International Journal of Pharmaceutics, 16 (1983) 319–325
Elsevier

The influence of carbohydrates and polyhydric alcohols on the stability of cephalosporins in aqueous solution

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(Received January 25th, 1983)
(Accepted March 25th, 1983)

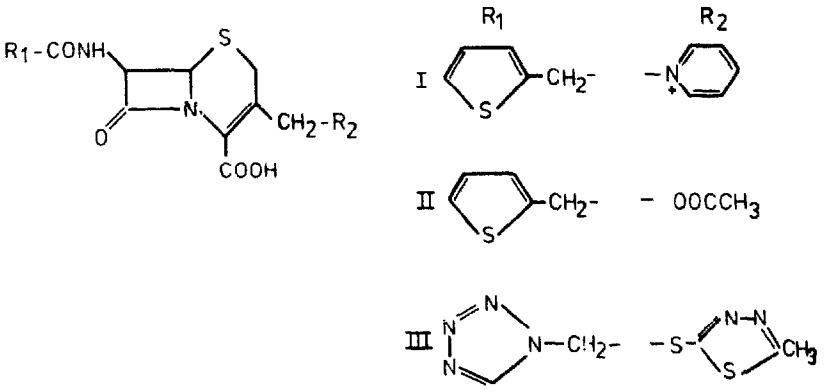
Summary

The kinetics of the degradation of cephaloridine, cephalothin and cefazolin in aqueous solutions containing various carbohydrates (glucose, fructose, sucrose and dextrans) or polyhydric alcohols (sorbitol, mannitol and glycerol) was studied over the pH range 5–12 at 35°C. The degradation rate increased linearly with the hydroxy compound concentration up to 15% at constant pH for pH > 6.5. The rate-accelerating effect of the compounds was directly proportional to the hydroxide ion activity up to pH about 11 above which value the rate of reactions levelled off with increasing pH as demonstrated for glucose. Maximal stability of the cephalosporins in solutions containing carbohydrates or polyhydric alcohols is attained at pH 5–6.5. It is proposed that the rate-accelerating effect of the hydroxy compounds is due to a nucleophilic reaction mechanism involving opening of the cephalosporin β-lactam moiety by an alkoxide ion derived from proton ionization of one of the hydroxyl groups in the compounds.

Introduction

Penicillins and cephalosporins are often dispensed or administered in solutions containing various carbohydrates or polyhydric alcohols, e.g. infusion, mixture and syrup preparations. Since these β-lactam antibiotics are known to undergo facile cleavage of their β-lactam bonds, it is of practical importance to have information about the effects of such hydroxy compounds on the stability of drugs. Whereas the effect of various carbohydrates and alcohols on penicillin stability has been studied

rather extensively (Landersjö et al., 1977; Stjernström et al., 1978; Bundgaard and Larsen, 1978a and b, 1979; Bundgaard 1980) only scanty information is available in the case of cephalosporins (e.g. Bornstein et al., 1974; O'Brien et al., 1979; Das Gupta and Stewart, 1981). The purpose of the present investigation was to provide basic information on the stability of cephalosporins in aqueous solutions containing various hydroxy compounds. To this end the kinetics of degradation of some representative cephalosporins (cephaloridine (I), cephalothin (II) and cefazolin (III)) in such solutions was studied over a broad range of pH and carbohydrate/alcohol concentrations.



Materials and Methods

Chemicals and apparatus

The cephalosporins studied were commercial products and were used as received (cf. Bundgaard, 1975). Carbohydrates and other chemicals used were of pharmacopoeial or reagent grade quality. Dextran 40 and dextran 70 with an average molecular weight of 41,000 and 64,400, respectively, were obtained from Pharmacia, Sweden.

A Zeiss PMQ II spectrophotometer and a Perkin-Elmer model 124 spectrophotometer were used for the ultraviolet spectral measurements. The pH measurements were made at the temperature of study using a Radiometer Type PHM26 instrument.

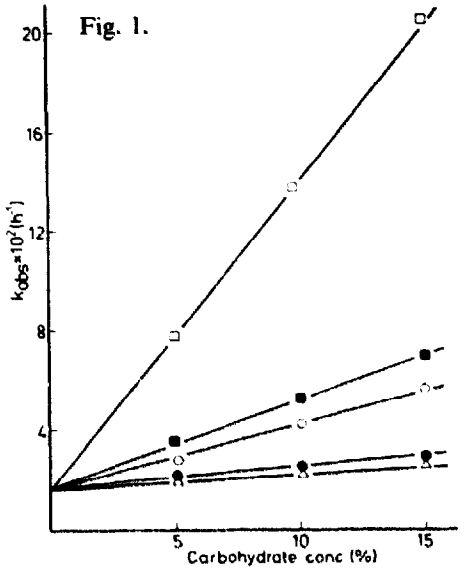
Kinetic measurements

All kinetic experiments were carried out at 35°C in aqueous buffer solutions (acetate, phosphate, borate or carbonate) containing varying amounts of carbohydrates and polyhydric alcohols. Disodium edetate (10^{-3} M) was added to the solutions in order to prevent any rate acceleration from possible traces of metals (cf. Bundgaard and Larsen, 1978b). For reactions at pH less than 10 the solutions were kept in a water bath in screw-capped test tubes. After addition of the cephalosporin compound to give an initial concentration of about 0.25 mg · ml^{-1}, samples of 1000 μl were taken at appropriate intervals and diluted 10 times with

water. The absorbance of the resultant solution was immediately read at 261 nm using 1-cm cuvettes. The degradation of the cephalosporins is accompanied by a large decrease in absorbance at 261 nm (Bundgaard, 1975; Yamana and Tsuji, 1976) and pseudo-first-order rate constants were obtained from the slopes of linear plots of $\log(A_t - A_\infty)$ against time, where A_t is the absorbance at 261 nm at time t and A_∞ is the absorbance at infinite time (corresponding to 8–10 half-lives). The rates of degradation of the cephalosporins in reaction solutions with pH greater than 10 were determined in a similar way except that the reaction solutions were kept in cuvettes placed in the thermostated compartment of the spectrophotometer. The initial cephalosporin concentration was 0.02–0.04 $mg \cdot ml^{-1}$ and the decrease in absorbance at 261 nm was recorded continuously.

Results and Discussion

The rates of degradation of cephaloridine, cephalothin and cefazolin in aqueous solutions containing various carbohydrates (glucose, fructose, sucrose and dextrans) or polyhydric alcohols (sorbitol, mannitol and glycerol) were measured at 35°C over the pH range 6.5–12. In the presence of excess carbohydrate or alcohol, and at constant pH, the observed pseudo-first-order rate constants (k_{obs}) for the degradation were found to be linearly dependent on the concentration of the hydroxy compounds at each pH and concentration range (0–15%) investigated. Typical plots of k_{obs} versus carbohydrate or alcohol concentration at various pH values are shown



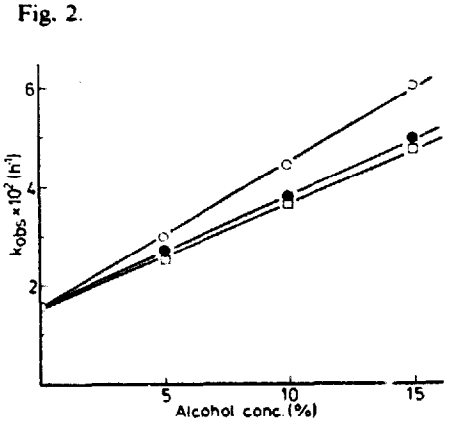


Fig. 1. Effect of various carbohydrates on the pseudo-first-order rate constant for the degradation of cephaloridine in 0.1 M phosphate buffer solution of pH 7.55 (35°C). Key: □, glucose; ■, fructose; ○, sucrose; ●, dextran 40; △, dextran 70.

Fig. 2. Effect of various polyhydric alcohols on the pseudo-first-order rate constant for the degradation of cephaloridine in 0.1 M phosphate buffer solution of pH 7.55 (35°C). Key: ○, mannitol; ●, sorbitol; □, glycerol.

Fig. 3.

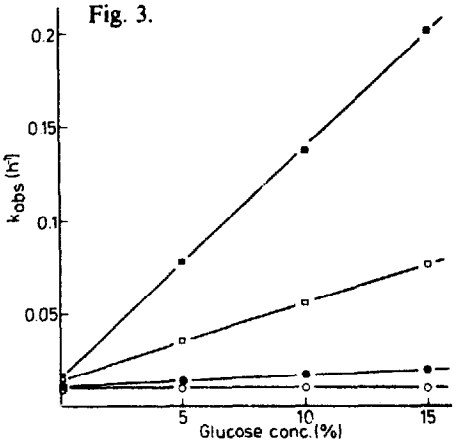


Fig. 4.

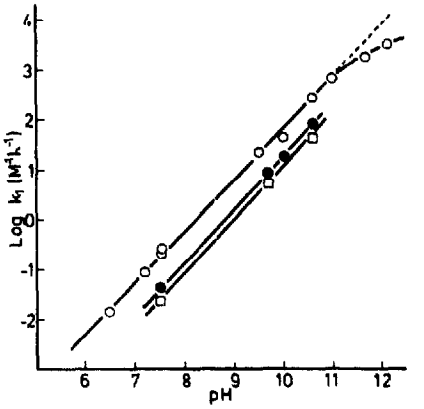


Fig. 3. Effect of glucose on the pseudo-first-order rate constant for the degradation of cephaloridine in aqueous buffer solutions at various pH values (35°C). Key: ■, pH 7.55; □, pH 7.20; ●, pH 6.50; ○, pH 5.00.

Fig. 4. The logarithm of the second-order rate constants (k_1) for the carbohydrate- and alcohol-catalyzed degradation of cephaloridine plotted as a function of pH. Key: ○, glucose; ●, fructose; □, sorbitol.

in Figs. 1–3. The results may be described by the following expression:

$$k_{obs} = k_{hyd} + k_1[ROH]_T \quad (1)$$

where $[ROH]_T$ represents the total molar concentration of the hydroxy compounds, k_1 is a pH-dependent second-order rate constant for the reaction of cephalosporin with the hydroxy compounds and k_{hyd} represents the pseudo-first-order rate constant for hydrolysis of cephalosporin in absence of carbohydrate or alcohol, i.e. equal to the intercept of the lines in Figs. 1–3.

When the logarithms of the slopes ($= k_1$) of the plots of k_{obs} against $[ROH]_T$ were plotted against pH, straight lines with slopes of 1.0 ± 0.05 were obtained at pH values up to about 11. This is shown for cephaloridine in Fig. 4. Thus in this pH range the reactions show a first-order dependence on hydroxide ion activity and Eqn. 1 may accordingly be written as:

$$k_{obs} = k_{hyd} + k'_1 a_{OH}[ROH]_T \quad (2)$$

where a_{OH} (the hydroxide ion activity) was determined at 35°C from the measured pH according to the following equation (Harned and Hamer, 1933):

$$\log a_{OH} = pH - 13.68 \quad (3)$$

Values of the apparent third-order rate constants, k'_1, for the reaction of the cephalosporins with the various hydroxy compounds are listed in Table 1; for comparison values of the rate constants for the reaction of benzylpenicillin at similar conditions (Bundgaard and Larsen, 1978b) are included.

TABLE 1

APPARENT THIRD-ORDER RATE CONSTANTS FOR THE REACTIONS OF CARBOHYDRATE AND POLYHYDRIC ALCOHOLS WITH SOME CEPHALOSPORINS AND BENZYLPENICILLIN IN AQUEOUS SOLUTION AT 35°C

Compound	$k_1' \times 10^{-5}$ ($M^{-2} \cdot h^{-1}$)			
	Cephaloridine	Cephalothin	Cefazolin	Benzylol-penicillin [a]
Glucose	3.4	0.29	0.33	0.69
Fructose	0.90	0.07	0.07	0.43
Sucrose	1.3			1.4
Dextran 40	47			41
Dextran 70	44			55
Sorbitol	0.57	0.06	0.07	0.38
Mannitol	0.74			0.34
Glycerol	0.26			0.10

[a] From a previous study (Bundgaard and Larsen, 1978b).

The examination of the pH dependence of the reaction of cephaloridine with glucose was extended to include pH values up to 12.1. As can be seen from Fig. 4, the reaction rate conforms to a first-order dependence on hydroxide ion activity below pH 11 in accordance with Eqn. 2, but at higher pH it increases less rapidly. As previously described for the reaction of penicillins with carbohydrates (Bundgaard and Larsen, 1978b), this behaviour may most likely be interpreted to imply that an alkoxide ion derived from proton ionization of a hydroxyl group in glucose is the species reacting with the cephalosporin. At 35°C the pK_a value for the glucose is 11.8 (Bundgaard and Larsen, 1978b) and therefore, at pH near pK_a, further increases in hydroxide ion concentration will not result in a corresponding increase in the glucose alkoxide ion concentration. According to this interpretation, Eqn. 2 may now be written as:

$$k_{obs} = k_{hyd} + k_n[RO^-] \tag{4}$$

or

$$k_{obs} = k_{hyd} + k_n \frac{K_a}{a_H + K_a}[ROH]_T \tag{5}$$

where $[RO^-]$ is the molar concentration of ionized carbohydrate or alcohol, K_a is the ionization constant for the hydroxy compound, a_H is the hydrogen ion activity and k_n is the pH-independent second-order rate constant for reaction of oxygen anion with cephalosporin. At pH about one unit or more below pK_a, i.e. $a_H \gg K_a$, Eqn. 5 can be written as:

$$k_{obs} = k_{hyd} = + k_n \frac{K_a}{K_w} a_{OH}[ROH]_T \tag{6}$$

where K_w is the ionization constant of water. Thus, for pH values up to about 11 this interpretation of the reactions conforms to the observed proportionality between hydroxide ion activity and rate of reaction of cephalosporin with the hydroxy compounds as expressed by Eqn. 2. From Eqns. 2 and 6 it is readily seen that values of k_n can be obtained from the following relationship:

$$k_n = k_1' K_w / K_a \tag{7}$$

The value of k_n for reaction of glucose with cephaloridine was calculated to be $4.5 \times 10^3\ M^{-1} \cdot h^{-1}$.

At pH values lower than 6.5 no significant effect of the hydroxy compounds on the rate of degradation of the cephalosporins was observed. At these pH values the spontaneous hydrolysis becomes the predominant degradation reaction.

The kinetics observed for the reaction of the hydroxy compounds with the cephalosporins is similar to that previously found for the analogous reactions of penicillins (Bundgaard and Larsen, 1978a and b). In the penicillin reactions the presence of a penicilloyl ester ester in the reaction pathway was demonstrated and it was shown that the rate-accelerating effect of the hydroxy compounds is entirely due to a nucleophilic reaction mechanism with an intermediate formation of penicilloyl esters which subsequently undergo hydrolysis to produce penicilloic acid. In view of the structural similarity between penicillins and cephalosporins the reaction of the latter with the hydroxy compounds may most likely occur by a similar mechanism rather than by a kinetically equivalent mechanism involving general base catalysis of hydrolysis by an alkoxide anion.

Inspection of the rate constants in Table 1 shows that the most remarkable difference between the reactions of cephalosporins and benzylpenicillin with the hydroxy compounds is the behaviour of glucose and fructose. While these carbohydrates are almost equally reactive with benzylpenicillin, glucose is 4–5 times more reactive than fructose with the cephalosporins. This difference may most likely be attributed to the more sterically hindered β-lactam carbonyl moiety in the cephalosporin molecule (Bundgaard, 1975). While the most acidic and therefore the most reactive hydroxyl group in glucose is the secondary hydroxyl group in the 1-position, the corresponding group in fructose is the C-2 hydroxyl group which is tertiary (Prince et al., 1982; Perrin et al., 1981). Thus, the reacting alkoxide anion is sterically more hindered in fructose than in glucose.

The greater reactivity of cephaloridine as compared with cephalothin and cefazolin (Table 1) is as expected; in reactions with both hydroxide ions and various amines cephaloridine exhibits a greater reactivity than the other two cephalosporins (Bundgaard, 1975).

In conclusion, it has been shown that in neutral and alkaline solutions various carbohydrates and polyhydric alcohols accelerate the rate of degradation of cephalosporins. The degradation rates increase linearly with the concentration of the hydroxy compounds and the rate-accelerating effect is directly proportional to the hydroxide ion activity up to pH about 11. The hydroxy compounds have no significant effect on the stability of the cephalosporins at pH less than 6–6.5. The

stability of most cephalosporins in aqueous solution is maximal at pH 5–6.5 (Yamana and Tsuji, 1976) and accordingly, such pH values would be optimal both as regards depression of the catalytic effect of hydroxy compounds and as regards overall stability of cephalosporins.

References

Bornstein, M., Thomas, P.N., Coleman, D.L. and Boylan, J.C., Stability of parenteral solutions of cefazolin sodium. Am. J. Hosp. Pharm., 31 (1974) 296–298.

Bundgaard, H., Chemical studies related to cephalosporin allergy. I. Kinetics of aminolysis of cephalosporins and effect of C-3 substituents on β-lactam reactivity. Arch. Pharm. Chem., Sci. Edn., 3 (1975) 94–123.

Bundgaard, H., Pharmaceutical aspects of penicillin allergy: polymerization of penicillins and reactions with carbohydrates. J. Clin. Hosp. Pharm., 5 (1980) 73–96.

Bundgaard, H. and Larsen, C., Kinetics and mechanism of the sucrose-accelerated degradation of penicillins in aqueous solution. Int. J. Pharm., 1 (1978a) 95–104.

Bundgaard, H. and Larsen, C., Kinetics and mechanism of reaction of benzylpenicillin and ampicillin with carbohydrates and polyhydric alcohols in aqueous solution. Arch. Pharm. Chem., Sci. Edn., 6 (1978b) 184–200.

Bundgaard, H. and Larsen, C., Piperazinedione formation from reaction of ampicillin with carbohydrates and alcohols in aqueous solution. Int. J. Pharm., 3 (1979) 1–11.

Das Gupta, V. and Stewart, K.R., Stability of cefamandole nafate and cefoxitin sodium solutions. Am. J. Hosp. Pharm., 38 (1981) 875–879.

Harned, H.S. and Hamer, W.J., The ionization constant of water in potassium chloride solutions from electromotive forces of cells without liquid junction. J. Am. Chem. Soc., 55 (1933) 2194–2206.

Landersjö, L., Stjernström, G. and Lundgren, P., Studies on the stability and compatibility of drugs in infusion fluids. IV. Factors affecting the stability of benzylpenicillin in carbohydrate solutions. Acta Pharm. Suec., 14 (1977) 293–308.

O'Brien, M.J., Portnoff, J.B. and Cohen, E.M., Cefoxitin sodium compatibility with intravenous infusions and additives. Am. J. Hosp. Pharm., 36 (1979) 33–38.

Perrin, D.D., Dempsey, B. and Serjeant, E.P., pK_a Prediction for Organic Acids and Bases. Chapman and Hall, London and New York, 1981.

Prince, R.C., Gunson, D.E., Leigh, J.S. and McDonald, G.G., The predominant form of fructose is a pyranose, not a furanose ring. Trends Biochem. Sci. 7 (1982) 239–240.

Stjernström, G., Olson, O.T., Nyqvist, H. and Lundgren, P., Studies on the stability and compatibility of drugs in infusion fluids. VI. Factors affecting the stability of ampicillin. Acta Pharm. Suec., 15 (1978) 33–50.

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