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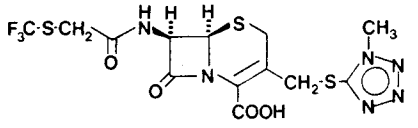
Cefazaflur: kinetics of hydrolysis in aqueous solution, acid dissociation constant and alkaline decomposition to fluorescent products

VINCENT P. IRWIN, RICHARD F. TIMONEY, *Department of Pharmaceutical Chemistry, School of Pharmacy, Trinity College, Dublin, Ireland*

Abstract—The values of the pseudo-first order hydrolysis rate constants in the pH range 1 to 10, and the pK_a, were determined for cefazaflur in aqueous solution at 37°C and ionic strength 0·2 M. A fluorimetric assay, based on alkaline hydrolysis at 100°C, was also developed for this compound. The results are consistent with previously reported related properties of other monoprotic cephalosporins.

Cefazaflur, a monoprotic monoionic cephalosporin antibiotic (Actor et al 1977), possesses a pH-independent chromophore in aqueous solution ($\lambda_{max} = 269$ nm; $\log \varepsilon = 4{\cdot}08$) which, in common with the chromophores of other cephalosporins (Yamana & Tsuji 1976; Schirmer 1982), disappears upon hydrolysis of the β-lactam ring. Measurements of UV absorbance have, therefore, been used previously as the basis of studies of the kinetics of hydrolysis of these compounds (Yamana & Tsuji 1976). We have



studied a series of buffered aqueous solutions of the heretofore uninvestigated cefazaflur sodium at ionic strength, I, of 0·2 M in the pH range 1 to 10. Thus, solutions of cefazaflur (initial antibiotic concentration 5×10^{-5} M) were incubated at 37°C and the absorbance, A, measured periodically at 269 nm. The values of the pseudo-first order hydrolysis rate constant, k_{pH}, as calculated using these A values by the method of Yamana & Tsuji (1976), are presented as a function of pH in Fig. 1. The profile is approximately U-shaped, this being typical of the monoprotic cephalosporins (Yamana & Tsuji 1976).

The value of the pK_a of cefazaflur, determined at 37°C and I = 0·2 M using the method of Streng (1978), was 2·45.

Stoichiometric degradations of β-lactam antibiotics to fluorescent products at elevated temperature in strongly acidic (Jusko 1971; Heald et al 1976) or strongly alkaline (Barbhaiya & Turner 1976; Yu et al 1977) aqueous solution have been employed previously in assay protocols for these compounds. In the present work, buffered solutions of cefazaflur sodium in the pH range 2 to 6 maintained at 100°C for up to 3 h did not generate fluorescence. Neutral and alkaline solutions (pH 7 to 14) did decompose to fluorescent products, however, (optimum reaction pH 13; optimum reaction time 75 minutes at 100°C; λ_{max} (excitation) 340 nm; λ_{max} (emission) 420 nm). These observations are consistent with the fact that acid-generated fluorophores have previously been described only as arising from the diprotic zwitterionic β-lactam antibiotics (Jusko 1971; Heald et al 1976), while the alkaline decomposition method has been reported to be applicable to many monoprotic cephalosporins also (Yu et al 1977). A concentration-fluorescence intensity plot was linear in the initial antibiotic concentration range 0 to 10^{-4} M (n = 5; r = 0·999) for cefazaflur solutions hydrolysed in excess alkali (pH 13) at 100°C, the fluorescence intensity being 1/20 of that generated from the highly fluorescence-active cephalosporin cephaloridine (Yu et al 1977) decomposed under the same conditions.

Correspondence to: R. F. Timoney, Department of Pharmaceutical Chemistry, School of Pharmacy, Trinity College, Dublin, Ireland.

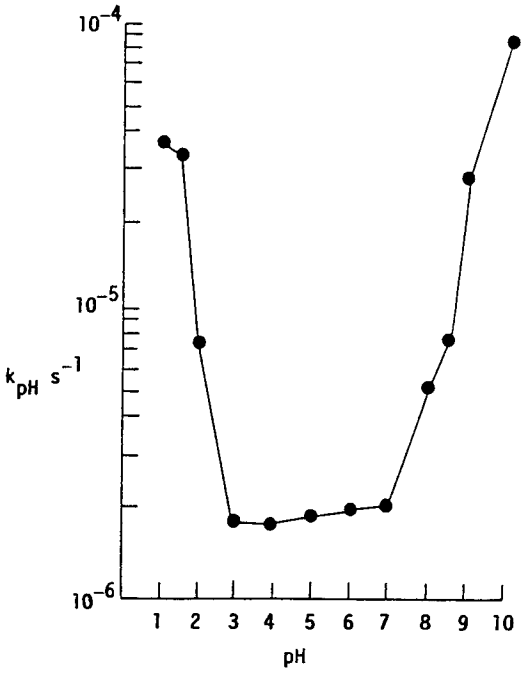


FIG. 1. pH-Hydrolysis rate profile for cefazaflur at 37°C and I = 0·2 M.

Materials

Antibiotic samples were generous gifts of Smith, Kline and French Laboratories, USA (cefazaflur sodium) and Glaxo Ltd, UK (cephaloridine). Aqueous buffers were formulated from analytical grade HCl/phosphate salts/borate salts/NaOH as appropriate (Dawson et al 1986), the ionic strength being adjusted by the addition of KCl.

References

Actor, P., Guarini, J. R., Uri, J., Bartus, H. F., Zajac, I., Weisbach, J. A. (1977) In vitro studies with cefazaflur and other cephalosporins. J. Antibiot. 30: 730–735

Barbhaiya, R. H., Turner, P. (1976) Fluorimetric determination of ampicillin and cephalexin. Br. J. Pharmacol. 58: 473p

Dawson, R. M. C., Elliott, D. C., Elliott, W. H., Jones, K. M. (1986) Data for Biochemical Research, 3rd. edn., Clarendon Press, Oxford. pp. 426–441

Heald, A. F., Ita, C. E., Schreiber, E. C. (1976) Fluorimetric determination of cephradine in plasma. J. Pharm. Sci. 65: 768–769

Jusko, W. J. (1971) Fluorimetric analysis of ampicillin in biological fluids. Ibid. 60: 728–732

Schirmer, R. E. (1982) Modern Methods of Pharmaceutical Analysis, Vol. 1, CRC Press, Boca Raton, Florida, pp. 65–67

Streng, W. H. (1978) Microionization constants of commercial cephalosporins. J. Pharm. Sci. 67: 666–669

Yamana, T., Tsuji, A. (1976) Comparative stability of cephalosporins in aqueous solution: kinetics and mechanisms of degradation. Ibid. 65: 1563–1574

Yu, A. H. C., Nightingale, C. H., Flanagan, D. R. (1977) Rapid sensitive fluorimetric analysis of cephalosporin antibiotics. Ibid. 66: 213–216