroxil and, above 10.0 μM, saturation effects begin to appear, most likely due to the generated hydroquinone derivatives adsorbing at the electrode surface. A limit of detection of 0.3 μM was estimated, using 3 times the standard deviation of the blank divided by the slope of the calibration curve. The limit of quantification, 3.33 times the value of the limit of detection, coincides with the concentration after the first addition of analyte.

Given the possibility of obtaining a calibration plot for the determination of cefadroxil in aqueous media with a high recovery value of 96.5%, this procedure was used to determine cefadroxil in commercial samples.

3.4.2 Analysis of commercial samples

The active principle present in commercial samples from Duracef^R was determined using DPV in 0.040 M BR buffer pH 7.0 at bare GCE, and the results compared with UV-vis experiments using the same stock solution and the standard addition method, but in a different linear range due to the difference in sensitivity of the two techniques.

Table 2 - Determination of cefadroxil in commercial samples by DP voltammetry and UV-visible spectrophotometry (*n*=3).

Sample	Method	Linear Range (μM)	Specified (mg)	Found (mg)
A	DPV	1 - 5	500.0	450.0
	UV-vis	5 - 50	500.0	450.5
B	DPV	1 - 5	500.0	499.0
	UV-vis	5 - 50	500.0	480.8

In the electrochemical measurements, the excipients present in the capsule did not interfere with any of the oxidation processes, but saturation of the surface from adsorbed species was greater than when using pure cefadroxil reagent. This was seen from the larger shift in peak potential after each and the values of current, which began to show saturation effects at lower concentrations of the compound.

Assuming that all of the active principle was dissolved, since the amount of water used was higher than that necessary according to the solubility of the compound (1.11 g/L in aqueous solution [47]), the values determined from two different capsules, Table 2, indicated that the amount of active ingredient was different in each capsule and significantly less than specified for sample A, given that the uncertainty of the electrochemical measurement is only 3.5 %.

4. Conclusions

The electrochemical characterization of cefadroxil was carried out at bare glassy carbon electrodes in aqueous media using different voltammetric techniques. An ECE mechanism was observed for the oxidation of the phenol moiety of the compound, generating catechol and resorcinol as sub-products that adsorbed on the electrode surface and hindered further oxidation processes in subsequent cycles. Comparison between the electrochemical profile of cefadroxil and amoxicillin allowed identification of the oxidation of the sulphur heteroatom present in the cyclic structure close to the β-lactam for both antibiotics which, for cefadroxil, occurred at less positive potentials with a better peak resolution than for amoxicillin. The diffusion coefficient for cefadroxil in 0.04 M BR buffer was obtained (D_0 = 4.2 ·10^{-5} cm^2 s^{-1}), which agrees with the diffusion coefficients reported in the literature for other cephalosporins. Formation of a complex between Cu(II) ions and cefadroxil or amoxicillin was confirmed using cyclic voltammetry. Calibration plots for determination of the two antibiotics were

constructed based on oxidation of the phenol moiety and the method was successfully applied to the quantification of cefadroxil in commercial samples.

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Highlights

- Electrochemical characterization of cefadroxil at glassy carbon in aqueous media
- Oxidation of the phenol moiety by ECE mechanism generating catechol and resorcinol
- Comparison between electrochemical profile of cefadroxil and amoxicillin
- The phenol oxidation process was used to obtain a limit of detection of 0.3 μM
- Phenol oxidation was used to measure the amount of cefadroxil in commercial samples