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The effect of protein on the dissolution of phenytoin

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Ultrafiltration of a continuous flow system was used to follow the dissolution of phenytoin powder in buffered solutions of pH 1, 4 and 7·4. The presence of either human serum albumin (HSA) or casein caused changes in both the absolute solubility and the dissolution rate. Normal protein binding cannot be responsible for the effects noted. It would seem likely that within the diffusion layer a higher degree of protein binding occurs than is possible in the bulk aqueous solution. The drug solubilized in this way is then released in solution in a form that is at least metastable with respect to phenytoin in normal solution. The effect of protein on phenytoin dissolution may well affect the bioavailability of the drug.

Dissolution of a drug involves the diffusion of drug molecules from the dissolving surface to the bulk solution. Any process which increases the concentration gradient involved will have an effect on the dissolution rate. However, the effect may only become apparent, and potentially important, in cases where the drug is only sparingly soluble so that changes in concentration gradient become significant. One method of increasing the concentration gradient is by binding the dissolved drug to some other substance present in solution. If the drug is sufficiently highly bound, then the concentration gradient is kept at a higher than normal level until the binding system is saturated.

We investigated this rationale using phenytoin as our model drug and human serum albumin (HSA) and casein as potential binding agents. The latter was chosen to simulate the possible effects of food on dissolution of an oral solid dosage form.

In order to establish the dissolution profile in the presence of protein it was convenient to measure dissolution using an Amicon Diaflo cell and membrane. It was felt that this would enable comparable studies to be made with and without protein present.

MATERIALS AND METHODS

The phenytoin used for all experiments was from the same source (Parke Davis and Co. Ltd Batch No. 6289). Particle size analysis showed an average length of 14·6 ± 4·4 μm and breadth 8·2 ± 1·8 μm. Pycnometric density measurements gave a value of 1·271 g cm^{-3}. Human serum albumin (HSA) was from Behringwerke and casein from Sigma. Control of the pH was by use of 0·1 M HCl for pH 1, M/15 phosphate/citrate buffer (pH 4) and M/15 phosphate buffer (pH 7·4).

Dissolution of phenytoin powder was followed using an Amicon Diaflo apparatus (Fig. 1). A known weight of phenytoin powder (10 or 30 mg) was added to a known volume (ca 160 ml) of solvent in the Diaflo cell. The cell was then pressurized with nitrogen and the cell volume maintained constant with the effluent passing through a molecular filter (Amicon PM10) balanced by the influx of fresh solvent from a reservoir. The cell was stirred throughout at constant speed (300 rev min^{-1}). The concentration of drug in the effluent was measured using a spectrophotometer and a flow-through cell. Under these conditions, if the added solid is greatly in excess of the amount required to saturate the solvent in the cell at any given time, a pseudo steady state concentration of dissolved drug is obtained, the value of this concentration being a function of cell volume, flow rate and solubility. The dissolution rate constant, k, can then be obtained either by analysis

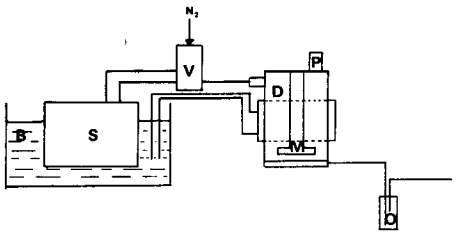

FIG. 1. Diagrammatic representation of the experimental apparatus. D is the ultrafiltration cell fitted with a stirrer M. A reservoir S containing solvent is kept in a constant temperature bath B, water from which also circulates around D. The switch V allows pressure to be applied to S, D or both. Immediately below the stirrer M is a membrane supported on a scintered disc. The emerging solution is passed through a flow cell O.

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of the approach to steady state or by comparison of the steady state values realized using different flow rates and volumes.

RESULTS

Dissolution in the absence of protein

Values of the solubility and dissolution rate constant for phenytoin under various conditions of pH, initial amount added and temperature are shown in Table 1. These solubilities agree well with those reported previously for pH 1 (Bastami & Groves 1978; Yamamoto et al 1976) and pH 7·4 (Schwartz et al 1977).

Table 1. Solubilities and dissolution rate constants of phenytoin powder (Batch No. 6289).

Temp. °C	pH	Solubility ($\mu g\ ml^{-1}$)	Dissolution constant rate (min^{-1})	Flux ($\mu g\ min^{-1}$)
21	1	9·7	0·06*	
36	1	20·8	0·04*	
21	1	9·7	0·141	23·26 ± 1·24
21	4	21	0·168	46·28 ± 6·62
21	7·4	31	1·10	

* Using 10 mg phenytoin powder. In all other cases 30 mg powder was used.

The dissolution rate constant quoted is a pseudo first order rate constant incorporating a term for the surface area. This dependence on surface area is seen in the change in value of the rate constant as the amount of powder is increased (Table 1).

The product of the steady state concentrations reached and the flow rates which produce them gives the total flux J_{tot}. From Fick's Law

$$J_{tot} = D.C_s/h$$

where C_s is the solubility. Taking the diffusion coefficient D as $7 \times 10^{-6}\ cm^2\ s^{-1}$ (cf. Mooney et al 1981) values are obtained for the diffusion layer thickness, h, of $2{\cdot}59 \times 10^{-2}$ at pH 1 and $2{\cdot}81 \times 10^{-2}$ at pH 4.

Dissolution in the presence of protein

Dissolution in the presence of protein was studied using the same methods as in its absence. Human serum albumin (HSA) and casein were used as model proteins. At pH 1 the dissolution profile obtained when protein was present was similar in nature to that in its absence; the dissolved drug concentration rose exponentially to a steady state value controlled by the constant volume and the flow rate.

The main characteristic of the dissolution behaviour of phenytoin in the presence of either protein at pH 4·0 or 7·4 was that maximum rather than steady state concentrations were reached. A slow decline in level was apparent after the maximum had been obtained. Dissolution rates in the presence of protein were calculated from analysis of the curves leading to the maximum. Rates were measured at the two pH values using various concentrations of either HSA or casein.

At pH 1 HSA had no effect on phenytoin solubility, though it did increase the rate of dissolution. At the same pH casein apparently caused a slight lowering of the solubility of phenytoin (Fig. 2A and Table 2), although it, too, increased the dissolution rate. The effect of changing the amount of added protein on the dissolution rate constant is shown in Fig. 3A, where it can be seen that maximum enhancement occurs at about 2 mg % casein.

At pH 4 addition of either protein caused significant increases in both solubility (Fig. 2B and Table 2) and dissolution rate constant (Fig. 3B), both properties increasing with increase in added protein until a plateau level is reached when the adduct is at a level of about 20 mg % (w/v).

At pH 7·4 the two proteins caused a solubility increase of about the same magnitude as at pH 4 which again rose to a plateau level. However the

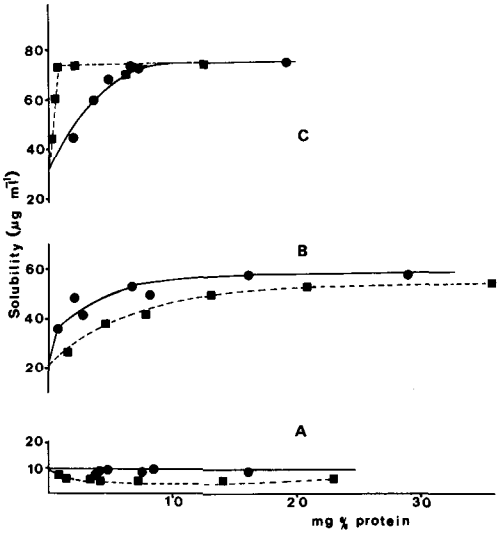

FIG. 2. Solubility of phenytoin at various pH as a function of concentration of added protein. ●——● HSA; ■ - - - ■ casein. A at pH 1; B at pH 4; C at pH 7·4.

Table 2. Maximum solubilities and dissolution rate constants of phenytoin powder (Batch No. 6289) reached at 21 °C in the presence of HSA and casein at various pH values.

pH	Protein	Conc. (mg%)	Solubility ($\mu g\ ml^{-1}$)	Dissolution rate constant (min^{-1})*
1	HSA	4	9·7	0·37
1	casein**	1·3	5·0	0·56
4	HSA	17	58	0·97
4	casein	21	55	0·70
7·4	HSA	6	74	1·33
7·4	casein	0·5	74	1·70

* Using 30 mg phenytoin powder.
** At pH 1 casein caused a drop in phenytoin solubility at the same time as an increase in dissolution rate.

plateau was reached at much lower concentrations of HSA and very much lower concentrations of casein (Fig. 2C and Table 2). The change in dissolution rate constant with amount of added protein was more variable than at pH 4. Somewhat similar to the result at pH 1, when the amount of added casein is less than 2 mg % (w/v) there is an increase in the dissolution rate constant. Above 2 mg % this increase disappears and then becomes negative (Fig. 3C). HSA at pH 7·4 has little effect on the dissolution rate,

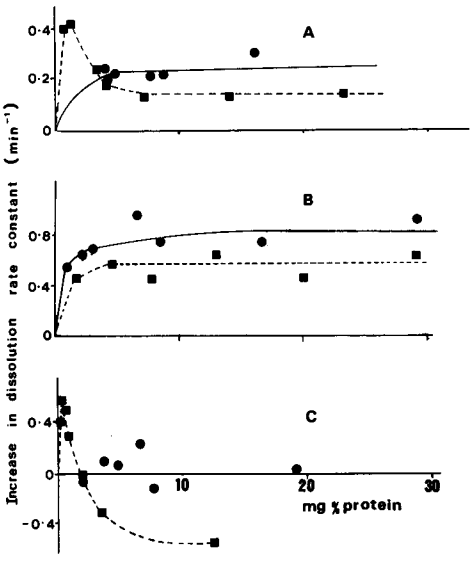

FIG. 3. Increase in dissolution rate constant of 30 mg phenytoin powder at various pH as a function of concentration of added protein. ●——● HSA; ■ - - - ■ casein. A at pH 1; B at pH 4; C at pH 7·4.

though, like casein, increasing concentrations lead to a maximum enhancement which then falls back to zero. For both casein and HSA a plot of solubility against amount of added protein shows that once the maximum enhancement of solubility has been reached it shows no sign of dropping, despite the changing shapes of the dissolution rate constant/amount of added protein graphs shown in Fig. 2C.

DISCUSSION

The effect of protein on the dissolution was variable, depending on the pH, the nature of the protein and the protein concentration. Both solubility and rate of dissolution were followed as functions of protein concentration and they will be considered separately in this discussion so far as is possible.

At pH 1 HSA had a negligible effect on the solubility of phenytoin, while casein appeared to cause a decrease in solubility. Similar decreases have been reported by Corrigan & Timoney (1975) for hydroflumethiazide and by Gibaldi & Weintraub (1968) for salicylic acid solubility at pH 1 in the presence of up to 2% w/v PVP.

At pH 4 and 7·4 both HSA and casein were capable of causing a 2·5-fold increase in phenytoin solubility, the actual value of the increase depending on the concentration of the protein used. Preliminary experiments with other additives (fibrinogen, PVP K-30, PVP K-90) gave curves very similar to those for HSA and casein.

The solubility values found in the presence of protein represent a true supersaturation of the solvent. Binding of phenytoin to HSA and to casein was investigated separately in aqueous solution at the three values of pH and was shown to be much too small to account for the increase in solubility found (Macheras & Rosen, unpublished). In any case, the method used for following the dissolution measures only the free solute, so that material which remains bound to the additive will not appear in the analysis of the effluent from the cell. Indeed, this effluent will underestimate the true solubility by the amount bound.

Solubility effects in the presence of PVP are well documented (Higuchi & Connors 1965; Simonelli et al 1970, 1976; Sekikawa et al 1979). Simonelli et al (1976) attributed the increased solubility shown by co-precipitates of sulphathiazole and povidone to a high energy form of the drug stabilized by the povidone. In the present instance phenytoin concentrations have been measured specifically so as to ensure the absence of any macromolecular species,

so that the ultrafiltrate must be in at least metastable equilibrium.

Higuchi (1967) and Dombrow & Touitou (1978) have derived an equation to account for dissolution rates in complexing media. This equation shows a linear dependence of the dissolution rate on the diffusion coefficients of the various species involved and on the association constant for complex formation.

As indicated previously, under normal conditions the binding of phenytoin to HSA or casein is insufficient to account for the effects found. The association constant for binding of phenytoin to HSA would need to be about 1000-fold greater than normally measured for the Higuchi equation to predict the rate enhancement found. However the terms of the Higuchi equation apply to association which occurs within the diffusion layer. The layer will be characterized by a solute concentration gradient which is initially at concentrations much above those in the bulk solution. It is highly probable that the behaviour of this diffusion layer is not that of the bulk medium; the dielectric constant will be reduced and the solvent properties generally will be altered. In these circumstances it is not difficult to conceive that an association originating within the diffusion layer might be much greater than occurs in the bulk medium and that it will be reversed when the complex enters the bulk medium. The additive then acts predominantly as a carrier which transports material from the solid surface to the liquid phase. The carrier might be showing a high association close to the solid surface which diminishes continuously as it crosses the diffusion layer; alternatively it may shed the bulk of the associated drug as it moves into the bulk layer. Whichever of these is true, the net effect will be an increase in diffusion rate of drug over the situation in the absence of additive.

The Higuchi equation implies a dependence of rate of dissolution on the concentration of additive. Dombrow & Touitou (1978) state explicitly that there will be a linear increase in dissolution rate with complexant concentration. That such a dependence exists is shown by Fig. 3 where the dissolution rate constants are plotted against protein concentration for various conditions of pH. However Fig. 3 shows that the function is not continuous but reaches an upper limit when the concentration of the complexing agent gets much larger than that of the dissolving species. At this stage all the solute in the diffusion layer is complexed with the additive. Any further increase in protein will be ineffective in terms of increasing drug transport across the diffusion layer, although a very much larger increase in protein concentration may cause a slowing down of the diffusional process as the viscosity of the bulk medium increases. This limit to the ability of protein to enhance dissolution is clearly seen using some rough calculations.

The average volume of a phenytoin particle in the sample used is $7{\cdot}7 \times 10^{-10}$ cc. A 6·9 mg% solution of HSA, which is of the order of concentration giving a maximum dissolution rate, contains 6×10^{14} molecules per cc. On the assumption of a uniform distribution, the number of HSA molecules in a particle volume is $4{\cdot}6 \times 10^{5}$. As the surface area of the particle is $4{\cdot}8 \times 10^{-6}$ cm^2, it may be assumed that each HSA molecule can cover an area of $(141 \times 42).10^{-16}$ cm^2, where the dimensions are those of the long and short axes of the ellipsoidal HSA (Wright & Thomspon, 1975). Then the HSA present is sufficient to cover 5–10% of the particle surface area. This is sufficiently close to indicate that the upper limit of protein involvement comes when the concentration is sufficient to coat each individual particle.

The effect of pH on the dissolution rate constants as they change with the nature and concentration of the added protein (Fig. 3) cannot be explained with any degree of certainty. Probably they arise from conformational changes within the proteins. Thus the change in effect of casein at pH 7·4 with increasing amount of casein might be related to the tendency of casein to undergo a conformational change and form aggregates as its concentration increases (Andrews et al 1979; Evans & Phillips 1979). HSA is subject to conformational changes and would be expected to be in the expanded form at pH 1, predominantly in the F form at pH 4 and at around the N–B transition at pH 7·4. These forms show different binding characteristics under normal conditions (Rosen & Mitchell 1984) and would therefore be expected to do the same in a diffusion layer, with the complication that the various conformers may be stabilized or destabilized by concentration effects.

In conclusion, then, the work reported here shows the following features: (i) in the presence of protein the solubility of phenytoin can be increased significantly, probably by formation of a high energy metastable form. (ii) the effect of protein on the dissolution rate of phenytoin is much higher than can be accounted for by normal binding. It is suggested that protein binding within the diffusion layer is of a different order to that in the bulk solution. (iii) the equation for the effect of a complexing agent on dissolution rate shows the latter to increase continuously with concentration of complexant. The results

reported here show clearly that this is not so, and would indicate that the upper limit of effect is reached when sufficient complexant to coat the dissolving surface is present.

Whatever the reasons, the fact that added protein can affect both solubility and dissolution rate of phenytoin has significant implications for the dosage regimen of the drug. It provides a good reason for the reported increase in bioavailability of phenytoin in the presence of food (Melander et al 1979) and may indeed be of more general application in rationalizing the effect of food on bioavailability.

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