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DETERMINATION OF THE MACRO- AND MICRO-IONIZATION CONSTANTS OF A DIPOLAR ZWITTERIONIC CEPHALOSPORIN: CEFADROXIL

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SUMMARY

The macro- and micro-ionization constants of a zwitterionic cephalosporin are calculated using potentiometric and spectrophotometric techniques.

This compound can undergo chromophoric changes depending upon the solution pH. By utilizing the micro-ionization constants the relationship of zwitterion to uncharted species were calculated and were seen to have a maximum concentration between pH 9 and 11.

INTRODUCTION

Cefadroxil is a semi-synthetic cephalosporin recently introduced into clinical practice and is destined for oral use. Its chemical structure, shown in Fig. 1 is 7-D-(–)α-amine-α-(4 hydroxyphenyl)acetamide-3 methyl, 3 cephem-4-carboxylic acid.

The aim of the survey is to determine the ionization constants of this beta-lactam antibiotic which has various ionizable functional groups in its structure. In this kind of substance, the ionization constants calculated potentiometrically may correspond to the simultaneous ionization of two groups and are thus really hybrid constants and are usually known as macroscopic constants. The term microscopic constants is reserved for those corresponding to individual functional groups.

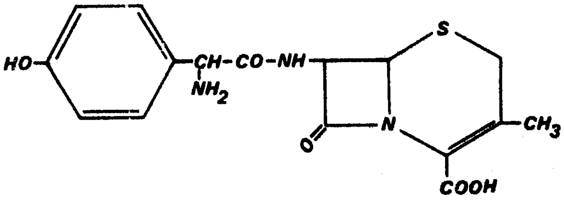


Fig. 1. Structure of cefadroxil.

THEORETICAL

The dissociation equilibrium of a doubly protonated acid may be expressed as:

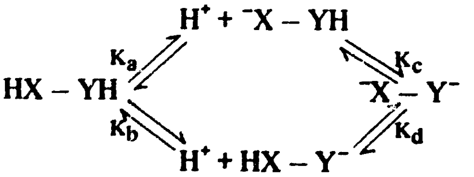
(SCHEME I)

$$RH_2 \overset{K_2}{\rightleftharpoons} H^+ + RH^- \overset{K_3}{\rightleftharpoons} R^= + H^+$$

A potentiometric measurement in which the pH is determined directly as a function of the number of dissociated protons by the addition of hydroxyl ions allows us to establish the macroscopic constants, K_2 and K_3, (Edsall et al., 1933).

If, however, there are two acidic protons present, 4 equilibria need to be considered instead of two, according to the following scheme:

(SCHEME II)



where RH_2 represents the non-ionized form or the HX–YH; RH^- represents the form with only one proton ionized and corresponds indifferently to $HX{-}Y^-$ and $^-X{-}YH$ and K_a, K_b, K_c and K_d are the microscopic constants which are not independent of each other since the dissociation of a proton can follow either of the two paths.

The macro- and microscopic constants are expressed by the following equation:

$$K_2 = \frac{[H^+] \cdot [RH^-]}{[RH_2]} \qquad K_3 = \frac{[R^{2-}] \cdot [H^+]}{[RH^-]}$$

$$K_a = \frac{[H^+] \cdot [^-X - YH]}{[HX - YH]} \qquad K_b = \frac{[H^+] \cdot [HX - Y^-]}{[HX - YH]}$$

$$K_c = \frac{[H^+] \cdot [^-X - Y^-]}{[^-X - YH]} \qquad K_d = \frac{[H^+] \cdot [^-X - Y^-]}{[HX - Y^-]}$$

The macroscopic constants are related to the microscopic constants as follows:

$$K_2 = K_a + K_b \; ; \quad \frac{1}{K_3} = \frac{1}{K_c} + \frac{1}{K_d} \; ; \quad K_2 \cdot K_3 = K_a \cdot K_c = K_b \cdot K_d$$

This kind of compound may simultaneously contain positive and negative charges and is thus amphoteric. The relative amount of zwitterion and uncharged molecules depends on the magnitude of the microconstants.

These amphoteric substances may or may not be zwitterionic in nature, depending on their pK values. In the case of cefadroxil, according to the test proposed by Albert for the determination of these (Albert and Serjeant, 1971) it was seen that the antibiotic is in fact zwitterionic in nature.

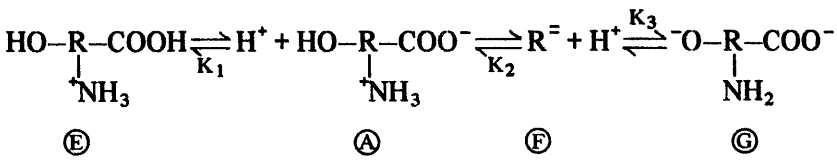
From the macroscopic constants, K_2 and K_3, of the degree of ionization (α) and the concentration of $[H^+]$ the value of the microscopic constants may be established according to the following expression (Edsall, 1933):

$$K_a = ([H^+] + K_2) - \frac{K_2 \cdot K_3}{[H^+]} \cdot (1 - \alpha)$$

The calculation of the microconstants may also be carried out by the method of linear regression (Niebergall et al., 1972); by plotting $\alpha \cdot ([H^+] + K_2)$ along the ordinate against $\alpha/[H^+]$ along the abscissa a straight line is obtained where the ordinate at the origin is K_a and where the slope is $K_2 \cdot K_3$. This method has the advantage that it is only necessary to know one macroconstant in order to calculate the other.

In the particular case of cefadroxil, the dissociation equilibrium from which the potentiometrically determined macroscopic constants are established is shown in the following scheme:

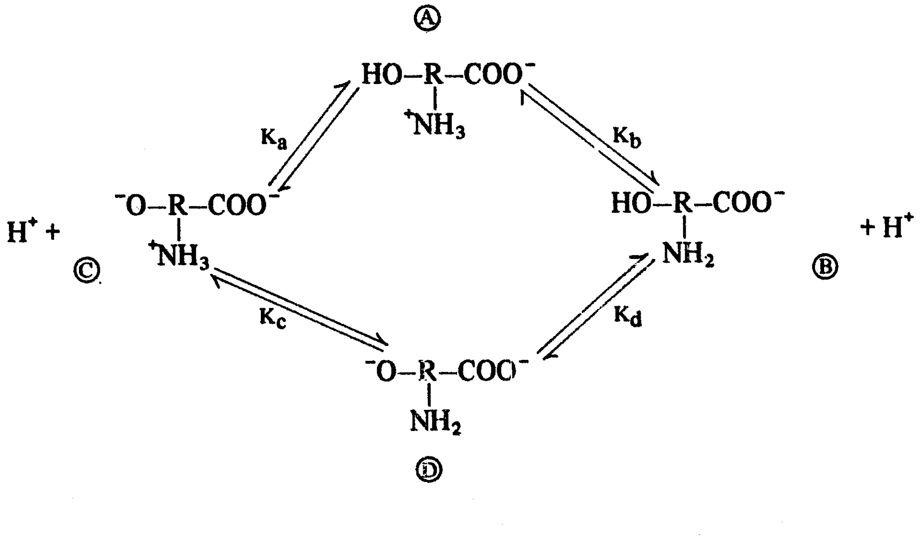
(SCHEME III)



$$\underset{Ⓔ}{\underset{{}^+NH_3}{HO{-}R{-}COOH}} \underset{K_1}{\rightleftharpoons} H^+ + \underset{Ⓐ}{\underset{{}^+NH_3}{HO{-}R{-}COO^-}} \underset{K_2}{\rightleftharpoons} \underset{Ⓕ}{R^=} + H^+ \overset{K_3}{\rightleftharpoons} \underset{Ⓖ}{\underset{NH_2}{{}^-O{-}R{-}COO^-}}$$

The microconstants which participate in the dissociation equilibrium of this beta-lactam antibiotic are shown in the following scheme:

(SCHEME IV)



Not all these microconstants can be determined by experimental methods and thus only the constants for the specific ionization of the phenol group have been determined. Once these microconstants have been established, together with the macroconstants K_2 and K_3, the rest of the microconstants may be established on the basis of the aforementioned considerations.

Having determined the microconstants, it is then possible to establish, according to the pH values, the proportion in which the various species are found (Streng et al., 1976) by the following equations:

$$\%A = 100\Big/\left(1 + \frac{K_a}{[H^+]} + \frac{K_b}{[H^+]} + \frac{K_b \cdot K_d}{[H^+]}\right)$$

$$\%B = 100\Big/\left(1 + \frac{[H^+]}{K_b} + \frac{K_d}{[H^+]} + \frac{K_a}{K_b}\right)$$

$$\%C = 100\Big/\left(1 + \frac{[H^+]}{K_a} + \frac{K_c}{[H^+]} + \frac{K_b}{K_a}\right)$$

$$\%D = 100\Big/\left(1 + \frac{[H^+]}{K_c} + \frac{[H^+]}{K_d} + \frac{[H^+]^2}{K_b \cdot K_d}\right)$$

MATERIALS AND METHODS

Antibiotic

Micronized cefadroxil monohydrate (Bristol Myers, Italy).

Potentiometric determination

The ionization macroconstants were determined by a potentiometric method using the apparatus shown in Fig. 2. The temperature was maintained at 20 ± 2°C. A small amount of cefadroxil was dissolved in redistilled water and a few drops of concentrated HCl were added in order to obtain the corresponding hydrochloride, this being measured

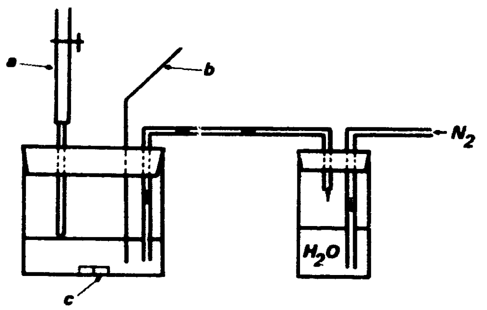


Fig. 2. Apparatus used for determination of ionization constants by potentiometry. (a) micrometer syringe; (b) miniature glass electrode; (c) magnetic agitator.

with 0.01 M KOH. The incorporation of the titrant was carried out with extreme care using a Dosimat automatic burette. After each addition of titrant, the pH was measured with a digital [1] pH meter fitted with a glass combined electrode [2].

Spectrophotometric determinations

In order to determine the ionization microconstants, the evolution of the absorbance of the phenol group was followed, in the UV region of the spectrum using a double-beam spectrophotometer [3]. A series of solutions of cefadroxil in buffer at pH concentrations of 6–12 was prepared and adjusted with KCl to an ionic strength of 0.05. Spectra were obtained over the 200–300 nm range using the appropriate buffer solution as a reference.

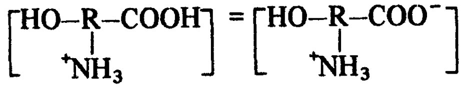
Studies carried out in this laboratory, the results of which await publication, have shown that the antibiotic is stable under these conditions for the time of duration of the experiments.

RESULTS AND DISCUSSION

Firstly, the determination of the macroscopic constants was carried out from the curves evaluating a solution of cefadroxil in water and water only. Fig. 3 shows these curves, together with the differential curve from the inflection points of which it has been possible to establish in an approximate way the pK values shown in Table 1.

In order to make a more accurate determination, we evaluated a solution of cefadroxil as its hydrochloride, registering the variation in pH of the solution after the addition of known volumes of 0.01 M KOH. Plotting ΔpH/ΔV against the volume (V), it is possible to obtain the curve shown in Fig. 4, in which the final point of evaluation may be established with a reasonable degree of accuracy.

The first equilibrium is reached when



$$\left[\mathrm{HO{-}\underset{{}^{+}NH_3}{\underset{|}{R}}{-}COOH}\right] = \left[\mathrm{HO{-}\underset{{}^{+}NH_3}{\underset{|}{R}}{-}COO^{-}}\right]$$

In order, therefore, to evaluate half of the existing acid, half the volume used would have been necessary, the moment at which the pH reached neutrality would correspond to the value of the pK$_1$. Along a similar line of reasoning, the values of pK$_2$ and pK$_3$ are obtained at the second and third point of equilibrium respectively. The pK values thus obtained are shown in Table 1 where it may be seen that there are no significant differences with respect to the values obtained with the above-mentioned method.

The determination of the ionization microconstants was carried out from the variations in absorbance of the phenol group with the pH of the solution. Fig. 5 shows the spectral changes of cefadroxil as a function of the pH. The degree of dissociation of the

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[1] Crison-501.

[2] Ingold 405-M-5.

[3] Varian Thechtrom-635.

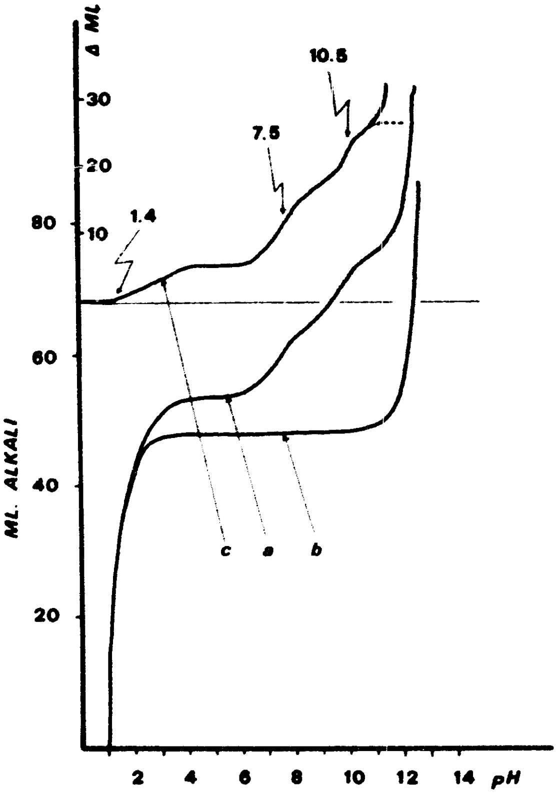

Fig. 3. Titration curves for cefadroxil. (a) Curve for the titration of the acid. (b) Curve for the titration of the solvent. (c) This curve represents the equivalents of $[H^+]$ found as a function of pH. It is obtained when the solvent titration curve is subtracted from the acid titration curve.

TABLE 1

VALUES OBTAINED FOR THE MACROSCOPIC CONSTANTS

	pK_1	pK_2	pK_3
(1)	1.4	7.5	10
(2)	1.38	7.55	10.10

(1) From the differential curve of the evaluation curves of the drug and of the solvent. (2) Potentiographically.

TABLE 2

VALUES OBTAINED FOR THE MICROSCOPIC CONSTANTS

	pK_a	pK_b	pK_c	pK_d
(A)	8.21	7.66	9.44	9.99
(B)	8.35	7.63	10.37	11.09

(A) Edsall's method. (B) Linear regression method.

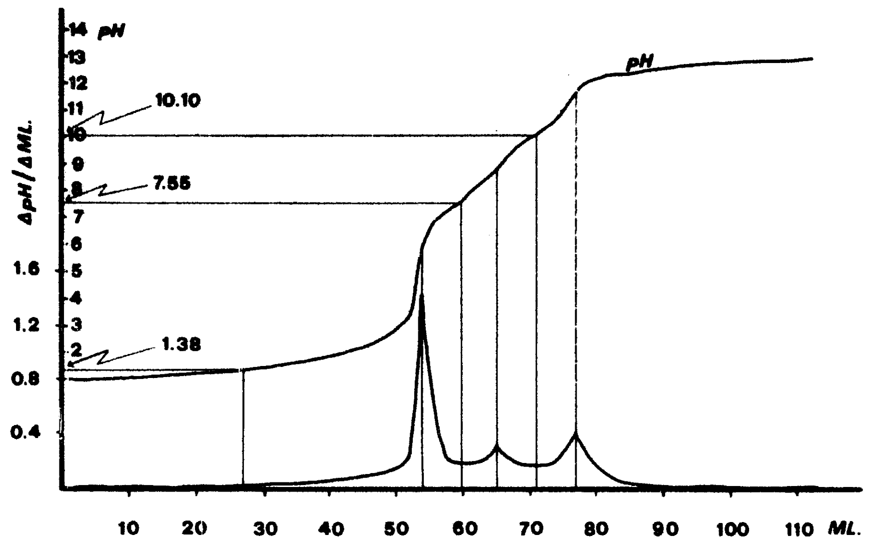

Fig. 4. Plot obtained by the continuous treatment of the cefadroxil solution with KOH and with continuous pH measurement by a potentiograph. A potentiographic curve together with its corresponding differential curve were obtained.

phenol group was determined from the absorbance values at 250 nm, where the absorption maximum is produced. At pH = 6, the phenol group is unionized; the degree of ionization increases in parallel to the increase in pH until, at pH = 12.0, it is seen to be totally ionized.

From the values of K_a it is possible to determine the remaining microscopic constants by applying the above-mentioned methods. The maximum zwitterion concentration

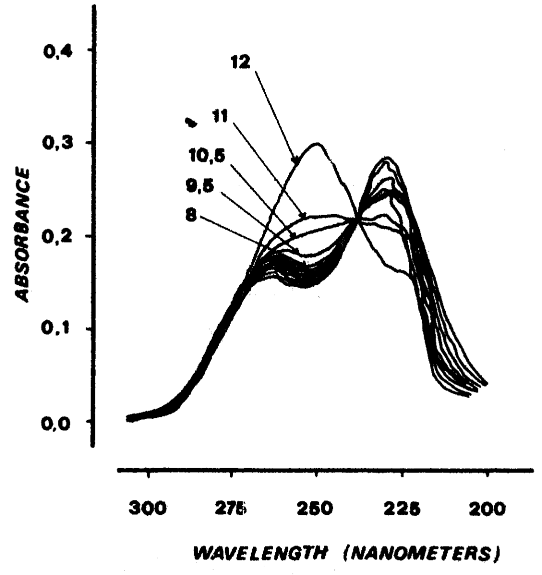

Fig. 5. Spectral changes of cefadroxil as a function of pH.

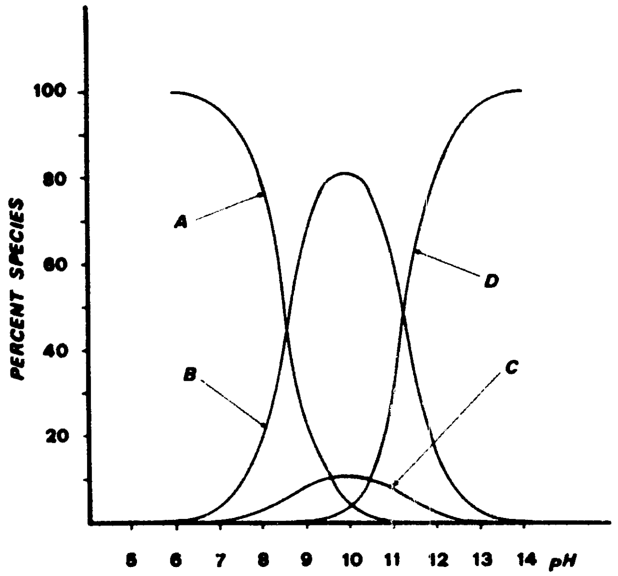

Fig. 6. Specific profiles for cefadroxil.

(Streng, 1976) is found at a particular pH value, according to the expression:

$$\mathrm{pH} = \frac{\mathrm{pK_a} + \mathrm{pK_c}}{2} = \frac{\mathrm{pK_b} + \mathrm{pK_d}}{2} = 9.36$$

From the values of the ionization microconstants, the proportion is obtained in which the various species, as a function of pH, are found as shown in Fig. 5. It may be seen how with cefadroxil, which fulfills the zwitterion criterion (Albert et al., 1971), the 4 ionized species coexist over the pH range of 6.8–11.8.

The value obtained for $\mathrm{pK_1}$ is significantly smaller than that observed for other cephalosporins and even smaller than that established for the carboxylic acid group when the $-\mathrm{CH_3}$ group is present (Flynn, 1972). The presence of the 4-hydroxyphenyl, which confers the molecule with its special physicochemical properties, is outstanding due to its statistically significant differences.

The values of K_a and K_b are similar to those obtained for the macroscopic constant, K_2, and the values of K_c and K_d are similar to the macroscopic constant, K_3. From what has been reported above, it may be deduced that these functional groups influence each other mutually, thus affecting the microscopic constant.

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