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Polarographic determination of clavulanic acid

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Abstract: A method is proposed for the determination of clavulanic acid by differential pulse polarography. The electroactive product was obtained by hydrolysis in sulphuric medium. It shows a reduction peak, that can be used analytically, at −0.75 V (vs SCE). The optimum conditions for the polarographic signal were determined and a study was made of the different parameters affecting the electrochemical process. A polarographic procedure is proposed for the determination of clavulanic acid in a concentration range of $8.0 \times 10^{-6} - 1.4 \times 10^{-4}$ M. The detection limit is about 2×10^{-6} M and the relative standard deviation is 1.1%. The method was applied to the determination of clavulanic acid in the presence of amoxicillin.

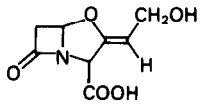
Keywords: *Differential-pulse polarography; clavulanic acid.*

Introduction

The β-lactam antibiotics, which include the penicillins and cephalosporins, have now become the largest single group of antibiotics in clinical practice. The penicillins and cephalosporins, however, are potentially subject to inactivation by enzymes collectively referred to as β-lactamases. Clavulanic acid is a naturally occurring inhibitor of β-lactamases which is capable of rendering penicillin- and cephalosporin-resistant organisms sensitive to the action of these drugs. Clavulanic acid is generally commercialized in formulations with amoxicillin, in which the antibacterial activity of the latter is potentiated by the acid.

The use of electrochemical techniques for the determination of compounds of pharmaceutical interest is continually gaining in importance. The inherent sensitivity and high selectivity of the techniques allow very simple determinations, both in commercial samples and body fluids.

In the present paper we propose a method for the determination of clavulanic acid (**I**) by differential pulse polarography, based on the measurement of its acid hydrolysis products.



I

Although clavulanic acid has already been determined by spectrophotometric [1, 2], microbiological [3, 4], enzymatic [5, 6] and chromatographic [7–11] methods, there are no references in the literature to the possible application of electrochemical techniques for its determination.

Experimental

Apparatus

The apparatus used consisted of the following: a Metrohm Polarecord E 506 polarograph and an E 505 device, a Metrohm EA 1029/1 dropping mercury electrode, a Metrohm EA 285 platinum electrode, a saturated calomel electrode, and a Crison pH meter with an Ingold 9811 electrode.

Reagents

Britton–Robinson buffers (0.1 M, pH 2–12) were used. The ionic strength was adjusted with KCl (Panreac). Clavulanic acid (potassium salt) and amoxicillin (trihydrate) were supplied from Antibiotics SA and used without further purification. Stock solutions (1.0×10^{-3} M) of clavulanic acid were prepared daily. Sulphuric acid was supplied by Panreac. The commercial formulation analysed was Augmentine (Beecham, S.A.)

Polarographic measurement

The experiments were carried out using a

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differential pulse polarograph. The instrumental parameters were as follows: applied potential range, −0.4 to −1.2 V; scan rate, 4 mV s^{-1}; drop time, 2 s; pulse amplitude, −50 mV.

Results and Discussion

Influence of pH *on the electrochemical response*

Aqueous solution of the potassium salt of clavulanic acid, with potassium chloride as the supporting electrolyte did not afford a peak in differential pulse polarography within the range of potentials permitted by the medium. However, in acid medium at pH values lower than 4, two peaks are seen at potentials close to −0.75 and −0.90 V, the first being more intense and better defined. The height of both peaks depends on the acidity of the medium and on time. Figure 1 shows the effect of both these variables on the first peak; with values of intensity that are practically constant for times above 100 min at room temperature. In successive studies, all measurements were carried out at least 2 h after preparing the samples.

To study the effect of pH on the potentials and peak intensities, the pH was adjusted between 4.0 and 1.7 with Britton–Robinson buffer, and pH values lower than 1.7 with sulphuric acid. The peak appearing at less negative potentials, of greater analytical interest and on which the present study focuses, is defined over the pH range considered, whereas the other peak can only be observed at pH values below 2.5. The intensity of both peaks decreases as pH increases (Fig. 2).

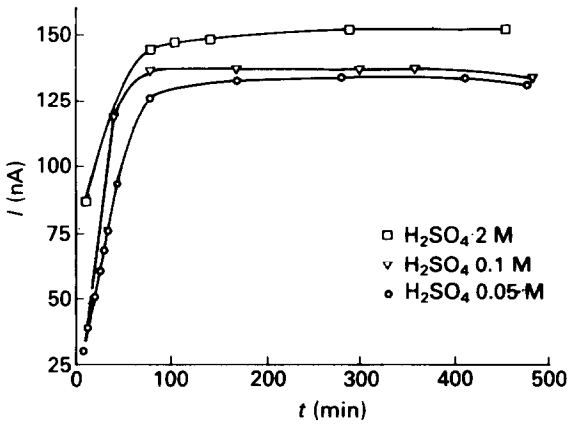


Figure 1
Effect of time and concentration of sulphuric acid. First peak E = −0.75 V; 8.0 × 10^{-5} M clavulanic acid.

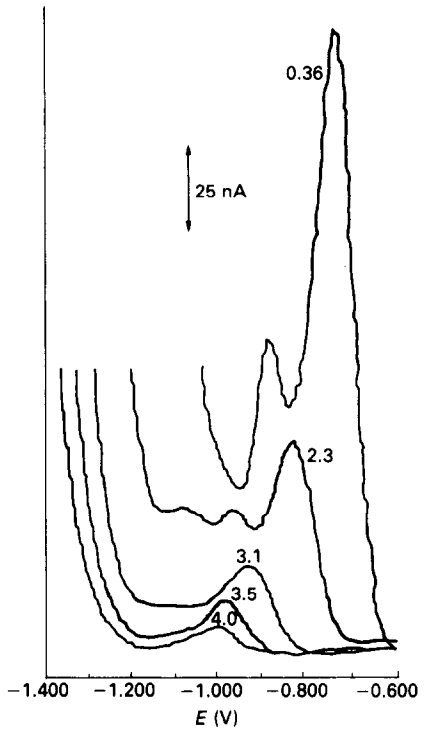


Figure 2
Polarograms at different pH values. 8.0 × 10^{-5} M clavulanic acid; 0.5 M KCl; t = 2 s; ΔE = −50 mV.

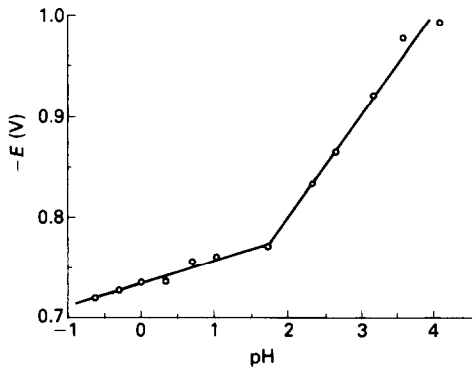
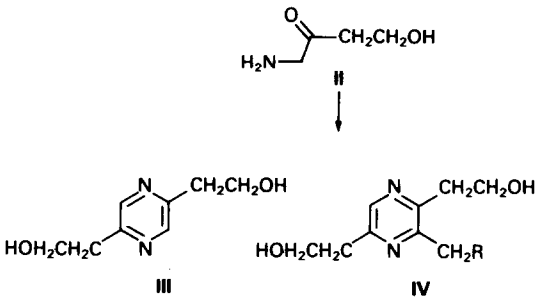


Figure 3
Effect of pH on peak potential.

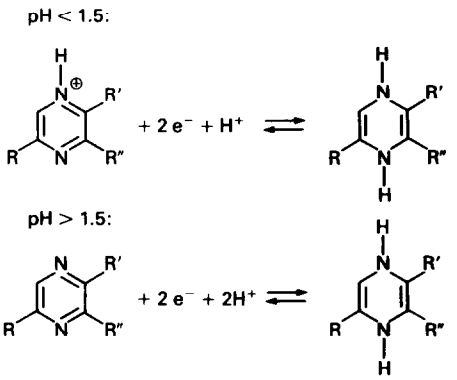
On representing the peak potential against pH two almost straight lines were obtained, with different slopes, that cross at a pH value close to 1.5 (Fig. 3). This indicates the participation of a different number of hydrogen ions in the electrodic reactions.

The degradation of clavulanic acid in acid medium [12] gives rise, among other compounds, to 1-amino-4-hydroxybutane-2-one (**II**). Although the polarographic peaks obtained are probably due to this species, its dimerization and later oxidation may give rise

to different derivatives of pyrazine [13], such as compound **III** and **IV**:



which could be reduced according to the following scheme, similar to that proposed for other pyrazines [14]:



Characteristics of the electrochemical response

All studies were carried out on 8.0×10^{-5} M solutions of clavulanic acid in 0.1 M sulphuric medium and in the presence of 0.5 M KCl. The results refer to the peak obtained at the less negative potentials.

Pulse amplitude was varied between ±5 and ±100 mV, linearity being observed with respect to peak intensity for values below ±60 mV.

Drop time was studied for values ranging between 0.4 and 5 s. A linear relationship is seen between peak intensity and $t^{2/3}$, suggesting that one is dealing with a diffusion-controlled process. The same conclusion is reached on studying the effect of temperature, since temperature coefficients of about 2% are obtained.

The study of the reversibility of the system was conducted using the criterion of Birke *et al.* [15] based on the difference between the cathodic (E^c_p) and anodic (E^a_p) peak potentials and on the intensity ratio, I^a_p/I^c_p, according to whether the pulse amplitude applied is positive or negative. The results obtained show that the I^a_p/I^c_p ratio is 1.26 and the difference $E^c_p - E^a_p$ is less than the pulse amplitude, such that the process can be thought of as quasi-reversible.

Linearity, detection limits and precision

The effect of the concentration of clavulanic acid on peak intensity was studied by measurement on the polarograms of different solutions of clavulanic acid within a concentration range of 8.0×10^{-6} and 2.0×10^{-4} M. For concentrations below 1.4×10^{-4} M good linearity is observed between i_p and concentration:

$$i_p(\text{nA}) = 0.52 + 9.66 \times 10^5 C\ (\text{M}); \quad r = 0.999.$$

In view of the linearity between the peak intensities and the concentration of clavulanic acid, the following procedure is proposed for the determination of the compound.

Solutions are prepared containing clavulanic acid (8.0×10^{-6} and 2.0×10^{-4} M), 0.1 M sulphuric acid and 0.5 M KCl as supporting electrolyte. After about 2 h, the solution is placed in the polarographic cell, bubbling through a stream of nitrogen for 5 min, then recording the polarogram with a pulse amplitude of −50 mV, and measuring the intensity of the peak appearing at −0.75 V.

The detection limit, calculated by the expression $3s/m$ (s = standard deviation of blank; m = slope of calibration straight line) was 1.9×10^{-6} M. The precision of the method was determined by applying the above procedure to 10 samples, each containing a concentration of clavulanic acid of 6.0×10^{-5} M. The relative standard deviation was 1.1%.

Application to the determination of clavulanic acid in pharmaceutical formulations

Most pharmaceutical preparations of clavulanic acid are commercialized together with amoxicillin with a view to enhancing the antimicrobial activity of the latter owing to the inhibition of β-lactamases by the acid. Polarographic determination of clavulanic acid can be carried out in the presence of amoxicillin since the latter, under the present working conditions, shows almost no signal close to −0.75 V (Fig. 4).

The procedure was applied to the determination of clavulanic acid in the products Clavucid (Lab. Zambeletti, S.A.) and Augmentine (Lab. Beecham, S.A.), both containing an amoxicillin–clavulanic acid ratio of

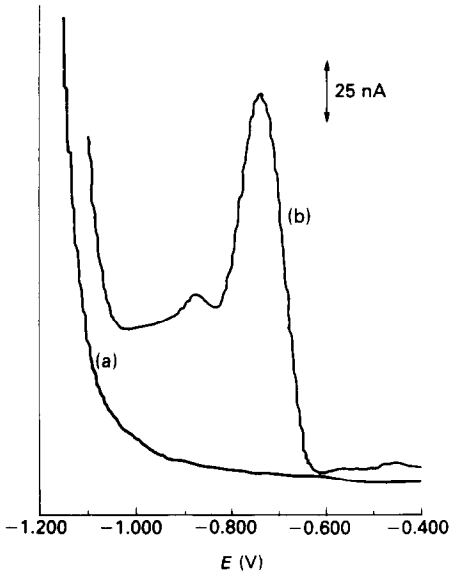


Figure 4
Polarograms of clavulanic acid and of amoxicillin. (a) 0.8 × 10^{-4} M amoxicillin; (b) 1.2 × 10^{-4} M clavulanic acid. Medium, 0.1 M H_2SO_4; 0.5 M KCl; t = 1.2 s; ΔE = − 50 mV.

500:125. Preparation of the samples was carried out simply by shaking the finely ground product vigorously with water and applying the described procedure. Determination was carried out by the standard addition method, obtaining for both products a content in clavulanic acid of 124.5 mg.

Conclusions

The method described for the polarographic determination of clavulanic acid requires a minimum of manipulations and preparation. Furthermore, it involves the use of only a few common reagents. The present procedure should be at least as selective (amoxicillin does not interfere) and sensitive as the spectrophotometric assay of clavulanic acid by reaction with imidazole [1].

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