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Dissolution and bioavailability of the anhydrate and trihydrate forms of ampicillin

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The solubilities and intrinsic dissolution rates of ampicillin anhydrate and trihydrate in distilled water and dilute hydrochloric acid at 37° have been measured. The dissolution rates of these materials from loose filled hard gelatin capsules were determined and the *in vivo* bioavailabilities of the compounds compared. Small differences in the *in vitro* behaviour of the anhydrate and trihydrate forms were recorded but the *in vivo* availability of the two compounds was the same.

Ampicillin occurs in crystalline anhydrate and trihydrate forms (Austin, Marshall & Smith, 1965). The biological availability after oral administration to animals and man has been reported to be related to the hydrated state of the ampicillin molecule (Poole & Bahal, 1968; Poole, Owen & others, 1968). An enhanced *in vivo* availability of the anhydrated product is claimed to be due to its greater solubility and dissolution rate in distilled water.

Ampicillin is amphoteric and sparingly soluble in water (solubility of anhydrate 10 mg ml^{-1} and trihydrate 8 mg ml^{-1} in distilled water at 37°, Poole & others, 1968). In dilute hydrochloric acid however, the hydrochloride formed has an increased solubility and dissolution rate, and dissolution of ampicillin in gastric juice should therefore be rapid and not rate limiting in gastrointestinal absorption.

We have measured the solubilities and intrinsic dissolution rates of the anhydrate and trihydrate forms in distilled water and dilute hydrochloric acid at 37° and their dissolution rates from loose filled gelatin capsules. We also compared their *in vivo* bioavailabilities.

MATERIALS AND METHODS

Materials

Ampicillin trihydrate was from a batch purified for analytical reference. The anhydrate was prepared by refluxing trihydrate from this batch in a mixture of 15% v/v water in isopropanol. The cooled suspension was filtered, and the precipitated anhydrate was washed in isopropanol and dried under vacuum at room temperature. Both materials were micronized and their particle size distributions determined. The identity of the samples was investigated by X-ray diffraction and infrared spectroscopy.

Methods

1. *Identification.* X-ray diffraction patterns were obtained on a Hilger and Watts diffractometer, using nickel-filtered Cu K α radiation and a proportional counter and infrared spectra on a Hilger and Watts 'Infrascan' spectrophotometer, using a 1% potassium bromide disc.

2. *Particle size distribution.* A Coulter Counter model A was used, with a 5% solution of ammonium thiocyanate in isopropanol as suspending medium. The samples were sized using the 100 μm aperture.

3. *Solubility.* The solubilities of anhydrate and trihydrate were determined in

distilled water and 0·053 N hydrochloric acid (pH 1·2) at 37° ± 0·5°. An excess of powder was stirred in the solvent for 3 h. At suitable times the supernatant was sampled and assayed for penicillin content by the acid degradation method (Smith, De Grey & Patel, 1967). In water, saturation was reached in 1 h, the concentrations remaining unchanged over the remaining 2 h. As slight decomposition occurred in the acid solution, solubility was obtained by plotting the logarithm of the concentration against time, and extrapolating to zero time.

4. *Intrinsic dissolution.* The intrinsic dissolution rates were measured by a method based on that of King (1935). A disc of the test material was prepared by compressing the powder in a 13 mm diameter die using 6000 kg cm^{-2} pressure. The disc was attached to a Perspex plug by a suitable adhesive and the plug was inserted into the wall of a dissolution cell (see Fig. 1). In position, the surface of the disc was flush with the internal surface of the cell. The cell assembly was mounted horizontally in

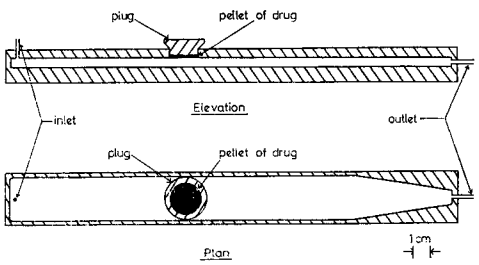

FIG. 1. Flow cell used for the determination of intrinsic dissolution rates.

a water bath at 37° ± 0·5°, and distilled water or 0·053 N hydrochloric acid at 37° was passed through the cell at a controlled rate. The solvent flow was seen to be laminar across the surface of the disc by injection of dye into the solvent stream. The effluent was assayed by measuring the absorbance at 262 nm on a Unicam SP 500 spectrophotometer, with reference to a standard curve. The dissolution rates (μg cm^{-2} s^{-1}) were independent of solvent flow rate (ml min^{-1}) over the range studied (for flow rates of 19·4, 12·4, 11·5 and 6·8 the respective dissolution rates were 35·8, 35·4, 35·4 and 35·7) and values were obtained at two convenient rates and averaged. The intrinsic dissolution values were calculated from the surface area of the disc, the flow rate of the solvent and the equilibrium concentration of the effluent solution.

5. *Dissolution from capsules.* The apparatus used for capsule dissolution measurement was similar to that described by Poole & others (1968). It consisted of a 1 litre round-bottomed flask (Quickfit No. FR 1LF) immersed in a water bath at 37° ± 0·5°. A stirrer, fitted with twin-bladed Teflon paddles set at right angles, one at the bottom of the shaft, the other 6 cm higher, was positioned centrally in the flask and the height was adjusted so that the top of the uppermost paddle was just below the surface of the liquid. Powder equivalent to 250 ± 5 mg of ampicillin was loosely-filled by hand into size 0 hard gelatin capsules, and the content confirmed by microbiological assay. Single capsules were fitted into a loose stainless steel wire coil and dropped into the flask containing 1 litre of solvent preheated to 37°. The stirrer was rotated at 30 rev min^{-1} and the solvent circulated continuously at about 100 ml min^{-1} through a glass fibre filter, then through a flow-cell of a Unicam SP.500 spectrophotometer, and back into the flask. The absorbance of the solution at 262 nm was recorded continuously and the dissolution rates calculated from the tracings.

6. *In vivo absorption.* 21 male and 6 female healthy volunteers were selected for assessing bioavailability. Ages ranged from 21 to 59 years and body weights from 51 to 99 kg. A random cross-over design was used.

The subjects fasted overnight and were dosed in the fasting state from 9.00 a.m. on the day of the study. The time of dosing was staggered to ensure an accuracy of ± 1 min in the sampling time. 300 ml of fluid was given at the time of dosing and a further 300 ml at the 2 and 4 h intervals. A standard breakfast was taken after the 2 h blood sample.

Each subject received one capsule from the batch prepared for the dissolution tests described. Blood samples were withdrawn at zero time and at the 0·33, 0·67, 1·0, 1·5, 2·0, 4·0 and 6·0 h intervals. The volunteers emptied their bladders before the test and all urine was collected from the time of dosing over the 6 h period.

The blood samples were allowed to stand for 1 h before the serum was separated. The concentrations of ampicillin in the serum and in the urine specimens were determined microbiologically by a conventional large-plate agar diffusion method similar to that of Knudsen, Rolinson & Stevens (1961). All assays were plated within 3 h of sampling and the plates were read 18 h later.

RESULTS

1. *Identification.* The infrared absorption spectra corresponded with those of the trihydrate and anhydrate published by Austin & others (1965).

The X-ray diffraction pattern for the trihydrate resembled that published by Shefter, Fung & Mok (1973). The diffractogram of the anhydrate was the same as that of the form I anhydrate polymorph described by these authors; this form they found to be stable in aqueous suspension for two days, while a second polymorph converted to the trihydrate very rapidly (during preparation of the slurry). In the solubility determination in the present study, the anhydrate sample was stable for up to 3 h, and this further supports its identification as the anhydrate polymorph form I. The anhydrate used by Poole & Bahal (1968) and Poole & others (1968) was stable in water over several hours, and may therefore also be the same form I polymorph.

2. *Particle size distributions.* These are shown in Fig. 2. The distributions were narrow, and the weight undersize curves were almost identical, with a mean average diameter of 6·5 μm.

3. *Solubility and intrinsic dissolution rate.* The results are shown in Table 1. The solubility and intrinsic dissolution rate of each antibiotic in distilled water were low

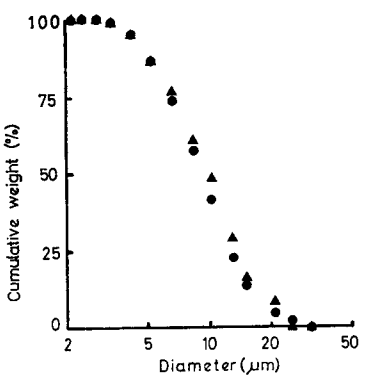

FIG. 2. Particle size distribution of ampicillin anhydrate (▲) and ampicillin trihydrate (●).

and the solubility values agreed closely with previously reported figures (Poole & others, 1968). The solubility and dissolution rate were many times greater in dilute hydrochloric acid than in distilled water. The solubility and intrinsic dissolution rate of the trihydrate expressed in terms of anhydrous material were less than those for the anhydrate in both solvents.

4. *Dissolution rate from capsules.* The dissolution rates of anhydrate and trihydrate from loose-filled gelatin capsules were: half lives (with s.d.) in water anhydrate 18·1 (1·0) and trihydrate 23·8 (4·2) and in dilute HCl anhydrate 3·6 (0·25) and trihydrate 3·9 (0·36) n = 6 in all cases. Dissolution followed first order kinetics in both solvents after a lag period due to the dissolution of the capsule shell, but the rates were governed by the nature of the solvent. In water, the dissolution rate of the trihydrate was slow compared with that of the anhydrate and the capsule to capsule variation was high. In acid solution, the dissolution of both forms was rapid and the behaviour of the two materials was identical.

Table 1. *Solubility and intrinsic dissolution rates of ampicillin anhydrate and ampicillin trihydrate at 37° ± 0·5°.*

	Water			0·053 N Hydrochloric acid		
	Ampicillin anhydrate	Ampicillin trihydrate	Trihydrate as anhydrous free acid	Ampicillin anhydrate	Ampicillin trihydrate	Trihydrate as anhydrous free acid
Solubility in mg ml^{-1}	10·2	8·4	7·0	34·5	35·1	29·5
* Intrinsic dissolution rate in $\mu g\ cm^{-2}\ s^{-1}$	3·94	3·85	3·23	35·0	32·6	27·4
(standard deviation)	(0·06)	(0·07)	(0·07)	(0·3)	(0·1)	(0·1)

* Mean values of 4 determinations each conducted at two flow rates.

5. In vivo *availability.* The results for the bioavailability of the anhydrate and trihydrate from loose-filled gelatin capsules are shown in Table 2. The results were submitted to analysis of variance, and no significant difference was found between the two presentations in the mean serum concentrations at any sampling time, or in the mean percentages of dose recovered from the urine, at the 5% level. This was true also of the means of individual peak serum concentrations, which had the values 3·69 (standard deviation 1·71) $\mu g\ ml^{-1}$ for the anhydrate, 4·00 (1·81) for the trihydrate. The means of individual peak times were almost identical, 91 (19) min for the anhydrate, and 89 (25) min for the trihydrate.

The close similarity of mean values for each of the measured and derived parameters leads to the conclusion that the bioavailability of ampicillin from the two forms is equivalent.

DISCUSSION

The dissolution rate of drugs which are not readily soluble in the gastrointestinal tract may be rate-limiting in the absorption process. When a difference in bioavailability of two different physical forms of a drug is attributed to that physical difference, it is essential, in any comparison, to render the forms as alike as possible in all respects apart from that specifically under investigation; and formulation factors should be minimized.

Table 2. *Results of a bioavailability study in which 250 mg of ampicillin, as anhydrate and trihydrate, was administered in loosely-filled capsules to 27 fasting volunteers.* Mean ampicillin serum concentrations, and percentage of dose recovered from the urine as ampicillin in the period 0 to 6 h after dosing (standard deviation in brackets).

	Serum concentrations, μg ml^{-1}								Recovery in the urine, %
Hours after dosing	0	0·33	0·67	1	1·5	2	4	6	
Anhydrate	0·03	0·04*	1·58 (1·33)	2·80 (1·72)	3·37 (1·62)	3·14 (1·46)	1·03 (0·54)	0·35 (0·18)	46·4 (20·9)
Trihydrate	0·03	0·21*	2·21 (1·53)	3·31 (2·00)	3·51 (1·71)	3·37 (1·55)	1·21 (0·72)	0·35 (0·18)	47·3 (17·4)

* Approximate values.

The physical properties of a drug which may affect bioavailability include surface area (related to particle size distribution), polymorphism, and solvation; but while there are numerous examples of the influence of the first two properties, solvation is rarely implicated when administration is by the oral route. It has been reported (Poole & Bahal, 1968; Poole & others, 1968) that suspension and capsule formulations containing ampicillin anhydrate exhibited superior bioavailability to formulations of the trihydrate, and the authors suggested that the greater aqueous solubility of the anhydrated form was probably the major factor responsible. However, the difference in aqueous solubility (10 mg ml^{-1} for the anhydrate, 8 mg ml^{-1} for the trihydrate, at 37°), is small. Also, the solubilities of the two forms in acidic conditions more relevant to drugs administered orally, are significantly greater, and the rates of dissolution are rapid and similar (Table 1).

The comparisons made by Poole and his colleagues were with formulated products from different manufacturers, and the results were therefore subject to processing and formulation factors. In the experiments we describe, the two forms of ampicillin were prepared in as simple and similar a manner as possible, by milling to the same particle size, and filling into identical capsule shells loosely, by hand, with no added excipients. Dissolution rates from the capsules in acidic solution were rapid and virtually identical. The *in vivo* results now reported (Table 2) confirm that anhydrate and trihydrate exhibit similar bioavailability after oral administration.

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