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# The release of a model low-dose drug (riboflavine) from hard gelatin capsule formulations

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The in vitro release of a model low dose drug, riboflavine, from hard gelatin capsules, formulated with a range of diluents, in the absence and presence of magnesium stearate (0·5, 1·0 and 2·0% w/w), has been assessed by a dissolution technique. Comparison of the values of the time for 50% of the drug content of the capsule to appear in solution T50, by analysis of variance, indicated that the type of diluent significantly influenced the drug release. Irrespective of the magnesium stearate content, the diluents could be ranked in the following order of effectiveness: Primojel >sodium bicarbonate >Avicel ≃ Dri-flo starch ≃ lactose > Emcompress ≃ kaolin >starch. Correction of the T50 values for possible adsorption of riboflavine onto the water insoluble diluents, using experimentally determined adsorption isotherms, altered the relative order of effectiveness of the diluents to Primojel >sodium bicarbonate >kaolin ≃ lactose >Avicel ≃ Dri-flo starch >Emcompress >starch. Comparison of the urinary excretion of riboflavine, after administration of capsule formulations containing lactose, Emcompress or kaolin as the diluent, to volunteers, suggests that the dissolution results not corrected for adsorption provide a better indication of the in vivo performance of the formulations.

The formulation of a low-dose drug in a hard gelatin capsule presents the problem of ensuring a uniform distribution of the drug within a diluent and the additional problem of possible retardation of drug release by the diluent. These problems contrast with those of high dose drug formulations which contain no additives and particle size and packing are the controlling features (Newton & Rowley 1970). The choice of a diluent is of fundamental importance to the in vitro release of drug from capsule formulations (Whithey & Mainville 1969; Newton & Razzo 1974), but there appears to be no systematic classification of diluents for use with low-dose drugs. The present work attempts to grade commonly used diluents and a highly adsorbent model diluent, kaolin, according to their influence on the release rate of such a drug, in the presence and absence of a lubricant, magnesium stearate. Riboflavine was chosen as the model low-dose drug for the following reasons: (a) it is absorbed mainly from the upper part of the gastrointestinal tract, i.e. the proximal small intestine (Jusko & Levy 1967); (b) it has a short biological half-life, (1·1 h) in man (Levy & Jusko 1966); (c) it is excreted primarily, if not solely, in its unchanged state (Jusko & Levy 1967); (d) it is virtually non-toxic; (e) it can be determined accurately and easily in body fluids at low concentrations.

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## MATERIALS AND METHODS

### *Materials*

The riboflavine (Roche Products Ltd.) was of B.P. quality; the geometric mean volume diameter was 6·8 μm with a geometric standard deviation of 2·5 μm, determined by Coulter Counter model TA. The diluents used and the mean surface diameters of their particles, determined by Fisher subsieve analyser, were respectively: Avicel PH 101 (Honeywill-Stein Ltd.) 12·0 μm, Dri-flo Starch (Laing National Ltd.) 35·4 μm, lactose B.P. (Whey Products, regular grade) 38·4 μm, light kaolin B.P. (Evans Medical Ltd.) 2·0 μm, Primojel (Kingsley and Keith (Chemicals) Ltd.) 41·3 μm, sodium bicarbonate Ph. Eur. (ICI Ltd., extra fine grade) 17·7 μm and starch Ph. Eur. (Evans Medical Ltd.) 23·4 μm. Also used was Emcompress from Kingsley and Keith (Chemicals) Ltd., the median diameter of its particles, determined by sieve analysis, being 136 μm. The lubricant, magnesium stearate from The Boots Company Ltd., consisted of particles with a mean surface area diameter of 4·4 μm, determined by a Fisher sub-sieve analyser. The hard gelatin capsules were opaque, white and of size '0' and were donated by the Elanco Division of Eli Lilly and Co.

### *Methods*

#### *Experimental design*

The formulation combinations of drug, diluent and magnesium stearate formed an 8 × 4 factorial design

with the 8 diluents forming the qualitative factors and the 4 concentrations of magnesium stearate (0, 0·5, 1·0 and 2·0% w/w) forming the quantitative factors.

*Preparation of hard gelatin capsules*

Appropriate quantities of the drug and, if present, magnesium stearate were gradually diluted with diluent by a process of hand mixing with a spatula on white demy paper. The mixed powder was then passed through a B.S. 85 mesh sieve, remixed, then further mixed by rotating within a glass bottle at 30 rev $min^{-1}$ for 15 min. Analysis of random samples of the blends gave 1·00 ± 0·05% w/w riboflavine, indicating an adequate uniformity of mixing.

The capsules were filled from a dosator nozzle assembly from a Zanasi capsule filling machine attached to a moveable crosshead within a metal frame. The nozzle was carefully lowered into the bed of powder contained within a cylindrical aluminium container (4 cm diameter and 7 cm high). The quantity of powder placed in the container was determined by trial and error to give a capsule weight of 500 mg. To standardize the bulk density, the powder was subjected to 100 displacements through 2·5 cm at the rate of 1 every 30 s. When the dosimeter nozzle was within the powder bed, the central plunger of the Zanasi nozzle was depressed twice by a plunger connected to an air pressure cylinder fed from a compressed air supply at 552 kPa. The nozzle was removed from the powder by withdrawing the crosshead and the plug of powder was then ejected into an empty capsule by activation of the air cylinder. The weight of the content of each capsule was determined and was found to be 500 ± 20 mg.

*In vitro dissolution tests*

The dissolution rates of 4 replicate capsules into 2·0 $dm^3$ of 0·1 mol $dm^{-3}$ hydrochloric acid were determined at 37 °C (Newton & Razzo 1974). Samples were taken after 5, 10, 20, 40 and 80 min and their riboflavine concentration was determined from the absorbance at 267 nm measured by a Cecil 292 ultraviolet spectrophotometer. The mean time for 50% of the contents of the replicate capsules to be released into the solution, designated T50, was interpolated from a linear plot of percentage of drug dissolved presented on a probability scale against the log time (Wagner, 1969).

*Adsorption studies*

The adsorption isotherm of riboflavine in aqueous solution onto each water-insoluble diluent at 37 °C was determined as follows. An accurately weighed quantity of the diluent was shaken with 50 $cm^3$ of water containing a known concentration of riboflavine in a 100 $cm^3$ glass-stoppered flask at 37 °C. When equilibrium had been attained, after an adequate time shown by a preliminary experiment, an aliquot was removed, filtered rapidly at 37 °C and analysed for riboflavine at 267 nm as described above.

*In vivo evaluation of capsules*

Capsules containing riboflavine with lactose, or Emcompress, or kaolin were separately administered to assess the in vivo significance of the in vitro dissolution tests, no lubricant was present. 200 $cm^3$ solution of riboflavine in phosphate buffer, pH 4·8, was administered as a standard. The protocol involved administration of 10 mg of riboflavine equally distributed in two capsules, plus 200 $cm^3$ of water, to each of four male volunteers on an empty stomach on two separate occasions. Urine was collected immediately before and, at regular intervals up to 8 h after administration, the output was maintained by regular intake of fluid. A small quantity of food was allowed 2 and 6 h after administration of the capsules. The riboflavine content of the urine was determined by a method based on the U.S.P. XVIII monograph, using a Farrand spectrophotofluorimeter.

## RESULTS AND DISCUSSION

The presence of a large proportion of a so-called 'inert' diluent within a capsule formulation could influence the release of a low-dose drug in several ways. A hydrophobic diluent could hinder the penetration of fluid into the powder mass. Even with hydrophilic diluents penetration of fluid and subsequent de-aggregation cannot be completely assured. The access of fluid to the powder bed does not necessarily ensure release of the drug. In addition, water-insoluble diluents could provide sites for adsorption of the drug from solution, an effect which could hinder release of the drug from the formulation. If the consequences of the addition of a hydrophobic lubricant, magnesium stearate, are added to these effects, it is perhaps not surprizing that the results for the in vitro drug released from the capsules, as assessed by a dissolution technique, do not conform to a simple regular pattern (Table 1). Analysis of variance of the time

Table 1. The average time (min) for 50% (T50) of the riboflavine content of the capsule to dissolve in vitro.

| Diluent | Lubricant concn (% w/w) T50 | | | |
|---|---|---|---|---|
| | 0 | 0·5 | 1·0 | 2·0 |
| Avicel | 32·0 | 63·0 | 8·0 | 90·0 |
| Dri-flo | 52·0 | 36·0 | 18·0 | 21·0 |
| Emcompress | 111·0 | 233·0 | 1622·0 | 1065·0 |
| Kaolin | 572·0 | 579·0 | 565·0 | 415·0 |
| Lactose | 3·4 | 6·5 | 16·0 | 243·0 |
| Primojel | 1·5 | 1·5 | 1·5 | 1·5 |
| Sodium bicarb. | 3·7 | 6·2 | 5·7 | 9·5 |
| Starch | 603·0 | 335·0 | 474·0 | 476·0 |

for 50% of the drug content of the capsule to appear in solution (Table 2) indicates that the diluent type has a significant effect on drug release, but there is also a significant interaction between the diluent type and the lubricant which apparently prevents the lubricant effect from being significant. The source of this interaction can be observed in Table 1 which presents the results for the individual formulation combinations. It is clear that the effect of lubricant concentration on dissolution time is dependent on the type of diluent. In some instances there occurs an anticipated extended dissolution with increasing lubricant content, in others, no change is seen, while for other diluents there appears to be an optimum concentration of lubricant capable of giving the shortest T50 value. The hydrophilic soluble diluents, lactose, Primojel and sodium bicarbonate, which gave low T50 values, are not seriously affected by the magnesium stearate (except at 2% lubricant with lactose). The hydrophilic insoluble, organic materials, Dri-flo starch, ordinary starch and Avicel show irregular behaviour while the inorganic insoluble diluents, kaolin and Emcompress are only slightly affected. Thus diluents that are soluble and hydrophilic, can generally counter the hydrophobic nature of magnesium stearate, but insoluble diluents provide a less predictable trend, so it cannot be held that the presence of magnesium stearate retards the dissolution process of all formulations.

Table 2. Analysis of variance of the effect of diluent type and lubricant concn on the time for 50% (T50) of the riboflavine content of the capsule to dissolve in vitro.

| Source of variation | Sum of squares | Degrees of freedom | Mean square | Variance ratio, F |
|---|---|---|---|---|
| Diluent D | 9 657 797 | 7 | 1 379 685 | 5·60* |
| Lubricant L | 678 902 | 3 | 226 300 | 0·92 |
| Interaction DL | 5 165 382 | 21 | 245 970 | 7·54* |
| Residual | 3 132 624 | 96 | 32 631 | |

* Significance at 1% level.

The analysis of variance suggests that it should be possible to rank the diluents in order of effectiveness irrespective of the presence of magnesium stearate. However, different concentrations of magnesium stearate give different rank orders of diluent effectiveness. To assess whether these different orders are significant, the Spearman ranking correlations (see Snedecor & Cochran 1967) were calculated for each of the four concentrations of magnesium stearate. The results indicate that there is no significant difference between the rankings for the four different concentration of lubricant. Consequently, the following average order of effectiveness of the diluents may be stated: Primojel >sodium bicarbonate >Avicel ≃Dri-flo starch ≃lactose >Emcompress ≃kaolin >starch.

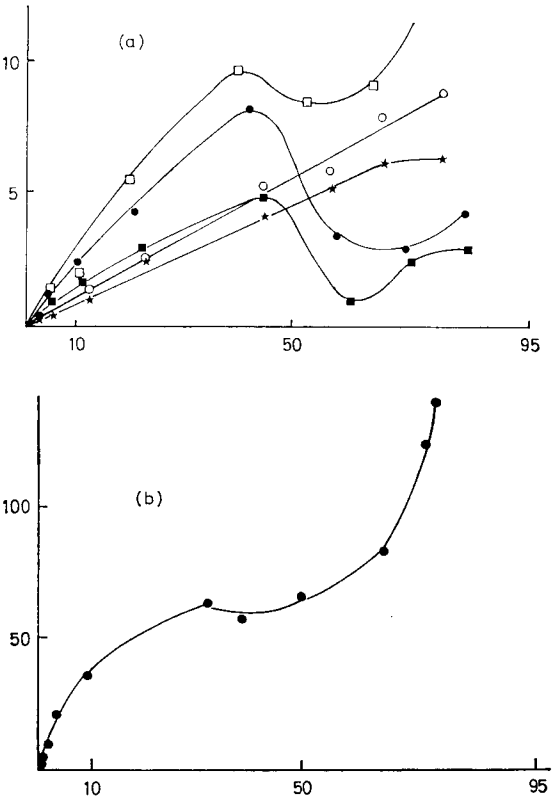


FIG. 1. Adsorption isotherms of riboflavine onto insoluble diluents used in capsule formulations: (a) Avicel ■; Dri-flo ●; Emcompress □; Primojel ○; starch ★. (b) kaolin ●. Ordinate: Riboflavine adsorbed per unit mass (x/m. $10^{-3}$). Abscissa: Equilibrium concentration of riboflavine (mg $dm^{-3}$).

A possible cause of the poor performance of the insoluble diluents may be their ability to adsorb the drug from solution. The adsorption isotherms for riboflavine, shown in Fig. 1, fall into the classification of Giles et al (1960). Dri-flo starch and kaolin give Langmuir types (L type) isotherms which suggest that, as more sites on the adsorbent are filled, it becomes increasingly difficult for a bombarding solute molecule to find a vacant site. This implies, according to Giles et al (1960), either that the adsorbed solute molecule is not vertically orientated or that there is no strong competition from the solvent during adsorption. The charge on the riboflavine molecule in acidic solution and the large, flat, tricyclic and aromatic component of the molecule probably accounts for the observed behaviour.

Avicel, Emcompress, Primojel and starch afforded, at low riboflavine concentrations linear constant-partition type (C type) isotherms which suggest that the number of adsorption sites remains constant. This means that, as the sites are filled, fresh sites are created. This situation is favoured by the following three conditions: (a) the adsorbent should be porous with flexible molecules and with regions differing in crystallinity, a condition fulfilled by each adsorbent used; (b) the solute should have a higher affinity for the adsorbent than has the solvent, a condition favoured by riboflavine, as discussed above; (c) the solute should have a greater penetrating power than the solvent into the crystalline regions of the adsorbent, by virtue of condition (b) or have a flat molecular geometry. Conditions (b) and (c) seem to be favoured by riboflavine for reasons given above.

The shapes of the adsorption isotherms distant from the origin enable the isotherms to be placed into certain subgroups (Giles et al 1960). The adsorption isotherm for riboflavine on starch, corresponding to subgroup 2, indicates that constant partition is followed by saturation of the surface at high concentrations of riboflavine. The isotherms for Emcompress, Avicel and Dri-flo starch give maxima, corresponding to subgroup mx. The maximum in each isotherm is followed by a minimum. The decrease in slope after the first inflexion indicates that the solute-solute attraction is increasing more rapidly than the adsorbent-solute attraction and corresponds to association of the solute at high concentrations. Indeed, the riboflavine molecule has a number of polar functional groups capable of enhancing association. The second rise suggests the development of fresh surface for adsorption. This fresh surface may result from penetration of the solute into more crystalline regions of the adsorbent or from re-orientation of adsorbed solute molecules.

Kaolin gives one ill-defined maximum but it is a very strong adsorbent because of its large specific surface area and its ability to attract polar molecules by strong chemical forces. Kaolin, of empirical formula $Al_2O_3$, $2SiO_2$, $2H_2O$, has cationic and anionic sites, dipoles and hydroxyl groups which can undergo various types of ion-ion, ion-dipole, dipole-dipole and hydrogen bond interactions with riboflavine in acid solution.

Irrespective of the complexities of the isotherms it is possible to calculate the amount of riboflavine adsorbed at any given time during the dissolution test.

To estimate from the absorption isotherms the amount of riboflavine adsorbed onto diluent, two extreme possibilities were considered. The first assumes that the bulk concentration obtained in a dissolution test represents the concentration of drug in equilibrium with the drug adsorbed onto the diluent. The second possibility considers adsorption to occur at the surface of the dissolving riboflavine particles, where the saturation concentration (i.e. the solubility) of riboflavine represents that concentration in equilibrium with the adsorbed drug. If the latter assumption is made, the calculated quantity of riboflavine adsorbed by kaolin would be greater than the riboflavine content of the capsule. Since this is untenable, the first assumption was adopted to correct for adsorption of the quantities of riboflavine dissolved in vitro. This provides a fresh set of dissolution results from which new T50 values were derived (Table 3).

Table 3. The average time (min) for 50% (T50) of the riboflavine content of the capsule to dissolve in vitro, corrected for adsorption.

| | Lubricant concn (% w/w) T50 | | | |
|---|---|---|---|---|
| Diluent | 0 | 0·5 | 1·0 | 2·0 |
| Avicel | 28·0 | 51·0 | 7·2 | 79·3 |
| Dri-flo | 43·3 | 31·8 | 8·7 | 16·0 |
| Emcompress | 107·0 | 159·5 | 650·0 | 942·5 |
| Kaolin | 11·5 | 8·1 | 9·3 | 8·8 |
| Primojel | 1·5 | 1·5 | 1·5 | 1·5 |
| Starch | 600·0 | 325·0 | 369·0 | 387·5 |

As before, statistical analysis (Table 4) indicates that the type of diluent has a significant influence whereas the influence of the lubricant is not

significant, but there is a significant statistical interaction between diluent and lubricant. It is therefore necessary to consider the ranking of the order of effectiveness of the diluents with respect to each concentration of lubricant present. Comparison of the rankings at each concentration of lubricant shows that statistically there is no significant difference between the relative effectiveness of the diluents at each lubricant concentration. An average order of dissolution rate can therefore be stated as: Primojel > sodium bicarbonate > kaolin and lactose > Avicel and Dri-flo > Emcompress and starch. Comparison of the rankings at each lubricant concentration with those derived from

Table 4. Analysis of variance of the effect of diluent type and lubricant concn on the time for 50% (T50) of the riboflavine content of the capsule to dissolve in vitro, corrected by adsorption.

| Source of variation | Sum of squares | Degrees of freedom | Mean square | Variance ratio, F |
|---|---|---|---|---|
| Diluent D | 4 351 885 | 7 | 621 698 | 6·48* |
| Lubricant L | 325 229 | 3 | 108 410 | 1·13 |
| Interaction DL | 2 074 983 | 21 | 95 952 | 7·90* |
| Residual R | 1 165 820 | 96 | 12 144 | |

* Significant at 1% level.

T50 values uncorrected for adsorption, indicates a highly significant difference. It is therefore clearly necessary to establish which of these two sets of T50 values reflects the in vivo situation.

*Assessment of riboflavine absorption in vivo*

To compare the influence of formulation on absorption of riboflavine, the cumulative quantity of drug excreted from 0 to 8 h, after administration of a particular formulation, was observed in four subjects, on two occasions. The formulations tested were: 1) The liquid formulation a 10 mg dose of riboflavine in 200 $cm^3$ of phosphate buffer, pH 4·8; 2) two capsules each containing 5 mg riboflavine in lactose and 3) two capsules each containing 5 mg riboflavine in Emcompress. The results (Fig. 2), when treated by analysis of variance, showed there was no significant differences between formulations or between subjects. There was, however, a highly significant interaction between formulation and subject, a possible source of which appears to be the particularly large inter-subject variation resulting from the administration of the solution formulation. When the results for the solution are excluded, there is a significant difference, assessed by analysis of variance, between the formulations at the early times. There is, however, still inter-subject variation. If the in vivo urinary excretion is compared with the in vitro assessment of the time for 50% of the drug content of the capsule to dissolve (Fig. 3), then, except for subject b, there is always greater excretion after administration of the capsule containing lactose as the diluent. Hence there seems to be some justification in considering that the in vitro effects of diluent type and lubricant effect do have some significance.

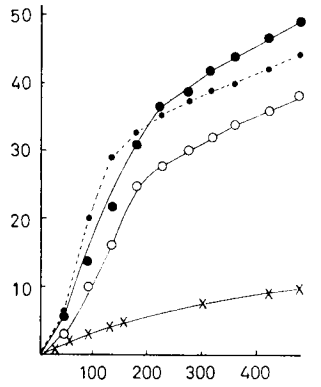


FIG. 2. Cumulative percentage amount of riboflavine excreted in 8 h from formulations containing 10 mg as: phosphate buffer solution, pH 4·8 ●- - ●; capsules containing Emcompress ○; capsules containing lactose ●—●; capsules containing kaolin ×. Ordinate: Cumulative percent riboflavine excreted in urine. Abscissa: Time (min).

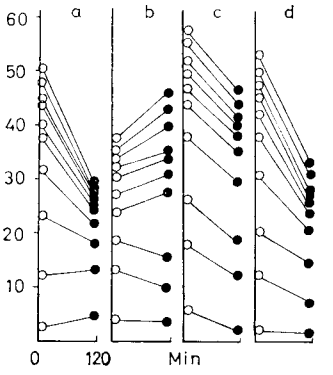


FIG. 3. Cumulative percentage of a 10 mg dose of riboflavine excreted at time intervals up to 8 h in four subjects (a–d) as a function of the time for 50% of the capsule formulation to dissolve in vitro. ○ Capsules containing lactose. ● Capsule containing Emcompress. Ordinate: Cumulative % of riboflavine excreted in urine. Abscissa: Time (min).

Part of the variability in response could be caused by variations in transit time of the formulation through the intestine. Since riboflavine is absorbed at a specific site in the upper part of the small

intestine (Levy & Jusko, 1966), the rate at which the formulation passes this site may be of greater importance than the solution rate. The solution formulation will pass more quickly through the gastrointestinal tract than the capsule formulations, thus accounting for the lower absorption from solution.

To assess whether the dissolution results should be corrected for adsorption, the urinary excretion of riboflavine from capsules containing kaolin was compared in two subjects. There is a clear reduction in the quantity of drug excreted (see Fig. 2) suggesting that the ranking of kaolin formulations should be based on the dissolution results uncorrected for adsorption. The lack of need for an adsorption correction may be because the adsorption corrections calculated for equilibrium adsorption are not valid in the case of the changing concentrations during the short period of the dissolution test. An alternative explanation is that adsorption of riboflavine onto kaolin is too tenacious for it to be readily removed from this diluent during passage down the gastrointestinal tract. Whatever the reason, the dissolution test uncorrected for adsorption provides a better indication of in vivo performance of the capsule formulations.

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