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Dissolution studies on ampicillin embonate and amoxycillin embonate

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Abstract: The dissolution behaviour of slightly water-soluble embonic acid salts of ampicillin and amoxycillin was studied quantitatively as a function of solution pH (1.15–8.00) using the rotating disk method combined with reversed-phase high-performance liquid chromatography. The dissolution rate of ampicillin embonate was greater than that of amoxycillin embonate at all pH values investigated. The graphs of pH-intrinsic rate of dissolution of the antibiotics from the salts were U-shaped, the minimum being close to the respective isoelectric points of the parent antibiotics. Embonic acid was not detectable below pH 5; above pH 5 the dissolution rate of embonic acid increased as a function of pH.

Keywords: *Ampicillin embonate; amoxycillin embonate; dissolution; rotating disk technique; reversed-phase HPLC.*

Introduction

Ampicillin and amoxycillin are amphoteric, very acid-stable penicillins that exhibit a broad spectrum of antibacterial activity. The main drawbacks to their use orally are the bitter taste and fast degradation in suspensions; in addition, these drugs often cause diarrhoea and allergic reactions. Almost water-insoluble embonic acid salts of ampicillin and amoxycillin (1:2) have been prepared to overcome the undesirable features of the parent penicillins (Fig. 1) [1, 2].

The use of a salt form of the drug, when designing a bioavailable dosage form, depends on the physicochemical properties of the salt, especially the dissolution rate and/or solubility. Inorganic salts have been commonly used to increase the solubility and bioavailability of the parent drugs; organic salts are often less soluble in water than are the parent drugs, and therefore are even more slowly absorbed [1, 3, 4]. However, some organic salts of drugs are freely or even highly water-soluble [5–9]; an increased or equal bioavailability has been described for some organic salts, compared with that of the parent drug [10–14].

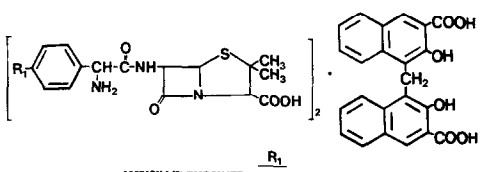

Figure 1
The structure of ampicillin embonate and amoxycillin embonate.

In addition to equilibrium solubility, the dissolution behaviour of organic and inorganic salts of organic drugs has been determined using, for example, the stoichiometric solubility method [5, 15] or the rotating disk method [16–19]. The relative solubilities or intrinsic rates of dissolution of the salts can be determined by these methods. Because of the instability of β-lactam antibiotics in water, however, the determination of the equilibrium solubility or the stoichiometric solubility of these antibiotics or their salts is not possible. The pH-dependent solubility of β-lactam antibiotics has been determined by measuring the amount of antibiotics dissolved within 2 h [20, 21].

In the present study, the dissolution behaviour of ampicillin embonate, amoxycillin embonate and amoxycillin trihydrate (reference compound) were examined using a fast, rotating disk method to avoid the error arising from degradation of the penicillins. The amounts of both salt components which dis-

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solved were assayed by a high-performance liquid chromatographic (HPLC) method earlier developed for ampicillin embonate and amoxycillin embonate [22].

Experimental

Materials

Amoxycillin trihydrate (Amphar BV, Amsterdam, Holland), ampicillin trihydrate (Fermion OY, Helsinki, Finland) and phenoxymethylpenicillin potassium (internal standard) (Biochemie, Kundel-Tirol, Austria) were kindly supplied by Orion Pharmaceutica. Embonic acid (99%) was obtained from Ega-Chemie (Steinheim, FRG). All other chemicals were of commercial analytical grade. Identification of the compounds and methods of preparation for ampicillin embonate (2:1) and amoxycillin embonate (2:1) have been reported previously [2].

Determination of dissolution rate

Dissolution from a constant surface area was studied using a slightly modified procedure of Nogami *et al.* [23]. Disks of 1.3-cm dia. weighing 200 mg were prepared by compressing the crystalline powders at about 225 MPa in a hydraulic press. Adhesion of the materials was reduced by lubricating the mould with toluene saturated with magnesium stearate. According to the IR spectra determined after compression (SP3-200 IR spectrophotometer, Pye Unicam), phase conversion had not occurred. The disk was fixed to a rotating shaft (114 rpm) with solid paraffin adhesives. The disk was submerged in 200 ml of solution to a depth of 4 cm below the solvent level in a 400-ml beaker. The temperature of each medium (Sörensen phosphate buffers pH 4.8–8.0, Clark and Lubs buffers pH 1.15–2.2 and HCl solutions pH 2.3–3.0) was maintained at 37°C in a water-bath. The ionic strength of the solutions was adjusted to 0.2 M with potassium chloride, and the pH was checked before and after the analysis with a pH meter (PHM 83 Autocal pHMeter, Radiometer). The solutions were sampled (5 ml) periodically (2 or 4 min); the same volume of the same solvent, preheated to 37°C, was added immediately after sampling. After cooling to room temperature, neutralization of acidic samples and appropriate dilutions with the same solvent, the penicillins and embonic acid were analysed on an RP-8 column at 240 nm using methanol–Sörensen phosphate buffer (pH 7.0) (38:62, v/v) as the mobile phase [22].

Results and Discussion

Intrinsic rates of dissolution are generally used as one of the criteria for salt selection during preformulation studies on drug substances. According to previous reports, however, intrinsic rates of dissolution tend to exaggerate the actual differences between the salts compared with the dissolution rates of their respective capsule or tablet formulations [16, 18]. Data for the intrinsic rates of dissolution for salts and parent drugs should therefore be evaluated very carefully.

Methods used for the determination of intrinsic rates of dissolution of salts formed from two organic substances have not been selective, e.g. merely recording the combined absorbance of both of the dissolved salt components [18]. In some cases the salt-forming agent may have a much larger molar absorptivity than the parent drug at the wavelength of measurement as well as different absorptivities at different pH values. This gives a misleading picture of the dissolution of the effective component of the salt. In the present study, for example, the salt forming agent, embonic acid, has a much higher absorption at all UV wavelengths than do ampicillin and amoxycillin; at 240 nm the molar absorptivity ratios of embonic acid to amoxycillin trihydrate and embonic acid to ampicillin trihydrate were about 10 and 45, respectively.

As the dissolution of the effective component is of primary importance, reliable determination of the dissolution rate of the organic salts of drugs requires either an analytical method that can simultaneously separate and detect the dissolved salt components, or specific analytical methods for the components after chemical separation. It is difficult, owing to the amphoteric character of ampicillin and amoxycillin, to extract quantitatively the embonate salt components for separate chemical analysis. Moreover, the solubilities of embonic acid and its antibiotic salts are very low in all common solvents. The developed HPLC method offered a very rapid analytical method for the separation and determination of very unstable penicillins and slightly soluble embonic acid after the dissolution process.

The structure of the disks was such that no

flaking or capping occurred during the dissolution test and the amount dissolved did not exceed half the weight of the disk; thus the surface area of the disks could be regarded as remaining constant, and the method was considered to be suitable for determining the intrinsic dissolution rates of the compounds investigated. The buffering capacity of all the dissolution media was sufficient because the pH remained practically unchanged throughout the process.

Dissolution studies were not performed at pH 3.5–4.5 owing to the lack of a suitable buffer solution. The amounts of antibiotics which dissolved within this pH range were very small and the absorption of the buffer components (the buffers of Clark and Lubs and of McIlvaine, were tested) disturbed the integration of the chromatograms of the antibiotics.

The amount dissolved within 0.5 h and the dissolution rate of ampicillin embonate were greater than those for amoxycillin embonate at all the pH values investigated (Figs 2 and 3). The results were in agreement with the ranking of the solubilities (after 2 h) of the parent antibiotics presented by Tsuji *et al.* [21]. It should therefore be possible to predict the relative solubilities of β-lactam antibiotics and their salts using their apparent solubility after 0.5 h; the error arising, for example, from rapid polymerization of the antibiotics [24, 25] can be minimized by shortening the time of dissolution.

The graphs of pH-solubility and pH-intrinsic rate of dissolution of the antibiotics from the salts were U-shaped, the minimum being close to the respective isoelectric points of the parent antibiotics (Figs 2 and 3) [21]. At pH 7.0 the amount of amoxycillin which dissolved from amoxycillin embonate was very close to that of amoxycillin trihydrate, even though the relative area occupied by amoxycillin in the amoxycillin embonate disk should be two-thirds of that in the amoxycillin trihydrate disk. It would appear that the rapid dissolution of embonic acid at this pH (Fig. 4) promotes the dissolution of amoxycillin from the salt. The solubility and dissolution rate of embonic acid increased as a function of pH above pH 5; embonic acid was not being detected below pH 5. When the amounts of antibiotics that dissolved from the disks at a pH below 3 (Fig. 2) are compared, it would appear that the insolubility of embonic acid decreases the dissolution

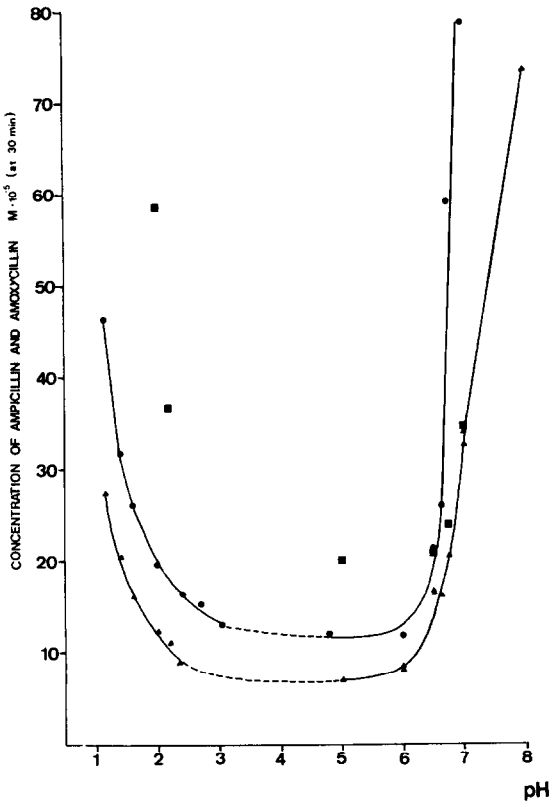

Figure 2
pH–apparent solubility profiles of penicillins. Ampicillin embonate (●); amoxycillin embonate (▲); and amoxycillin trihydrate (■).

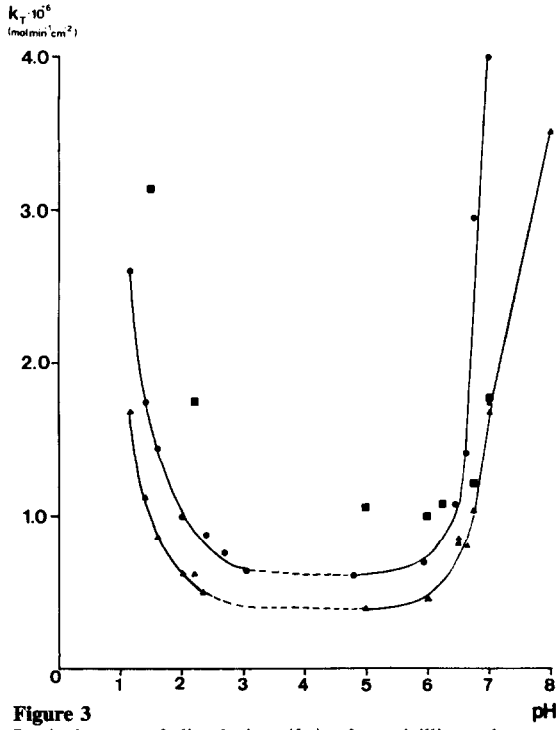

Figure 3
Intrinsic rate of dissolution (k_T) of ampicillin embonate (●), amoxycillin embonate (▲) and amoxycillin trihydrate (■) at pH 1.15–8.0 (calculated from the amounts of antibiotics dissolved).

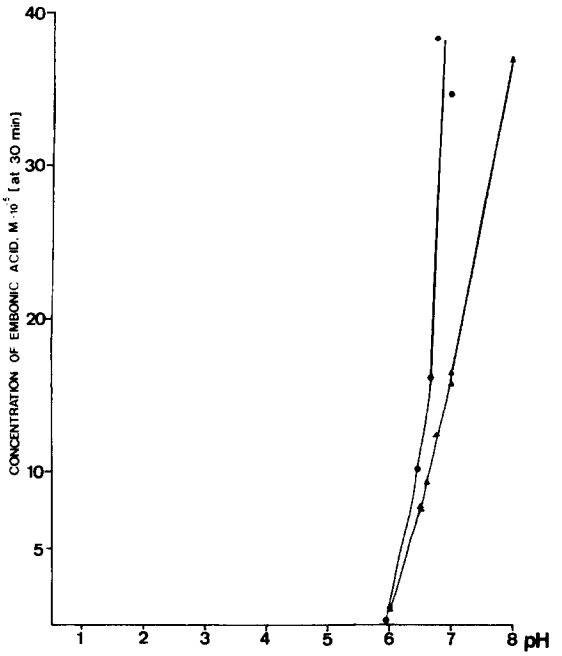

Figure 4
pH–apparent solubility profiles of embonic acid. Ampicillin embonate (●) and amoxycillin embonate (▲).

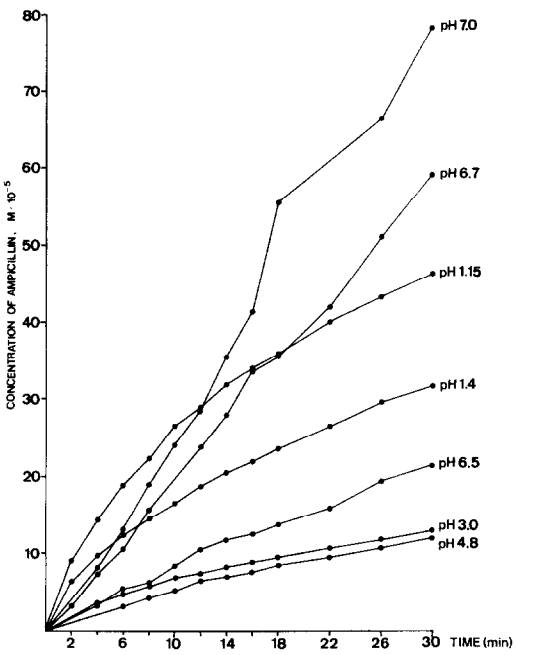

Figure 5(a)
Dissolution of ampicillin embonate at pH 1.15–7.0.

of the antibiotics. However, the amounts of antibiotics that dissolved from the salts were about the same at pH 1.6 and 6.5; the barrier of undissolved embonic acid did not appear to prevent dissolution of the antibiotic component of the salts. The basic character of the antibiotics is obviously strong enough to effect their release from these salts. According to the cumulative plots of the dissolution tests (Fig. 5), the dissolution rate of the antibiotics from the salt disks at acidic pH values decreases during the first 14 min, but it continues at a constant rate. The dissolution rate of the antibiotics is practically constant not only within the range of the isoelectric points, where the solubility of both embonic acid and the antibiotics is very low, but also at pH 6.0 and above, where embonic acid begins to dissolve rapidly.

Table 1 illustrates the dissolution of both salt components as a function of time at two different pH values. At pH 7.0 the components are released from the ampicillin embonate and amoxycillin embonate disks in proportions close to their stoichiometric ratio (embonic acid/antibiotic), i.e. a mean ratio of 0.52 and 0.48, respectively. At pH 6.5 the corresponding ratios are 0.41 and 0.45; the difference between these values appears to be due to the higher solubility of ampicillin.

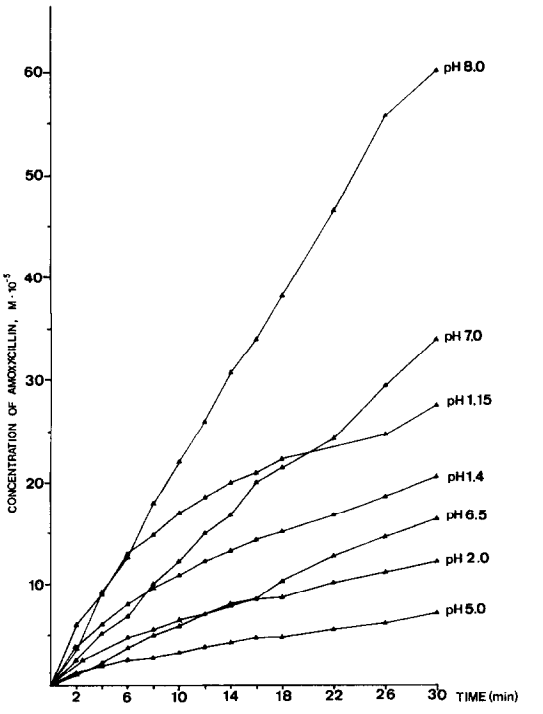

Figure 5(b)
Dissolution of amoxycillin embonate at pH 1.15–8.0.

Table 1
Amounts (mol $l^{-1} \times 10^{-5}$) of ampicillin (**I**), amoxycillin (**II**) and embonic acid (**III**) that dissolved from the disks after 4, 10, 16, 22 and 30 min at pH 6.5 and 7.0

	Time (min)	Ampicillin embonate I	Ampicillin embonate III	Amoxycillin embonate II	Amoxycillin embonate III
pH 6.5					
	4	3.2	0.9	2.2	0.9
	10	8.3	3.1	5.8	2.5
	16	12.1	5.3	8.6	4.1
	22	15.8	7.5	12.7	6.0
	30	21.4	10.3	16.4	7.8
pH 7.0					
	4	8.2	4.5	5.2	2.5
	10	24.1	12.8	12.3	5.8
	16	41.4	22.1	20.1	9.3
	22	64.6	30.2	24.4	12.4
	30	78.4	41.7	34.0	16.5

The primary goal of this study was to determine the extent to which the embonic acid component of the salt decreases the release of ampholytic β-lactam antibiotics, ampicillin and amoxycillin, from their embonic acid salts, particularly in an acidic solution. By combining HPLC with the rotating disk method the dissolution behaviour of these salts was determined quantitatively, thus providing information about both the dissolving compounds. The results showed that the antibiotics dissolved from the salts within the physiological pH range to give a U-shaped graph of pH-intrinsic rate of dissolution for both the antibiotics investigated in spite of the fact that embonic acid does not dissolve below pH 5.

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