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Short communication

Acidity constants of cefetamet, cefotaxime and ceftriaxone; the effect of the substituent at C3 position

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Abstract

Ionization constants of three cephalosporin antibiotics, cefetamet (CEF), cefotaxime (CFX) and ceftriaxone (CFTR) are determined using pH-potentiometric titrations at $I = 0.1$ M (NaCl) and $t = 25\,^{\circ}C$. Cefetamet and cefotaxime have three ionization groups: carboxylic, amide and aminothiazole. Besides those three, ceftriaxone possesses an hydroxytriazinone group as new and additional ionization center. In acid medium two overlapping acid–base processes are occuring with acidity constants being: pK_1 2.93 (COOH) and pK_2 3.07 (aminothiazole) for cefetamet, and pK_1 2.21 (COOH) and pK_2 3.15 (aminothiazole) for cefotaxime. In the case of ceftriaxone the situation is even more complicated, three overlapping processes coexist with pK_1 2.37 (COOH), pK_2 3.03 (aminothiazole) and pK_3 4.21 (hydroxytriazinone). Protolysis of amide group is happening in the alkaline medium as completely separated process from those in acid medium. The acidity constants which correspond to amide group are pK_3 10.65 (CEF), pK_3 10.87 (CFX) and pK_4 10.74 (CFTR).

The influence of the C3 substituent on the dissociation process of the neighboring ionization group, particularly carboxylic group, was considered. The differences in acidity of CEF, CFX and CFTR (pK_1: 2.93, 2.21 and 2.37, respectively) are likely to be caused by the stereoelectronic properties of substituents in the β-position to the carboxylic group due to the combined inductive, hyperconjugative and resonance effects.

Keywords: Cephalosporins; Cefetamet; Ceftriaxone; Cefotaxime; Acidity constants; Potentiometry

1. Introduction

The extensive study of electrochemistry of cephalosporins [1–8] led us to a conclusion that good knowledge of the acidity constants is of great importance both for understanding of the electrode process and establishing of the appropriate electroanalytical method for its determination.

According to the older literature data dealing with acidity constants of first and second generation of cephalosporins [9,10] acidity constants for cefalexin, cefaloglycin, cefaradine and cefachlor are reported. However, there are only few reports dealing with the pK values of the third generation cephalosporins [11,12].

Having that in mind, this paper deals with the acidity constants of some selected, third generation cephalosporins, cefetamet (CEF), cefotaxime (CFX) and ceftriaxone (CFTR).

It is interesting that for cefotaxime three ionization constants were already reported in 1985 [13], but unfortunately neither details of the experimental procedure nor the data treatment are given by the authors. By using the potentiometric method, the authors obtained two close pK values (2.1 and 3.4), as a consequence of overlapping processes in acid medium. The mean attribution of this paper is that the pK value of 3.4, was ascribed to aminothiazole ring due to the shift of its absorption maximum of the UV spectrum to

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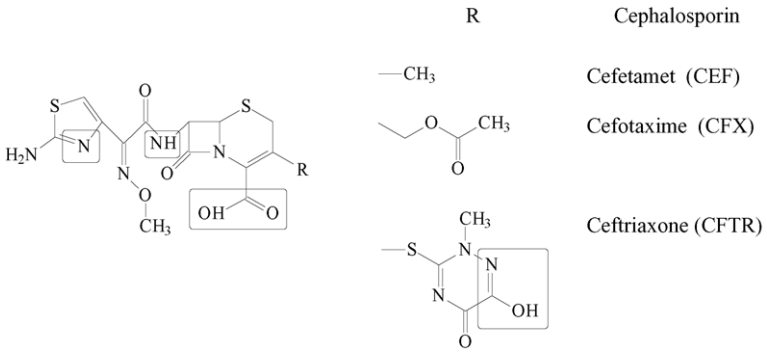


Fig. 1. Chemical structure of cephalosporins.

higher wavelengths caused by pH changes. The literature data regarded to cefetamet and ceftriaxone are still not complete, and acidity constants are not well attributed [14,15] to the corresponding ionization centers.

Those three compounds are chosen due to its same basic (cephem 2) structure and the same substituent at C2 and C7 position, but differentiating in substituent R at C3 position (Fig. 1). The presence of the substituent R at C3 position is considered as a potential new ionization center (ceftriaxone). Besides, its structure can influence the dissociation process of the neighboring ionization centers, particularly the carboxylic group.

The idea of this work was to determine the acidity constants of cefetamet and ceftriaxone under the same experimental conditions using cefotaxime as a model compound. Based on the results obtained, the attribution of the determined p*K* values to corresponding ionization centers was done, and the influence of the C3 substituent on p*K* values is discussed.

2. Experimental

2.1. Apparatus and reagents

Potentiometric titrations were performed on TIM900 Titration Manager-TitraLab (Radiometer Copenhagen) equipped with a combined pH electrode (GK2401B) and ABU901 autoburette (Radiometer Copenhagen).

Investigated drugs CFX (cefotaxime-Na) and CFTR (ceftriaxone-Na_2) are produced by SIGMA, and CEF (cefetamet-Na) is produced by Hoffman La Roche (Basel, Switzerland). All other reagents (HCl, NaOH and NaCl) were of analytical-reagent grade.

All solutions were prepared in doubly distilled water. Solutions of HCl (0.1030 M) and NaOH (0.1002 M) were standardized potentiometrically.

2.2. Determination of acidity constants

The acidity constants of cephalosporins, were determined at 25 ± 0.1 °C and constant ionic strength of 0.1 M (NaCl).

The solution of cephalosporin (1×10^{-3} M) was prepared in 0.1 M NaCl by adding standard HCl solution for acidifying solution. The obtained solution was titrated with standard NaOH solution. The function of formation ($\bar{n}$) was calculated according to equation:

$$\bar{n} = \frac{c_{\text{cephalosporin}} + c_{\text{HCl}} - c_{\text{NaOH}} - [\text{H}^+] + [\text{OH}^-]}{c_{\text{cephalosporin}}} \quad (1)$$

In Eq. (1) $c_{\text{cephalosporin}}$, c_{HCl} and c_{NaOH} represent the stoichiometric concentrations of the corresponding cephalosporin, HCl and NaOH in the solution, respectively. $[\text{H}^+]$ and $[\text{OH}^-]$ are equilibrium concentrations of hydrogen and hydroxyd ions obtained from the measured pH values [16].

3. Results and discussion

The chemical structure of the investigated cephalosporins, CEF, CFX and CFTR, are shown in Fig. 1. All three compounds have carboxylic group, as ionization center at C2 position, and the same C7 substituent bearing two ionization groups, amide and aminothiazole. The substituent at C3 becomes more complex from CEF to CFTR, and in the case of CFTR it posses hydroxytriazinone $\left(\text{—N=}\overset{|}{\text{C}}\text{—OH}\right)$ group as a new ionization center.

Since the acid–base equilibrium of CFTR is more complex compared to CEF and CFX, further consideration will be given regarded to this compound. In the pH interval from 0 to 14, CFTR is involved in four acid–base processes, i.e. it posses one basic (nitrogen of aminothiazole ring) and three acidic centers (carboxylic, hydroxytriazinone and amide group). Completely protonated form of CFTR (H_4C^+) is formed in the most acidic medium by protonation of nitrogen of aminothiazole group. By decreasing of acidity, the successive protolysis of carboxylic, protonated thiazolic group, hydroxytriazinone and amide group is occurring, which results in the following species: $H_3C^{\pm}$, H_2C^-, HC^{2-} and C^{3-}, respectively. The first three processes are happening in acid medium and are overlapping, while the

fourth one is completely separated and happening in alkaline medium. The corresponding acidity constants are defined as:

$$K_1 = \frac{[H_3C^{\pm}][H^+]}{[H_4C^+]} \quad (2)$$

$$K_2 = \frac{[H_2C^-][H^+]}{[H_3C^{\pm}]} \quad (3)$$

$$K_3 = \frac{[HC^{2-}][H^+]}{[H_2C^-]} \quad (4)$$

$$K_4 = \frac{[C^{3-}][H^+]}{[HC^{2-}]} \quad (5)$$

Acidity constants were determined potentiometrically, by using the formation function method ($\bar{n}$), i.e. the average number of bound protons [17]:

$$\bar{n} = \frac{\sum_{1}^{n} n[H_nC]}{\sum_{0}^{n}[H_nC]} \quad (6)$$

The charges in Eq. (6) are omitted due to the simplicity.

Constants K_1, K_2 and K_3 of CFTR are determined in the pH range 2–7 where the concentration of the anionic form $[C^{3-}] \rightarrow 0$. By combining the Eqs. (2)–(4), and Eq. (6) the following expression is obtained:

$$\bar{n} = \frac{4[H^+]^3 + 3K_1[H^+]^2 + 2K_1K_2[H^+] + K_1K_2K_3}{[H^+]^3 + K_1[H^+]^2 + K_1K_2[H^+] + K_1K_2K_3} \quad (7)$$

which shows that the formation function is a non-linear function of the solution acidity ($[H^+]$) in the investigated pH region. On the basis of experimentally determined $\bar{n}$ at each data point using pH-metric titration, the acidity constants given in Eq. (7) were determined by a non-linear curve-fitting analysis (Table 1).

At pH > 8 the constant K_4 of CFTR was evaluated regardless to the procesess occuring in the acid medium. Since only one acid–base pair (HC^{2-} – C^{3-}) exists in alkaline medium, the formation function is given by:

$$\bar{n} = \frac{[HC^{2-}]}{[HC^{2-}] + [C^{3-}]} \quad (8)$$

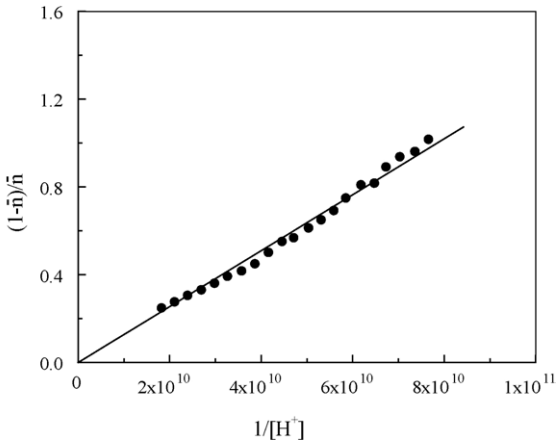


Fig. 2. Determination of the acidity constant K_4 of ceftriaxone by applying Eq. (9).

By combining Eqs. (5) and (8) the following dependence is obtained:

$$\underbrace{\frac{1-\bar{n}}{\bar{n}}}_{y} = K_4 \underbrace{\frac{1}{[H^+]}}_{x} \quad (9)$$

By applying the linear regression analysis the constant K_4 is calculated from the slope of the linear dependence given at Fig. 2.

Two acid–base processes are overlapping in acidic medium in the case of CEF and CFX, protolysis of carboxylic and protonated thiazolic group. Formation fuction in this case can be expressed by the following equation:

$$\bar{n} = \frac{3[H^+]^2 + 2K_1[H^+] + K_1K_2}{[H^+]^2 + K_1[H^+] + K_1K_2} \quad (10)$$

In order to obtain constants K_1 and K_2, Eq. (10) is further transformed to the linear dependence:

$$\underbrace{\frac{\bar{n}-2}{\bar{n}-1}[H^+]}_{y} = -K_2 + \frac{1}{K_1}\underbrace{[H^+]^2\frac{3-\bar{n}}{\bar{n}-1}}_{x} \quad (11)$$

on the basis of which K_1 and K_2 can be determined from the slope and intercept, respectively.

The obtained results are presented at Fig. 3 (representative diagram is shown) and in Table 1.

Table 1
Stoichiometric acidity constants of cephalosporins; $t = 25\,^{\circ}C$, $I = 0.1$ M (NaCl)

Ionization center	Carboxyl pK_1	Aminothiazole pK_2	Hydroxytriazinone	Amide pK_3
Cefetamet (CEF)	2.93	3.07		10.65
Cefotaxime (CFX)	2.21	3.15		10.87

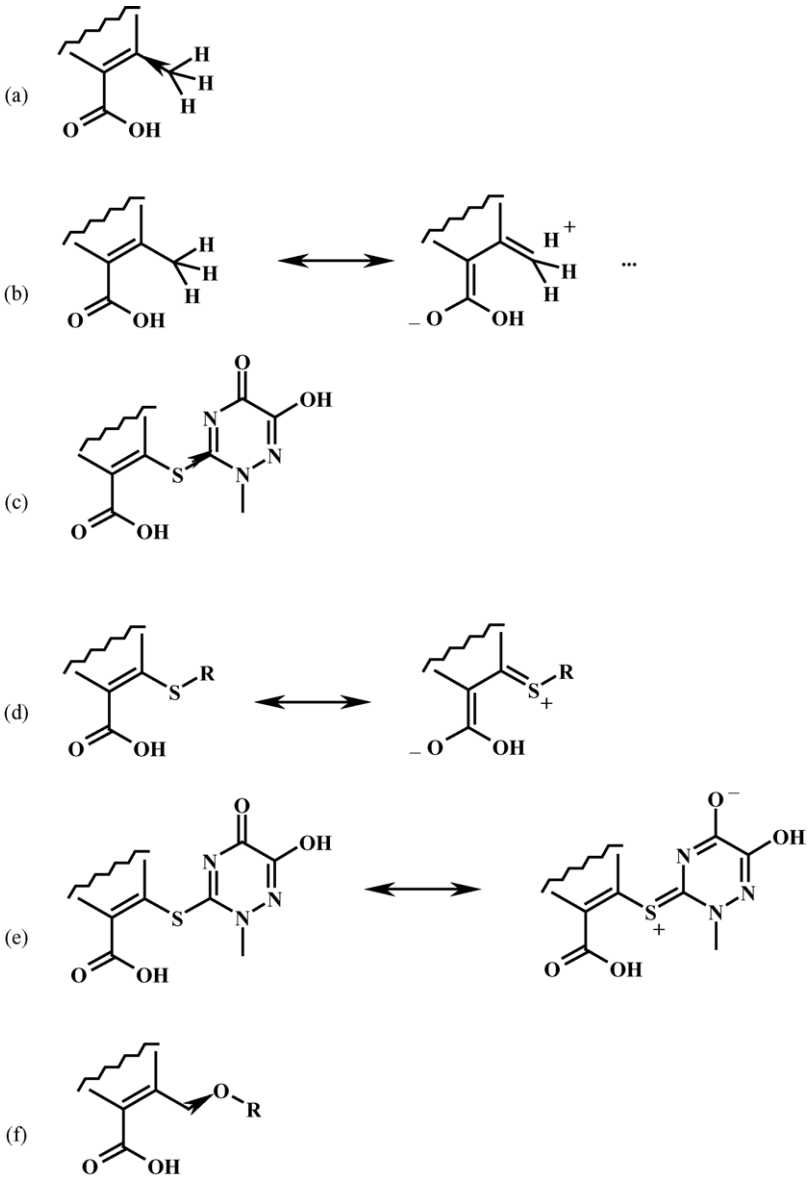
	pK_1	pK_2	pK_3	pK_4
Ceftriaxone (CFTR)	2.37	3.03	4.21	10.74

Acidity constant K_3 of CEF and CFX, which corresponds to amide group, was determined in identical manner as constant K_4 of CFTR.

The obtained pK values and the corresponding ionization centers are shown in Table 1. The pK values obtained for CFX in our experiments are in a good agreement with those previously determined [13] (2.1, carboxylic; 3.4, aminothiazolic and 10.9, amide). Since CEF has the same ionization centers as CFX, it was predictable to obtain three pK values, with two of them being very close. One of them, pK_2, corresponding to the protolysis of the protonated thiazolic group was determined for the first time. The acidity constant (pK_3 10.65) obtained in alkaline medium was attributed to protolysis of amide group, which was not previously done [14]. In the case of CFTR the overlapping effect was the most pronounced, since three constants are very close (pK_1 2.37, pK_2 3.03 and pK_3 4.21). Since the two first constants are in a good agreement with those obtained for CEF and CFX, those constants can also be ascribed to carboxilyc and aminothiazolic groups. The new acidity constant (pK_3 4.21) due to protolysis of group $(-N=\overset{|}{C}-OH)$ in C3 position is also defined. Besides, in alkaline medium, the acidity constant of amide group (pK_4 10.74) in position C7 was evaluated too.

As seen from Table 1, the greater differences were obtained between pK_1 values, than between pK_2 values. Therefore, the lower pK values obtained in acidic medium were ascribed to carboxylic group, rather than to aminothiazolic one, since the most pronounced influence of the C3 supstituent was on the ionization of the neighboring carboxylic group.

The differences in acidity of CEF, CFX and CFTR (pK_1) are likely to be caused by the stereoelectronic properties of



Scheme 1.

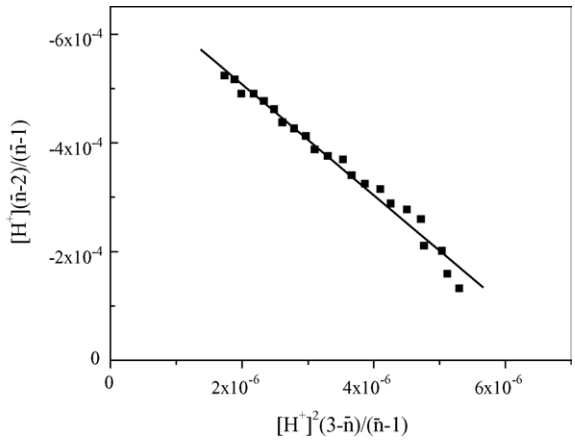


Fig. 3. Determination of the acidity constants K_1 and K_2 of cefotaxime applying Eq. (11).

substituents in the β-position to the carboxylic group. β-methyl derivate, i.e. CEF is the weakest acid (pK_1 2.93), which is probably result of combined inductive and hyperconjugative effect of the methyl substituent. Inductive effect (+*I*) of the methyl group, Scheme 1 (a) increases electron density on the carboxylic substituent deterring the –COOH dissociation process. A similar outcome is the result of the methyl group's hyperconjugative effect, Scheme 1. (b) CFTR is slightly stronger acid (pK_1 2.37). Although the *S*-triazinone substituent shows positive resonance effect (+*R*), Scheme 1 (d) which is expected to be more important than the inductive and hyperconjugative effects in the above case, there is no evidence of a significant acidity decrease. There are few reasons for this (+*R*) effect of sulphur is somewhat of weaker influence due to ineffective overlapping of C(2p) and S(3p) orbitals caused by the symmetry differences. In addition, the triazinone substituent also diminishes electron-donating properties of sulphur mainly due to its electron-withdrawing effect acting in the opposite way, as outlined in Scheme 1 (c and e). Finally, it is likely that *S*-triazinone substituent is out of plane of the α, β-conjugated moiety, which additionally reduces the electron-donor properties of the *S*-atom due to difficulties in overlapping of the p-orbitals of the neighboring C and S atoms. This makes (−*I*) effect of the *S*-triazinone substituent dominant, which increases acidity of the carboxylic group. The specific conformational properties of CFTR might be caused by steric interactions of the carboxylic and the *S*-triazinone substituent. In order to avoid this steric clash, one of the moieties has to adopt "out of plane" conformation. CFX is stronger acid (pK_1 2.21) compared to CEF and CFTR due to (–*I*) effect of the acetoxy substituent, Scheme 1 (f) which diminishes electronic density of the carboxylic group and increases the possibility of the –OH bond dissociation.

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