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Intrinsic dissolution rate and solubility studies on josamycin, a macrolide antibiotic

M. Skinner and I. Kanfer

School of Pharmaceutical Sciences, Rhodes University, Grahamstown 6140 (South Africa)

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Summary

The effect of pH on the intrinsic dissolution rate and solubility of josamycin, a macrolide antibiotic, has been investigated to determine the possible effects of the gastro-intestinal pH on absorption The intrinsic dissolution rate (G) was determined in various dissolution media over a pH range of 1.2–7.5 using a disc rotated at 50, 100, 200 and 300 rpm at each pH. The intrinsic dissolution rate at infinite rotation speed (G^{∞}) was determined using an extrapolation procedure described previously (Nicklasson and Brodin, *Acta Pharm. Suec*, 19 (1982) 109–118). A plot of log G^{∞} vs time was linear (linear regression equation $y = 1.4288 - 0.6007x$, correlation coefficient = 0.9904) with values of G^{∞} ranging by a factor of > 8500 from a maximum of 5 16 mg cm^{-2} s^{-1} at pH 1 2 to 5.81×10^{-4} mg cm^{-2} s^{-1} at pH 7.5. Furthermore, comparison of G^{∞} values with limits suggested by Kaplan (*Drug Metab. Rev*, 1 (1972) 15–34) indicates that the absorption of josamycin could be dissolution rate-limited from an environment of pH 5 4 to 7.0, and is highly likely to be dissolution rate-limited from intestinal fluid at pH values above 7 0 The solubility of josamycin was equally dependent on pH and ranged from 212 mg ml^{-1} (21%) at pH 5.45 to 0 18 mg ml^{-1} (0.018%) at pH 9.0. Josamycin has a solubility of approx 1% at pH 6.0 and decreases with increasing pH to approx 0 019% at pH 8 5 This suggests that the absorption of josamycin may also be solubility rate-limited particularly from an intestinal environment of pH 6 0 and above.

Introduction

Josamycin is a macrolide antibiotic which contains a 16-membered lactone ring and is reported to exhibit significant advantages over other similar antibiotics. For example, it does not induce resistance in susceptible microorganisms (Mitsuhashi, 1967; Ono et al., 1975; Lam and Baselka, 1985), has excellent tissue penetration and distribution (Fraschini et al., 1983; Periti et al., 1989), is relatively acid stable compared to erythromycin and is extremely well tolerated, with a low incidence of side effects. Despite these advantages the antibiotic has not achieved widespread usage. Bioavailability studies indicated that absorption from two commercially available tablet preparations was erratic (Skinner, 1991) and prompted investigations into two major physico-chemical aspects pertinent to drug absorption – namely the intrinsic dissolution rate and solubility. Kaplan

Correspondence to I. Kanfer, School of Pharmaceutical Sciences, Rhodes University, Grahamstown 6140, R.S.A.

(1972) suggested that drugs with an aqueous intrinsic dissolution rate of less than 0.016 mg cm^{-2} s^{-1}, as determined utilising Woods apparatus at 50 rpm, may exhibit dissolution rate-limited absorption whilst those with an intrinsic dissolution rate of less than 0.0016 mg cm^{-2} s^{-1} are highly likely to do so. Similarly, Kaplan (1972), and Smythe and Hottendorf (1980) suggested that solubility limited absorption may occur if the aqueous solubility of a compound is less than 1%. Intrinsic dissolution rate and solubility are often highly dependent on pH. As the pH of gastro-intestinal fluid can vary considerably, these two physico-chemical parameters were determined over the gastro-intestinal pH range to assess their possible influence on the absorption of josamycin at pH values likely to be encountered in the gastro-intestinal tract.

Materials and Methods

Josamycin powder was kindly donated by Yamanouchi Pharmaceutical Co. (Tokyo, Japan). Water used was HPLC grade purified by a Milli-RO 15 reverse-osmosis system and a Milli-Q system (Millipore, Bedford, MA, U.S.A.). All reagents were of analytical grade.

Intrinsic dissolution rate

Intrinsic dissolution rates were determined using a Pharmatest Dissolution Apparatus (Type PTW S, Hainburg, Germany). Discs of pure josamycin with a diameter of 11.5 mm were prepared by compression of 200 mg of josamycin powder at the required pressure in a Carver press using a tablet punch and die modified to produce a flat surfaced disc. After compression, discs were carefully removed from the die and coated on the underside and circumference with a water insoluble lacquer (Tipp-Ex Fluid, Tipp-Ex, Vertrieb, GmbH & Co. KG, Frankfurt, Germany) to ensure that the flat upper surface only was exposed to the dissolution medium. The discs were then mounted vertically onto a flattened portion on the edge of a circular perspex holder 40 mm in diameter and 10 mm thick. The perspex holder with affixed disc was screwed onto the end of a basket dissolution apparatus stirrer shaft, lowered into 100 ml of dissolution fluid (37°C) and rotated at various rotation speeds. Samples of dissolution medium were periodically removed from the bulk solution without replacement and analysed by HPLC.

The effect of the disc compression force on the intrinsic dissolution rate was assessed in order to determine the most suitable compression force for discs used in all subsequent experiments. Discs for these experiments were compressed at 0.5, 1.0, 1.5, 2.0 and 3.0 tonne for 30 s and the dissolution rate from each disc determined in dissolution medium of pH 2.2 at a rotation speed of 50 rpm.

To determine the influence of pH on the intrinsic dissolution rate, dissolution runs were carried out in duplicate at 50, 100, 200 and 300 rpm using discs compressed at 2.0 tonne for 30 s, in each of the following dissolution media: USP Simulated Gastric Fluid without pepsin (pH 1.2), McIlvaine's buffer consisting of 0.1 M citric acid adjusted to pH 2.2, 3.0, 4.0, 5.0 and 6.0 with 0.2 M disodium hydrogen phosphate, and USP Simulated Intestinal Fluid without enzymes (pH 7.5).

Calculation of intrinsic dissolution rates

The intrinsic dissolution rate from each disc was calculated utilising Eqn 1 (Nelson, 1957):

$$W = KSC_s t \quad (1)$$

where W represents the mass dissolved (mg), S is the surface area of disc (cm^{-2}), C_s denotes the solubility, t is the time (seconds) and K corresponds to a constant. The intrinsic dissolution rate, G (mg cm^{-2} s^{-1}), was obtained from the slope of a plot of W/S (amount dissolved per unit area) vs t. Intrinsic dissolution rates at infinite rotation speed, G^{∞} (mg cm^{-2} s^{-1}), in each dissolution medium were then determined using Eqn 2 (Nicklasson and Brodin, 1982):

$$\frac{1}{G} = \frac{1}{k_1} + \frac{k_2}{\omega} \quad (2)$$

where G is the intrinsic dissolution rate determined at a rotation speed of ω (rad s^{-1}), k_1

represents the rate at which molecules leave the solid surface when unhindered by molecules already in solution, i.e., the 'true' intrinsic dissolution rate where $k_1 = G^{\infty}$ when $\omega \rightarrow \infty$, and k_2 is a constant. G^{∞} is therefore equal to the inverse of the y-intercept obtained by linear regression of a plot of $1/G$ vs $1/\omega$.

Solubility

The solubility of josamycin was determined in McIlvaine's buffer of pH 4.0, 5.0, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 prepared as previously described, and in McIlvaine's buffer of pH 8.5 adjusted to pH 9.0 with sodium hydroxide pellets.

Excess josamycin was weighed into screw-capped test-tubes (Kimax, Kimble, Vineland, NJ, U.S.A.) followed by the addition of 2 ml of buffer. The powder was wetted by vigorous shaking and sonication for 20 s, after which the solution was allowed to reach equilibrium at 37°C whilst being shaken by a mechanical shaker. Samples were withdrawn after 8 h with a 1 ml syringe pre-heated to 37°C, and filtered through a 0.45 μm filter (Millipore, Bedford, MA, U.S.A.). The pH of each filtered solution was then determined and the samples immediately assayed. Solubility determinations were performed in triplicate.

Analysis

Intrinsic dissolution rate studies Josamycin concentrations were determined by HPLC with UV detection using the system previously described (Skinner and Kanfer, 1988). A 50 μl aliquot of dissolution medium was added to 200 μl of internal standard solution (oleandomycin phosphate, 0.5 mg/ml in an acetonitrile/0.1 M phosphate buffer mixture (1:5) at pH 7.5). After mixing by vortexing for 30 s, a 50 μl aliquot of the resulting solution was injected into the chromatographic system. Concentrations were determined from calibration curves constructed using peak height ratios over the concentration ranges of 0–1 mg/ml for studies at pH 1.2 and 2.2, from 0 to 0.1 mg/ml for studies at pH 3.0, 4.0, 5.0 and 6.0, and from 0 to 0.01 mg/ml for studies at pH 7.5.

Solubility studies Josamycin concentrations in samples from solubility studies were determined using a stability-indicating assay capable of separating josamycin from acid and alkali degradation products. The modular HPLC system consisted of a Beckman 114 constant-flow pump (Beckman Instruments Inc., Fullerton, CA, U.S.A.), a fixed loop SSI 3XL manual injector (Scientific Systems Inc., State College, PA, U.S.A.) fitted with loop disc No. 3, an M490 UV detector (Waters Associates, Milford, MA, U.S.A.) and a Hitachi 561 flat-bed recorder (Hitachi, Tokyo, Japan). The analyses were performed on an Ultrasphere-XL 3 μm C_{18} analytical column with guard cartridge (Beckman Instruments Inc., Altex Division, San Ramon, CA, U.S.A.) at ambient temperature. The mobile phase was acetonitrile/0.005 M phosphate buffer (65:45) at pH 4.0. The buffer was prepared by making 0.32 ml of phosphoric acid (85%) up to 1 l with water and adjusting the pH to 4.0 with sodium hydroxide pellets. The flow rate was 1.6 ml/min. The use of a highly precise fixed loop injector rendered the use of an internal standard unnecessary. Filtered samples (1 ml) of dissolution media of pH 4 and 5 were diluted 1 in 500; of pH 6.0, 1 in 250; of pH 6.5, 1 in 100; and of pH 7.5 and 9.0, 1 in 10 with an acetonitrile/water mixture (1:1) to ensure that concentrations were within the linear calibration range. A 2 μl aliquot of each dilution was then injected onto the analytical column and the concentration determined from a calibration curve from 0 to 0.1 mg/ml. The solubility was then determined by adjusting for the respective dilution.

Results and Discussion

Intrinsic dissolution rates

The influence of compression force on G for josamycin in dissolution medium of pH 2.2 is depicted in Fig. 1. G decreases slightly as the compression force increased from 0.5 to 1.5 tonne and decreased only marginally from 1.5 to 3.0 tonne. Surface cracks were, however, observed in discs compressed at 3.0 tonne. A compression force of 2.0 tonne was therefore selected for all subsequent studies.

Plots of W/S vs t were constructed for each

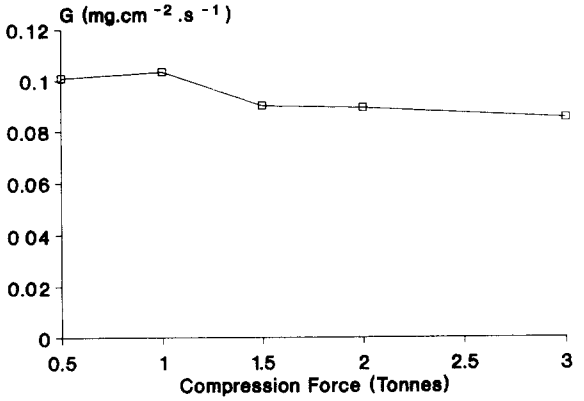

Fig 1 Effect of disc compression force on G (discs rotated at 50 rpm in dissolution medium of pH 2.2)

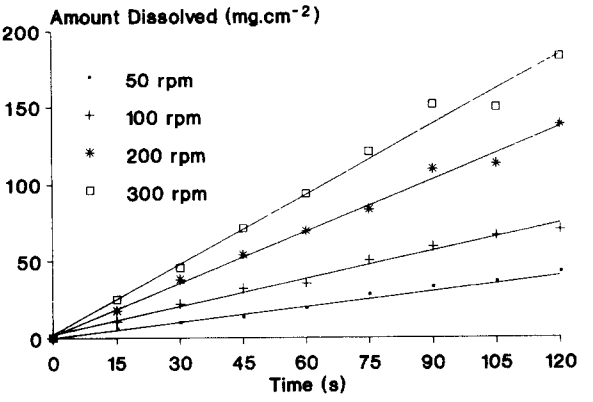

Fig 2 Typical plots of W/S vs t with disc rotation speeds of 50, 100, 200 and 300 rpm in dissolution medium of pH 1 2 for calculation of G

rotation speed at each pH to determine G. Typical plots, obtained at pH 1.2, are depicted in Fig. 2 which illustrate the influence of rotation speed on G. Data from these plots are tabulated in Table 1. G was influenced more strongly by rotation speed at low pH than at high pH which is illustrated in a three-dimensional plot of G at each rotation speed with respect to G at 50 rpm vs pH (Fig. 3). For example, at pH 1.2, G at 300 rpm was 4.28 times greater than at 50 rpm whilst at pH 7.5, G at 300 rpm was only 2.63 times greater than at 50 rpm. This makes the choice of a specific rotation speed for the comparison of G vs pH difficult. For instance, at 50 rpm G was 2002 times greater at pH 1.2 than at pH 7.5 but at 300 rpm, G was 3341 times greater at pH 1.2 than at pH 7.5. This anomaly can be attributed to the influence of the diffusion layer on the intrinsic dissolution rate. However, at infinite rotation speed, the diffusion layer theoretically no longer contributes to the dissolution process and the sole influence of pH on the intrinsic dissolution rate can be assessed by comparing the intrinsic dissolution rates at infinite rotation speed (G^{∞}) vs pH. Plots of $1/G$ vs $1/\omega$ at each pH tested were

TABLE 1

Values of G determined from plots of W / S vs t for excentrically mounted discs at pH 1 2, 2 2, 3 0, 4 0, 5 0, 6 0 and 7 5

pH of dissolution medium	G (mg cm^{-2} s^{-1})			
	50 rpm ω = 5 24 rad s^{-1}	100 rpm ω = 10 47 rad s^{-1}	200 rpm ω = 20 96 rad s^{-1}	300 rpm ω = 31 45 rad s^{-1}
pH 1 2	0 356	0 686	1.125	1 512
	0 333	0 600	1.115	1 516
pH 2.2	0 0852	0.162	0 241	0 345
	0.0950	0.158	0 249	0 375
pH 3.0	0.0454	0.0724	0.148	0 193
	0 0448	0 0838	0 134	0.221
pH 4 0	0 0183	0.0266	0.0546	0 0647
	0 0172	0.0247	0.0504	0 0603
pH 5 0	5.545×10^{-3}	0.0104	0 0161	0.0239
	5.766×10^{-3}	0.0101	0 0162	0 0221
pH 6 0	1.561×10^{-3}	2.818×10^{-3}	3.661×10^{-3}	4.625×10^{-3}
	$1\ 503 \times 10^{-3}$	2.611×10^{-3}	$3\ 595 \times 10^{-3}$	$4\ 823 \times 10^{-3}$
pH 7 5	$1\ 667 \times 10^{-4}$	2.535×10^{-4}	3.079×10^{-4}	$4\ 294 \times 10^{-4}$
	$1\ 766 \times 10^{-4}$	2.687×10^{-4}	3.757×10^{-4}	4.775×10^{-4}

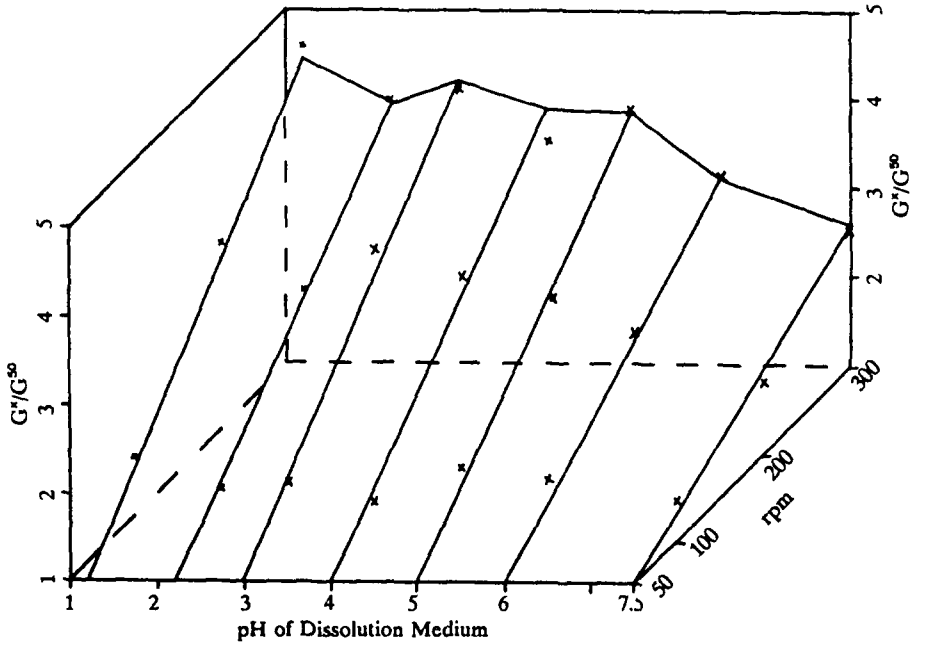

Fig 3. Three-dimensional plot of G^x/G^{50} vs rotation speed vs pH (where $G^x = G$ at either 50, 100, 200 or 300 rpm) showing the effect of rotation speed on G at each pH.

constructed (Fig. 4a–c) for the determination of G^∞ values, which were obtained from the intercepts on the y-axis of the linear regression lines of these plots as described by Nicklasson and Brodin (1982). Results of linear regression analyses of these plots together with values of G^∞ are listed in Table 2. A plot of log G^∞ vs pH is depicted in Fig. 5 and was found to be linear over the pH range studied ($y = 1.4288 - 0.6007x$, correlation coefficient = 0.9904). G^∞ is highly dependent on pH over the entire pH range studied and ranges from a maximum of 5.16 mg cm^{-2} s^{-1} at pH 1.2 to a minimum of 5.81×10^{-4} mg cm^{-2} s^{-1} at pH 7.5, i.e., G^∞ varies by a factor of > 8500 over the gastro-intestinal pH range. As G^∞ values are generally considerably higher than G obtained utilising a centrically mounted disc at 50 rpm, direct comparison between G^∞ values and the limits suggested by Kaplan to assess a drug's potential for absorption provides a very conservative estimate of the possible role of the intrinsic dissolution rate and pH on the absorption of josamycin. From the linear regression equation of the plot of log G^∞ vs pH (Fig. 5), G^∞

TABLE 2

Results of linear regression analyses of plots of $1/G$ vs $1/\omega$ for the determination of G^∞ at each pH

pH	Slope	y-intercept	Correlation coefficient	G^∞ (mg cm^{-2} s^{-1})
1 2	14 2244	0 1938	0 9962	5 1500
2 2	51.1826	1.3779	0 9938	0.7257
3 0	107 600	1 8976	0.9947	0 5270
4 0	258 740	8 9674	0 9791	0 1115
5.0	822.980	19 659	0 9985	0.05087
6 0	2 709 55	129.40	0 9952	0 007728
7 5	21 708.9	1 719 7	0 9854	0.0005815

at pH 1.2 is 5.10 mg cm^{-2} s^{-1} but decreases rapidly with increasing pH to 0.016 mg cm^{-2} s^{-1} at pH 5.4 and to below 0.0016 mg cm^{-2} s^{-1} at pH 7.0. Consequently, the absorption of josamycin could be dissolution rate-limited from intestinal fluid of pH 5.4 to 7.0 and is highly likely to be dissolution rate limited from intestinal fluid above pH 7.0. Furthermore, G^{∞} varied considerably (by a factor of 31) over the normal intestinal pH range of 5.0–7.5. The pH of the intestinal tract may therefore be critical in determining the bioavailability of josamycin after oral administration with small changes in the intestinal pH having a profound effect on the dissolution rate and subsequently the absorption rate.

Solubility

The pH-solubility profile of josamycin is depicted in Fig. 6. The solubility of josamycin was also highly dependent on pH and ranged from 212 mg ml^{-1} (21%) at pH 5.45 to 0.18 mg ml^{-1} (0.018%) at pH 9.0. Attempts were made to determine solubility between pH 1.0 and 5.0. However, the addition of a substantial amount of josamycin to all buffers below pH 5.0 resulted in the formation of a clear highly concentrated solution from which a viscous mass spontaneously precipitated leaving a saturated supernatant of 212 mg ml^{-1}, pH 5.45, irrespective of the initial buffer pH.

Since the solubility of josamycin is highly dependent on pH, the pH of the gastro-intestinal tract will significantly influence its absorption. Although josamycin is highly soluble at gastric pH values, the drug molecules will be highly ionised in the stomach (pK_a of josamycin = 7.1) suggesting that absorption from this region will be poor (Brodie and Hogben, 1957). On the other hand, josamycin, with a favourable lipophilicity (Wildfeuer and Lemme, 1985) and a pK_a of < 10 should be rapidly absorbed from the more alkaline medium of the intestinal tract (Brodie and Hogben, 1957). However, the solubility of josa-

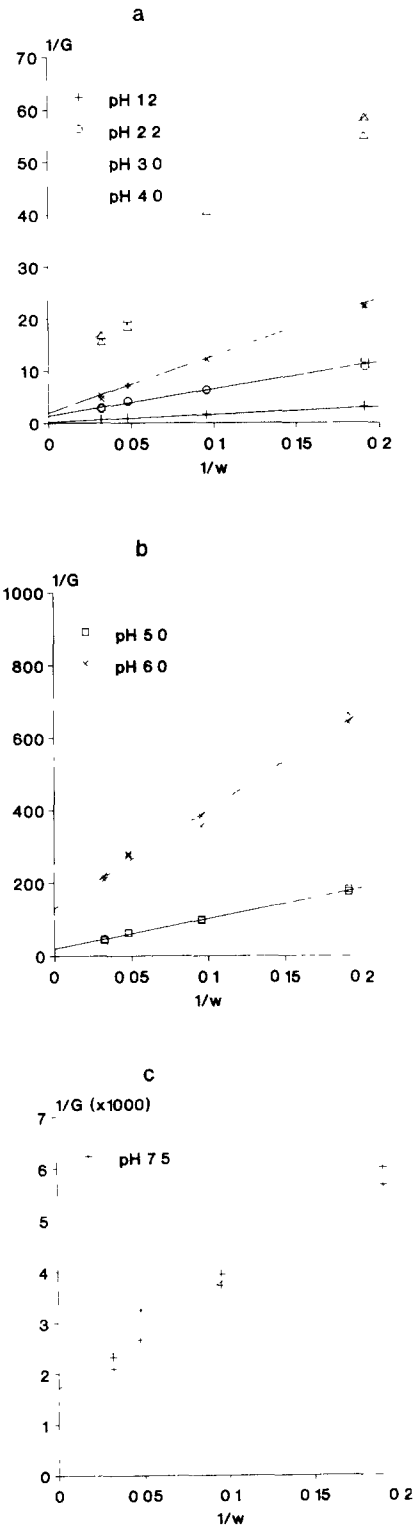

Fig. 4 (a–c) Plots of $1/G$ vs $1/\omega$ at (a) pH 1.2, 2 2, 3 0 and 4.0, (b) pH 5.0 and 6.0 and (c) pH 7.5 showing linear regression lines for the calculation of G^{∞}

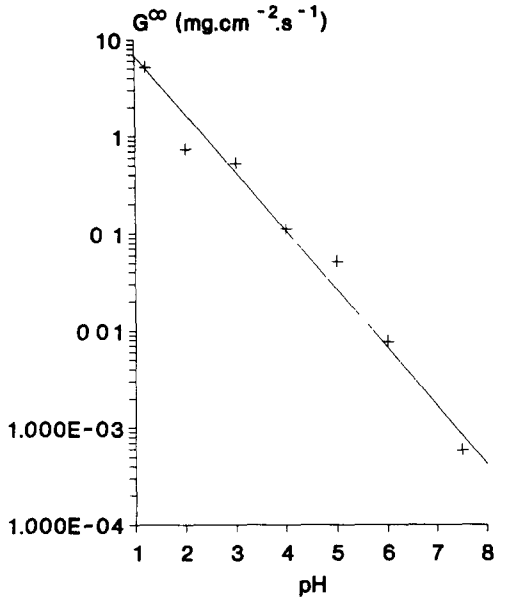

Fig 5. Plot of log G^{∞} vs pH illustrating the log-linear dependence of the intrinsic dissolution rate on pH Linear regression equation $y = 1.4288 - 0\,6007x$.

mycin at intestinal pH values ranged from 21% at pH 5.45 to 1% at about pH 6.0 and to as low as 0.019% at about pH 8.5. Absorption of josamycin could therefore be solubility limited from an intestinal environment of pH 6.0 and be increasingly hindered by poor solubility with increasing pH. The predisposition of josamycin towards dissolution rate and solubility limited absorption, compounded by the high sensitivity of these two parameters to pH, could help to explain the erratic absorption observed during the bioavailability studies conducted (Skinner, 1991). These data suggest that formulations designed to control the pH at the site of absorption in the intestinal tract in order to promote dissolution may considerably improve the bioavailability of josamycin, and warrant investigation.

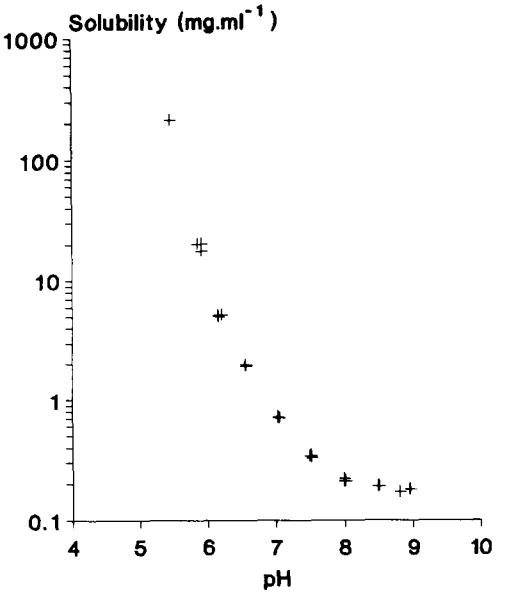

Fig 6 pH-solubility profile of josamycin in McIlvaine's buffer at 37°C (triplicate determinations)

Acknowledgements

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