International Journal of Pharmaceutics, 20 (1984) 25–41
Elsevier

IJP 00673

Controlled-release penicillin complexes: dissolution and stability of benzathine cloxacillin

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(Received November 11th, 1983)
(Modified version received January 3rd, 1984)
(Accepted January 13th, 1984)

Summary

The dissolution behaviour of benzathine cloxacillin is shown to depend upon pH. At pH 6.0, dissolution is governed by the solubility product of the complex and steady-state conditions are achieved. The degradation rate of cloxacillin becomes significant when dissolution is undertaken at pH 2.0. This prevents maintenance of the equilibrium concentration of cloxacillin and as time proceeds the solution contains an increasing proportion of benzathine. At pH 9.0 cloxacillin continues to degrade by a first-order process but, additionally, a bimolecular reaction involving benzathine and cloxacillin produces an amide adduct. This product results in the loss of benzathine from solution and eventually appears as a precipitate.

Introduction

The development of controlled-release dosage forms of penicillins has a long history which began in 1944 with the formulation of amorphous calcium penicillin G

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in oil and beeswax (Romanzky and Rittman, 1944). Such products were prone to adverse reactions following intramuscular injection and were soon superceded by alternative formulations. The most promising approach was the complexation of the penicillin with a strongly basic amine which provided a salt with a low aqueous solubility causing a slow-release of penicillin and enabling blood levels to be sustained for a prolonged period. Procaine penicillin G (Sullivan et al., 1948), for example, combined a local anaesthetic affect with low solubility (ca. 0.7%) and an even lower level (0.02%) was displayed by the complexation of N,N'-dibenzylethyl-

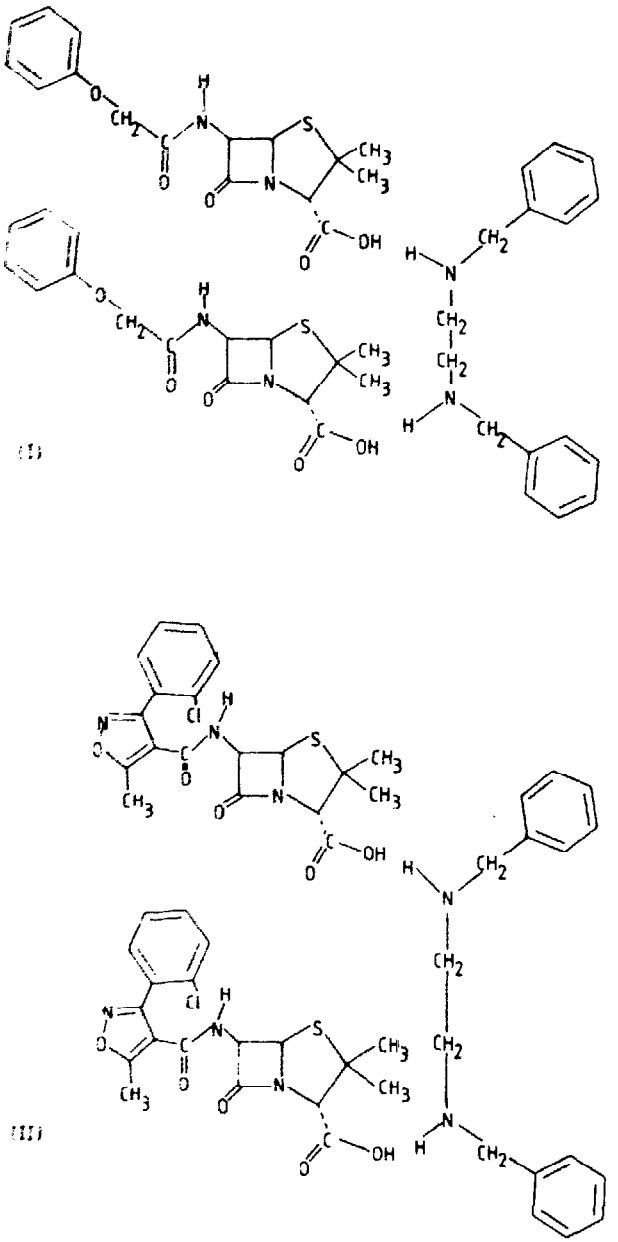


Fig. 1. Structures of benzathine penicillin V (I) and benzathine cloxacillin (II).

enediamine with penicillin G to give N,N'-dibenzylethylenediamine dipenicillin G or benzathine penicillin G (Elias et al., 1951). The low solubility of this latter product suppressed the overall degradation rate of penicillin and allowed the introduction of a commercial, oral penicillin suspension (Welch, 1953). A series of penicillin complexes, offering a range of depot products are now available and include benethamine penicillin G (Boger et al., 1954–55), Benzathine Penicillin V and Benzathine Cloxacillin (Szabo et al., 1951; Glassman et al., 1955–56).

These complexes vary in stoichiometry and their physicochemical properties related to pK_a and solubility. Although these data are essential to explain and predict the in vivo release characteristics of the salts, little information has appeared to date. Published studies include solubility determinations (Boger et al., 1954–55; Szabo et al., 1951; Scott et al., 1954; Weiss et al., 1957), degradation rates (Elias et al., 1951; Scott et al., 1954) and crystal structures (Lowe and Schwalbe, submitted for publication). Our interests in this field (Hempenstall et al., 1983; Irwin et al., 1984a and b; Irwin et al., 1983; Li Wan Po et al., 1983) have led us to study the affect of pH on the stability and dissolution profile of benzathine cloxacillin (Fig 1, II) a 1 : 2 complex used in the treatment of bovine mastitis.

Materials and Methods

Chemicals

Citrate buffer (pH = 2.0) contained citric acid (6.345 g), 1 M sodium hydroxide (60.4 ml) and 1 M hydrochloric acid (69.8 ml) in 1 litre; citrate buffer (pH = 6.0) contained citric acid (12.6 g) and sodium hydroxide (6.4 g) in 1 litre and the borate buffer (pH = 9.0; 1 litre) was prepared from boric acid (10.33 g), 1 M sodium hydroxide (83.5 ml) and 1 M hydrochloric acid (16.5 ml).

High-performance liquid chromatography

Analyses were performed using a high-performance liquid chromatogram (Irwin et al., 1984a and b) operated at a wavelength of 258 nm with a sensitivity of 0.02–0.04 AUFS (benzathine) and 0.16 AUFS (cloxacillin). The mobile phase consisted of acetonitrile (33%)–aqueous buffer (67%), containing potassium dihydrogen phosphate (4.54 g/l), with sodium heptane-1-sulphonate (6.3 g/l) as ion-pairing agent, and adjusted to an apparent pH of 3.40 (benzathine cloxacillin), or 3.50 (benzathine penicillin V), with phosphoric acid. The pre-filtered mobile phase (0.45 μm) was delivered at a rate of 1 ml/min and the column was thermostatted at 34°C to enhance column efficiency.

Degradation products from the reaction of benzathine with cloxacillin or with penicillin V were monitored using a mobile phase composed of acetonitrile (50%) in phosphate buffer (4.54 $g \cdot l^{-1}$ potassium dihydrogen phosphate; 6.30 $g \cdot l^{-1}$ sodium heptane-1-sulphonate) adjusted to an apparent pH of 3.55 with phosphoric acid.

Dissolution procedure. To the dissolution medium (230 ml), maintained at 34°C, was added a weighed amount of benzathine cloxacillin or benzathine penicillin V.

The medium was stirred at 150 rpm and samples (2–3 ml) were withdrawn periodically, and were filtered (Millipore type AA, 0.8 μm). Samples, where necessary to obtain the analytical concentration of benzathine, were immediately diluted with water; pH 2: 108 $mg \cdot l^{-1}$; pH 6 and 9: 250 $mg \cdot l^{-1}$. Dilution was not necessary at pH 6 and did not exceed 1 to 11 ml at pH 2 and 9.

Sieve analysis of benzathine cloxacillin. Sieve analysis was carried out using 33 mm diameter brass sieves with phosphor bronze mesh. Sieves were weighed empty before each analysis and 1.0 g of benzathine cloxacillin, placed in the top sieve, was mechanically agitated for 7 min. Each sieve was re-weighed individually at the end of this period to calculate the weight fraction retained.

Solubility measurements. The solubilities of benzathine and cloxacillin were measured by adding excess material to the appropriate buffer and stirring the solution rapidly. Where necessary, the pH of the resulting solution was adjusted to the original value by addition of either conc. HCl or conc. NaOH. The solution was stirred at 100 rpm for 2 days (benzathine) or 30 min (cloxacillin). After measurement of pH, the solution was diluted to the analytical concentration (approximately 700 $mg \cdot l^{-1}$ for cloxacillin and 200 $mg \cdot l^{-1}$ for benzathine) and assayed by HPLC against standards prepared in the same solvent.

Determination of the degradation rate of penicillin V at pH 9. 51.3 and 51.9 mg penicillin V potassium were dissolved in two 100 ml volumes of pH 9 borate buffer at 34°C. These solutions were assayed against freshly prepared penicillin V potassium standard dissolved in pH 9 borate buffer.

Reaction between benzathine and penicillins at pH 9

Benzathine and cloxacillin. 752 mg cloxacillin sodium was dissolved in 230 ml pH 9 borate buffer at 34°C and stirred at 150 rpm. After dissolution, 276 mg benzathine diacetate were added. The resulting solution was stirred, maintained at 34°C and sampled periodically. The samples were filtered (0.8 μm), diluted 1 in 6 with water, and assayed by HPLC against a freshly prepared series of standard mixtures (20–100%) prepared from 30 mg benzathine diacetate and 70 mg cloxacillin sodium in 100 ml sample solvent (30 ml pH 9 borate buffer added to 150 ml water).

Benzathine and penicillin V. 469.2 mg penicillin V potassium was dissolved in 230 ml pH 9 borate buffer at 34°C and stirred at 150 rpm. After dissolution, 217.4 mg benzathine diacetate were added. The resulting solution was stirred and kept at 34°C. Samples were filtered, diluted 1 ml to 11 ml with water and assayed by HPLC against a freshly prepared series of standard mixtures (20–100%) prepared from 25 mg benzathine diacetate and 50 mg penicillin V in 250 ml sample solvent (50 ml pH 9 borate buffer added to 500 ml water).

Preparation of benzathine cloxacillin crystals. Two solutions, one containing 4.6 g cloxacillin sodium in 900 ml water and the other 1.8 g benzathine diacetate in 100 ml water, were mixed slowly with rapid stirring, at ambient temperature. A fine precipitate immediately appeared. The mixture was left for 42 h, filtered (0.8 μm) and the crystals dried in a vacuum desiccator. The yield after drying was 5.4 g and the pH of the supernatant after filtration was 6.0.

Determination of the stability of benzathine

pH 2. 36.4 mg benzathine diacetate were dissolved in 230 ml pH 2 citrate buffer at 51°C. The solution was stirred at 150 rpm whilst maintaining the temperature, and sampled at 2, 10, 35 and 245 min. The samples were assayed against benzathine diacetate standard freshly prepared in pH 2 buffer.

pH 6 and pH 9. 120 mg benzathine diacetate were dissolved in 100 ml of the appropriate buffer at 34°C. The solutions were maintained at 34°C in a water bath sampled at 10, 60 and 180 min. The samples were assayed against freshly prepared benzathine diacetate standard, dissolved in the appropriate buffer.

Determination of the degradation rate of cloxacillin

pH 2 (without benzathine). 82.2 mg cloxacillin sodium were added to 230 ml of pH 2 citrate buffer at 34°C. The solution was filtered (Millipore type AA 0.8 μm) to remove precipitated degradation products and placed in a 250 ml flask in a water bath at 34°C. The solution was stirred at 150 rpm. Samples were withdrawn using a glass syringe, filtered (0.8 μm) to remove any precipitate and assayed against cloxacillin sodium standard in water.

pH 2 (with benzathine). 39.0 mg benzathine and 76.6 mg cloxacillin sodium were dissolved in 230 ml pH 2 buffer at 34°C, filtered (0.8 μm), then treated as above.

pH 6. 72.1 mg and 71.6 mg cloxacillin sodium were dissolved in two 100 ml volumes of pH 6 citrate buffer at 34°C. These solutions were placed in a water bath at 34°C and assayed against cloxacillin sodium standard dissolved in pH 6 citrate buffer, by HPLC.

pH 9. 326.1 mg cloxacillin were dissolved in 100 ml of borate buffer at 34°C. The solution was maintained at 34°C and assayed, after dilution (1 in 6) with water, against cloxacillin sodium standard dissolved in the same solvent.

Preparation of benzathine penicillin V. Solutions of 3.9 g penicillin V potassium dissolved in 700 ml of water and 1.8 g of benzathine diacetate dissolved in 100 ml water were slowly mixed with rapid stirring at ambient temperature. A fine precipitate immediately appeared. The mixture was left for 4 h, then filtered (0.8 μm) and dried in a vacuum desiccator. pH of the supernatant when filtered was 6.2. The yield after drying was 6.9 g.

Preparation of reaction product from benzathine and cloxacillin at pH 9. 2.5 g cloxacillin sodium and 1.8 g benzathine diacetate were dissolved in 900 ml and 100 ml, respectively, of pH 9 borate buffer. The two solutions were slowly mixed whilst being rapidly stirred, and then placed in a water bath at 34°C for 3 days. The precipitate produced was filtered (0.8 μm) and dried in a vacuum desiccator. The yield was 2.92 g and HPLC analysis indicated that this product was 95% pure, impurities being benzathine, cloxacillin and some degradation products of cloxacillin.

Reaction between benzathine and degraded cloxacillin at pH 9. 818 mg cloxacillin sodium were dissolved in 250 ml pH 9 borate buffer and left for 7 days at 34°C. HPLC analysis showed this solution contained 0.4% cloxacillin. 280 mg benzathine diacetate were added to 230 ml of this solution and the solution stirred at 150 rpm. Samples were taken at various time intervals and assayed, after dilution (1 in 6),

TABLE 1
PARTICLE SIZE DISTRIBUTION OF BENZATHINE CLOXACILLIN. MEAN OF 3 DETERMINATIONS

Sieve size (μm)	< 38	38	53	63	90	125	150	180	212	250	300	355	425	500
Weight retained (mg)	2	7	11	59	172	151	161	129	83	79	67	44	23	19
Confidence limits (95%)	2	5	10	24	27	18	38	38	18	15	23	16	7	9

against a series of standard solution (20–100%) from 25 mg benzathine diacetate in 100 ml sample solvent.

Calculations were undertaken in BASIC using the appropriate computer programs.

Mass spectra were determined by direct insertion probe using a Micromass MM12 mass spectrometer operated with a source temperature of 300 °C. an accelerating voltage of 3 kV and a trap current of 50 μA.

Results and Discussion

The benzathine cloxacillin was an amorphous material with a wide particle size range (Table 1). Approximately 30% of the material was within the range 125–180 μm which was selected for further study.

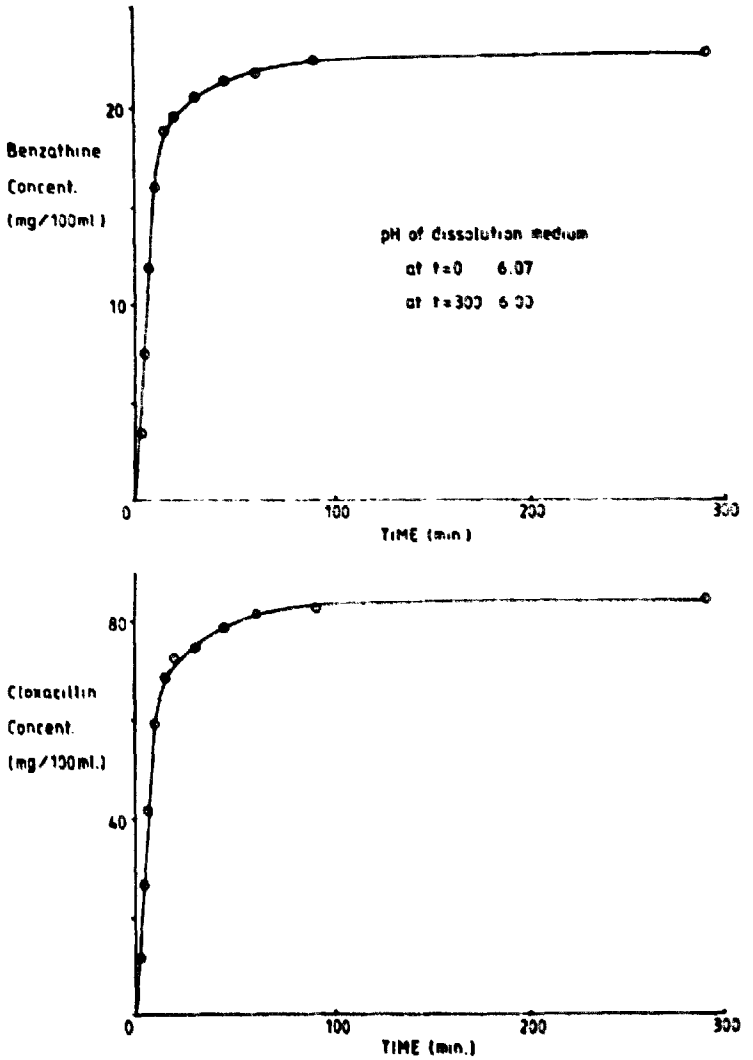


Fig. 2. Dissolution of benzathine cloxacillin at pH 6.0.

Dissolution at pH 6.0

The dissolution profile of benzathine cloxacillin at pH 6.0 is shown in Fig. 2. Individual levels of both benzathine and cloxacillin were followed and produce equivalent plots with equilibrium being attained after 300 min. This corresponds to 22.8 mg% (0.953 mM) benzathine and 85.0 mg% (1.877 mM) cloxacillin from the 1 : 2 complex. These data indicate a mean solubility of benzathine cloxacillin under the conditions to be 107 mg% (0.962 mM). Kinetic studies indicated that benzathine was stable at pH 6.0 and that cloxacillin degraded but slowly with a first-order degradation rate constant of 7.87×10^{-4} h^{-1}. A maximum fall in the cloxacillin concentration of 0.4% over the period of dissolution might be expected, a magnitude which causes little error in the estimation.

Benzathine cloxacillin (BP_2) is a 1 : 2 complex and the dissolution may be represented by Scheme 1:

$$\begin{array}{l} \quad B \overset{K_{a'}}{\rightleftharpoons} BH^{+} \overset{K_{a''}}{\rightleftharpoons} BH_2^{++} \\ BP_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} + \\ \quad 2P \overset{K_a}{\rightleftharpoons} 2P^{-} \end{array}$$

Scheme 1. Dissolution of benzathine cloxacillin at pH 6.0.

where B and P represent benzathine and penicillin, BH^+, BH_2^{++} and P^- are the ionized forms with ionisation constant $K_{a'}$, $K_{a''}$ and K_a.

The solubility of the complex will be determined by the solubility product (K_s), where B_s and P_s are the levels of unionised species at saturation:

$$K_s = [B_s][P_s]^2 \tag{1}$$

or

$$K_s = \alpha_B[B]\alpha_p^2[P]^2 \tag{2}$$

where α_B and α_P represent the fractions of unionised base and penicillin and B and P are the total amounts of each in solution.

An estimate of K_s may be obtained from the measured levels of total benzathine and cloxacillin in solution by calculating the fractions of ionized and neutral species (Martin et al., 1969) using the pK_a values of 9.2 and 6.2 for benzathine and 2.7 (Hou and Poole, 1971) for cloxacillin. This analysis gives values for unionized benzathine of 0.0398% and 0.0501% for unionized cloxacillin leading to an average value for K_s of 3.61×10^{-19} $mol^3 \cdot l^{-3}$.

The integrated form of the Noyes-Whitney equation (Notari, 1980) may be used to model the time course of dissolution. In the present example it is more appropriate to use the solubility product terms, K_s and the corresponding value K_t at time t, to estimate dissolution rates.

$$\ln\left[\frac{K_s}{(K_s - K_t)}\right] = k \cdot t \tag{3}$$

where k represents the apparent dissolution rate constant, but which is a composite term holding surface area, diffusion and volume components. When the data in Fig. 2 are treated in this way a linear plot is obtained from points collected after 10 min such that:

$$\ln\left[\frac{K_s}{(K_s - K_t)}\right] = 0.02663\,t + 0.4088 \qquad (r = 0.997,\ n = 7) \tag{4}$$

The dissolution rate constant is 0.02663 min^{-1} and the non-zero intercept probably represents a 'burst-effect' due to residual fines adhering to the main cut of particles.

Dissolution at pH 2.0

Dissolution of benzathine cloxacillin at pH 2.0 contrasted markedly to that at pH 6.0 with no steady-state levels being attained. Benzathine levels rose rapidly at first and then slowly increased throughout the run whereas, after an initial rapid increase, cloxacillin levels decreased with time (Fig. 3). To investigate the role of stability in

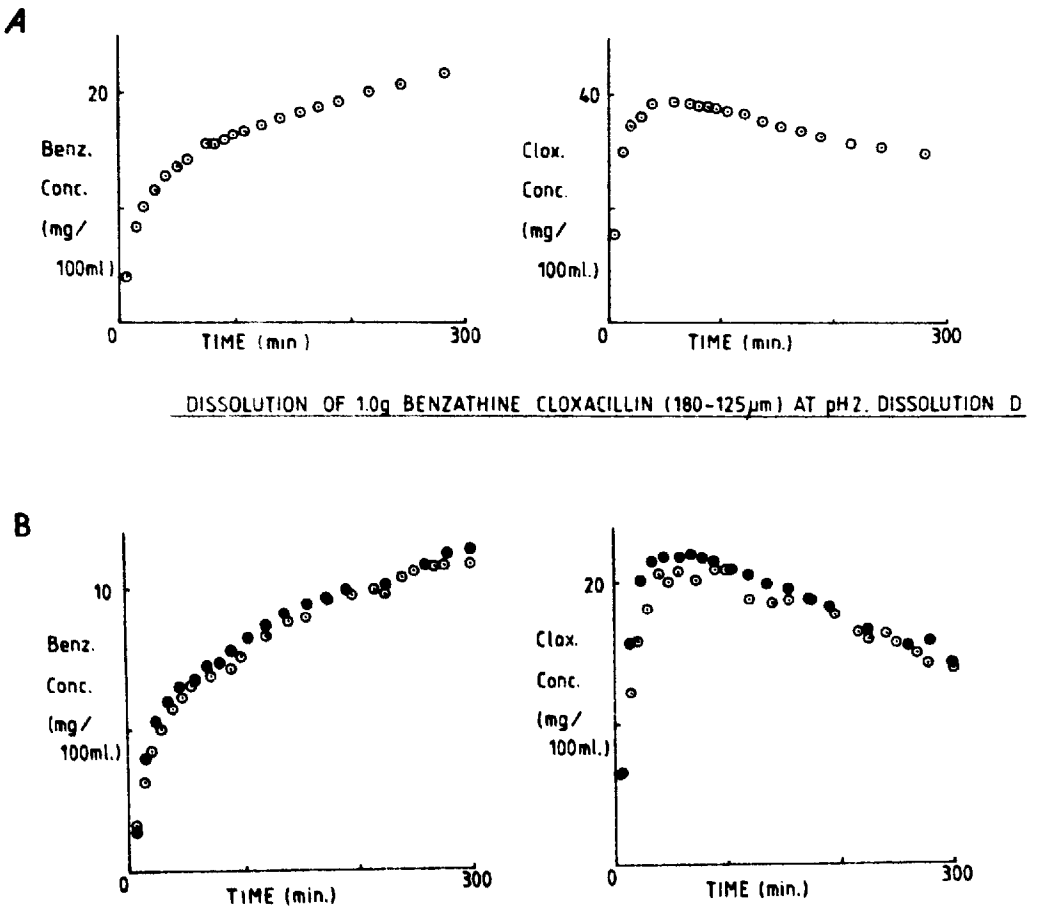


Fig. 3. Dissolution of benzathine cloxacillin at pH 2.0 (A, 1 g; B, 0.2 g, duplicate determinations).

this profile, the degradation of benzathine and cloxacillin was separately monitored in the pH 2.0 citrate buffer. Benzathine was stable under these conditions but cloxacillin, which undergoes first-order degradation in aqueous buffers between pH 1 and 11 (Bundgaard and Ilver, 1970), decomposed with precipitation to reveal a specific rate constant of 5.13×10^{-3} min^{-1}. When benzathine was added to this system, no significant change in the rate constant (5.07×10^{-3} min^{-1}) was observed.

The dissolution model in this case is better represented by Scheme 2

$$B \overset{K'_a}{\rightleftharpoons} BH^+ \overset{K''_a}{\rightleftharpoons} BH_2^{++}$$

$$BP_2 \underset{k_1}{\overset{k_1}{\rightleftharpoons}} + \qquad 2P \overset{K_s}{\rightleftharpoons} 2P^-$$

$$\downarrow k_2$$

$$D$$

Scheme 2. Dissolution and degradation of benzathine cloxacillin at pH 2.0.

to account for loss of cloxacillin (k_2) to degradation products (D). The concentration of cloxacillin does not reach a saturated equilibrium value at any point during the dissolution. This is because the dissolution of the complex is controlled by the

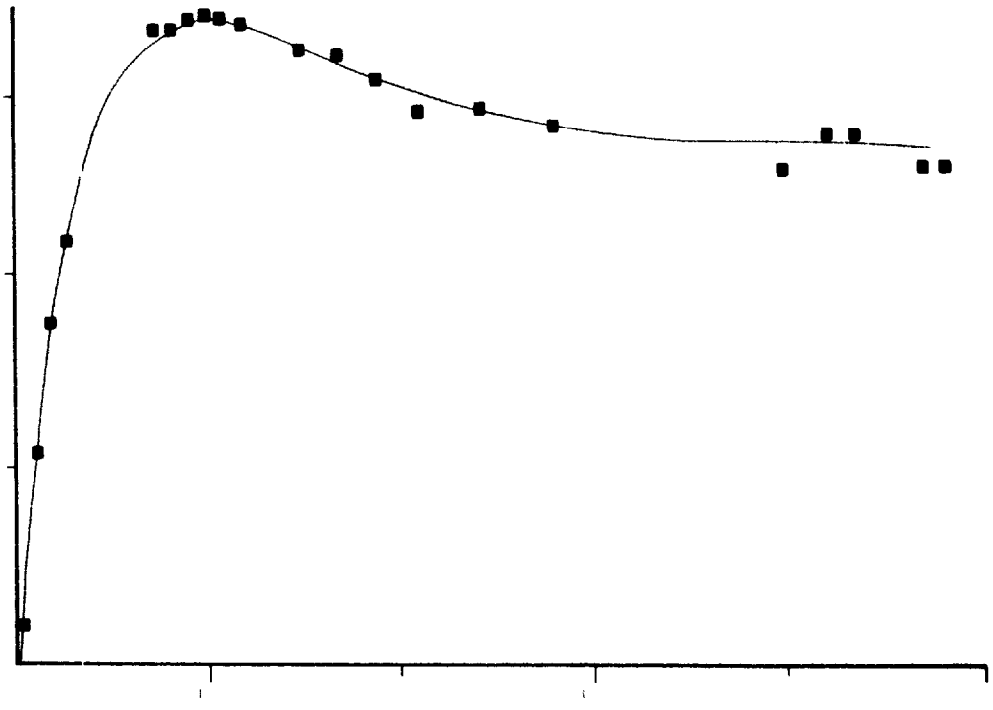


Fig. 4. Dissolution of benzathine cloxacillin at pH 2.0. Solubility product.

solubility product (K_s) as the dissolution proceeds, the level of benzathine increases progressively and so continually suppresses the level of cloxacillin which may simultaneously exist in solution.

This effect is illustrated in Fig. 4 which plots $K_t = [B_t][P_t]^2$ versus time for the data in Fig. 3A. The magnitude of K_t is difficult to assign for at pH = 2.0 benzathine is almost totally protonated and the level of residual free base cannot be calculated with certainty. Values of 3.98×10^{-10}% for free benzathine and of 83.36% for unionized cloxacillin have been used for further calculation, but at constant pH these represent scaling factors (Eqn. 2) and do not affect the overall shape of the curve. The slow fall in K_s values after 80 min is probably due to the secondary phenomenon of cloxacillin degradation which leads to precipitation of hydrolysis products during dissolution. This effect is revealed in Fig. 3B which shows that the peak levels of benzathine and cloxacillin are dependent upon the initial amount of complex with a smaller charge producing significantly lower levels of dissolved species. This phenomenon may be due to the coating of benzathine cloxacillin particles by less soluble degradation products, a process which reduces the surface area available for dissolution.

The level of benzathine assesses the extent of dissolution of the complex and may be used to estimate the total amount of cloxacillin released. The level of total cloxacillin ($[P^0]_t$) released after time t may be modelled by fitting an eleventh-order polynomial in time to the calculated, total cloxacillin levels:

$$[P^0]_t = 0.02202 + 0.7564\,t + 0.002742\,t^2 - 4.631 \times 10^{-4}\,t^3 + 1.006 \times 10^{-5}\,t^4 - 1.130 \times 10^{-7}\,t^5 + 7.637 \times 10^{-10}\,t^6 - 3.223 \times 10^{-12}\,t^7 + 8.321 \times 10^{-15}\,t^8 - 1.204 \times 10^{-17}\,t^9 + 7.472 \times 10^{-21}\,t^{10} \quad (5)$$

The residual, measured concentration ($[P]_t$), determined by degradation, may then be estimated from:

$$[P]_t = \mathrm{EXP}\left[\ln\left([P]_{t-1} + [P^0]_t - [P^0]_{t-1}\right) - k_2 t\right] \quad (6)$$

The cloxacillin levels calculated from this expression using 1 min intervals between the consecutive t and t − 1 points show an excellent fit between the theoretical and measured data. A value of k_2 corresponding to the first-order degradation rate constant for cloxacillin in solution (5.1×10^{-3} min^{-1}) confirms that the dissolution process is consistent with the mechanism proposed in Scheme 2.

Dissolution at pH 9.0

The dissolution profile of benzathine cloxacillin in borate buffer at pH 9.0 is illustrated in Fig. 5A. Wetting of the solid was noticeably more rapid than at lower pH values and solution of 1 g of complex in 230 ml of solvent, leaving a hazy solution, was effected within 1 h.

The maximum levels of cloxacillin and benzathine represented 91.3% and 90.6%

of the initial complex and both levels fell after solution was observed. Cloxacillin, in the absence of benzathine, was found to degrade with a first-order degradation rate of 5.70×10^{-4} min^{-1} under these conditions but benzathine alone was found to be stable although it disappeared at a rate faster than cloxacillin in the dissolution experiment. Crystalline benzathine cloxacillin was prepared from benzathine diacetate and cloxacillin sodium and dissolution of this material was followed but no significant difference between the profiles was evident. This eliminates solubility variation due to different physical forms.

As the dissolution experiment progressed, a long-retention time peak (Fig. 6) developed and, after some 2 h, opalescence and precipitation began. This phenome-

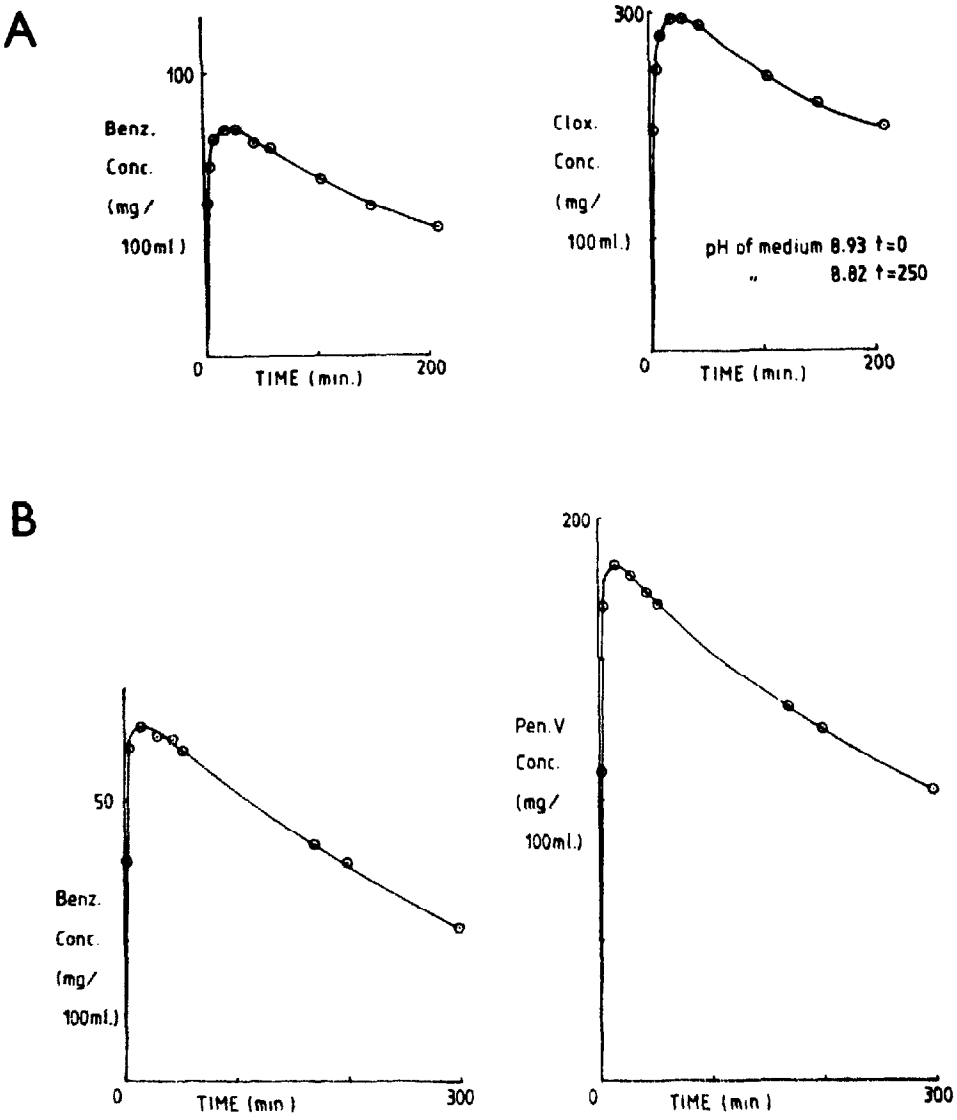


Fig. 5. Dissolution of benzathine penicillin complexes at pH 9.0 (A, benzathine cloxacillin; B, benzathine penicillin V).

non was also observed when benzathine and cloxacillin were dissolved in buffer and allowed to react but no significant loss of benzathine was observed when degraded cloxacillin was used. This suggests that reaction between benzathine and the lactam ring of cloxacillin is evident. Specific interactions with the side-chain of cloxacillin were not involved as benzathine penicillin V displayed a similar dissolution profile (Fig. 5B) and the development of a later peak in the chromatogram. In this instance, however, no precipitation was evident.

The isolated cloxacillin–benzathine reaction product melted at 175°C and displayed significant infrared absorption in the 2200–3500 cm^{-1} range indicating the presence of COOH and NH vibrations. The β-lactam absorbance band of cloxacillin, found in both sodium salt and benzathine complex at 1770 cm^{-1}, was absent. Mass spectrometry was consistent with a 1 : 1 amide structure involving the attack of a benzathine nitrogen atom at the β-lactam carbonyl group (Fig. 7), although the isomeric penamaldic acid amide cannot be excluded at this stage. The nucleophilic attack of the amine at the β-lactam carbonyl group is presumably promoted by the significant fraction of free base present at this higher pH.

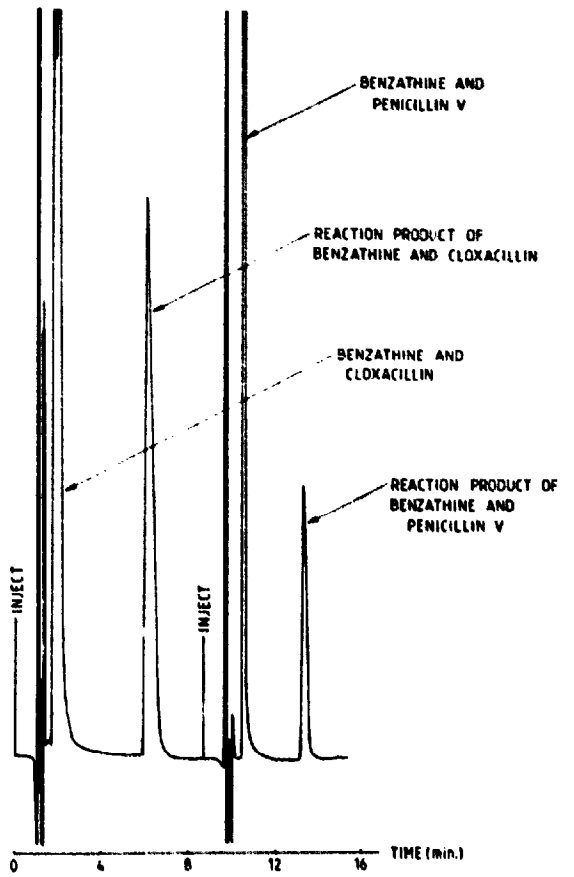


Fig. 6. HPLC of reaction products of benzathine with cloxacillin and penicillin V.

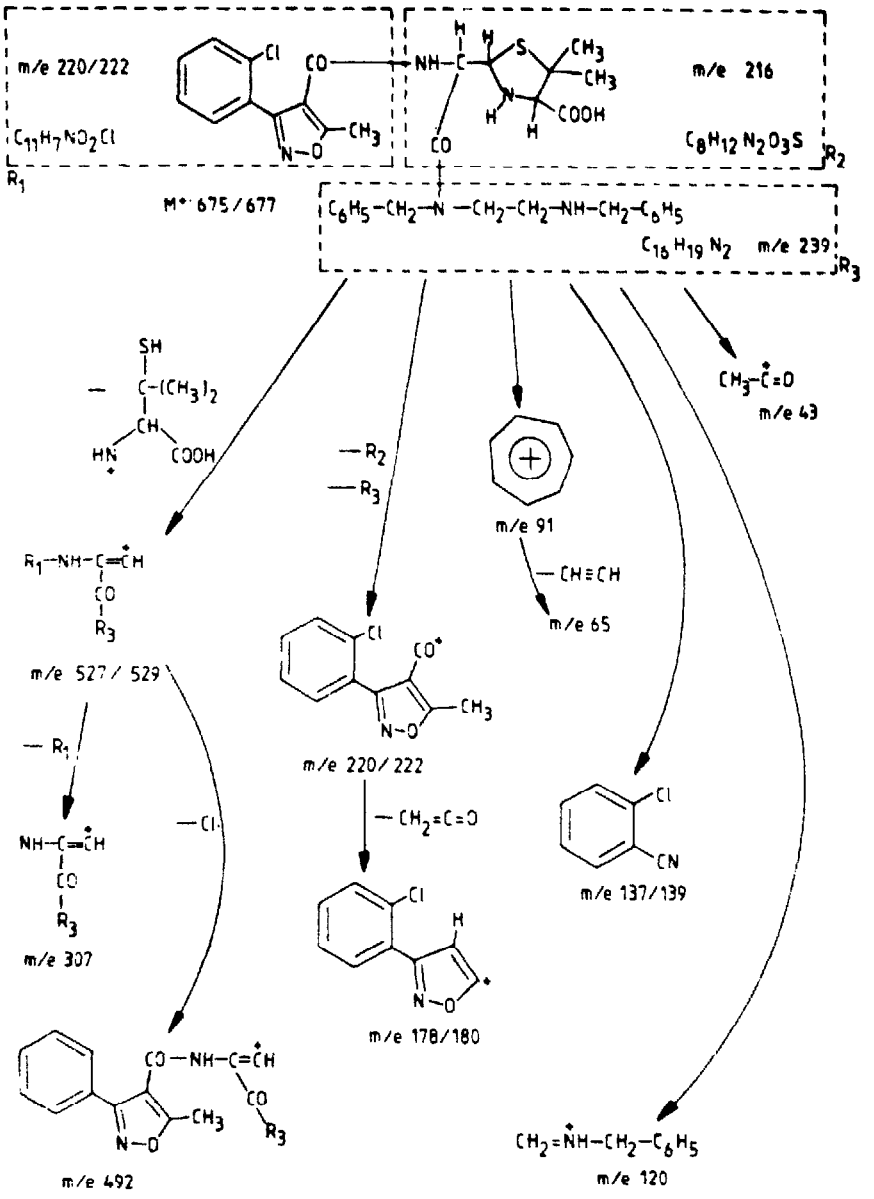
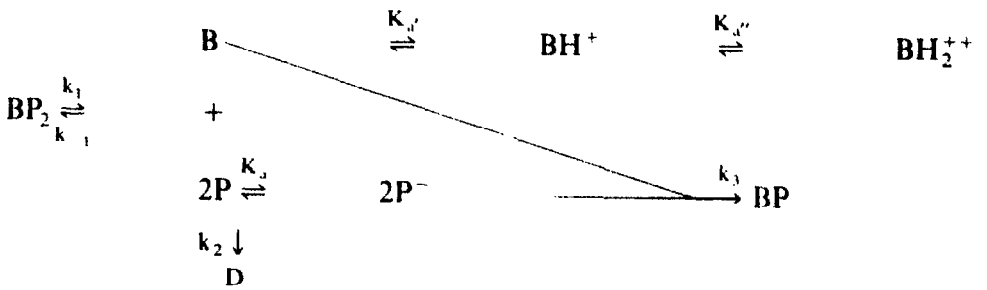


Fig. 7. Mass spectrometric fragmentation of benzathine-cloxacillin reaction product.

The overall dissolution process now may be represented by:



Scheme 3. Dissolution, degradation and reaction of benzathine cloxacillin at pH 9.

where BP represents the 1:1 reaction product and k_3 is the second-order rate constant for the production of BP.

This complex situation may be simplified for the purposes of the present discussion because the dissolution rates are much greater than the degradation rates and the reaction profile follows that of the solution experiments closely.

In this case, the degradation may be represented by Scheme 4:

$$\begin{array}{l} B + P \xrightarrow{k_2} BP \\ \quad\downarrow k_1 \\ \quad D \end{array}$$

Scheme 4. Reaction of benzathine and penicillin in solution at pH = 9.0.

The rate of disappearance of each species is now:

$$-\frac{dB}{dt} = k_2[B][P] \tag{7}$$

$$-\frac{dP}{dt} = k_2[B][P] + k_1[P] \tag{8}$$

and division to eliminate time yields:

$$\frac{dP}{dB} = \frac{k_2[B] + k_1}{k_2[B]} = 1 + \frac{k_1}{k_2[B]} \tag{9}$$

Separation of variables and summation to current levels of B and P leads to:

$$\int_{P_0}^{P} dP = \int_{B_0}^{B} dB + \frac{k_1}{k_2}\int_{B_0}^{B} \frac{dB}{[B]} \tag{10}$$

giving:

$$(P - P_0) = (B - B_0) + \frac{k_1}{k_2}\ln\left(\frac{B}{B_0}\right) \tag{11}$$

which may be linearized to:

$$(P - B) = \frac{k_1}{k_2}\ln(B) - \frac{k_1}{k_2}\ln(B_0) + P_0 - B_0 \tag{12}$$

A plot of ln(B) against (P − B) is thus expected to be linear with a slope providing the ratio k_1/k_2.

To test this result, the reaction in solution of benzathine diacetate (276 mg; B_0 = 3.33 mM) and cloxacillin sodium (752 mg; P_0 = 7.139 mM) was monitored and the levels of cloxacillin and benzathine were measured over a period of 260 min (Fig.

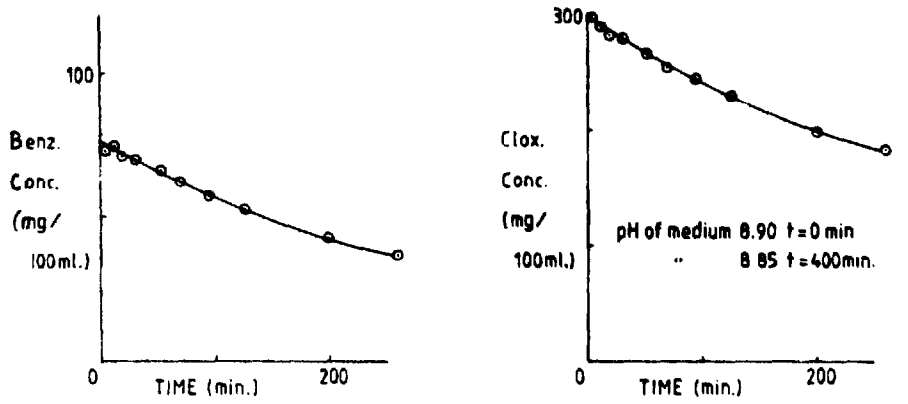


Fig. 8. Reaction profile of benzathine and cloxacillin in solution at pH 9.0.

8). These data indeed followed equation 12 and provided a slope of 1.255×10^{-3} mol $\cdot$ l^{-1} ($r = 0.994$, n = 10) from which the second-order rate constant of 0.4542 l $\cdot$ mol^{-1} $\cdot$ min^{-1} was determined, using the previously measured first-order rate constant of 5.70×10^{-4} min^{-1}. The calculated intercept of 1.0964×10^{-2} also compares favourably with the value of 1.0764×10^{-2} obtained from the linear regression analysis.

When the data from the dissolution experiments, using those points collected after complete dissolution was observed (ca. 45 min), were calculated in this way analogous results were obtained. The profiles in Fig. 5A led to an intercept of 1.087×10^{-2} and a slope of 1.231×10^{-3} mol. l^{-1} ($r = 0.991$, n = 7). This provides a value for the second order degradation rate constant of 0.463 l mol^{-1} $\cdot$ min^{-1}, a value in reasonable agreement with that obtained in the solution experiment.

Acknowledgements

We thank the SERC for provision of HPLC facilities and the SERC and Beechams Pharmaceuticals for the award of a CASE Studentship to J.M.H.

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