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An in vitro model for exploring CR theophylline–milk fat interactions

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Summary

The dissolution of the controlled-release theophylline formulations, Theodur tablet and Theodur sprinkle capsule, was studied in buffer of pH 6.5 and in milk with varying fat contents i.e. 0.1%, 2.0%, 5.0% and 7.5%. The topographical dissolution characterization made was based on a three-dimensional format with independent variables, time and percent fat content of milk. This plot revealed that the dissolution of capsule was markedly lower in all types of milk than in buffer. The dissolution of tablet was higher in milk with 0.1% fat content and slightly higher in milk with 2.0% and 5.0% fat content when compared to that in buffer. The dissolution of tablet in milk with 7.5% fat content was initially lower and then became progressively faster than observed in the aqueous medium. Binding experiments of theophylline to milk with 0.1% and 2.0% fat content, using concentrations encountered during the dissolution process, showed no significant interaction between theophylline and milk. The dissolution data clearly indicate a milk fat content dependent interaction between theophylline formulations and milk. In vivo data reported in literature and observed under "high fat meal" conditions were correlated with the dissolution data. Linear correlations were established in all cases. It was concluded that the 7.5% fat content milk mimics more closely the "high fat meal" conditions. This would aid the development of an appropriate dissolution test capable of simulating non-fasting conditions.

Introduction

The prolonged release of drugs from controlled-release (CR) formulations is most often accomplished by retarding the rate of drug's appearance in solution. Thus, the in vivo absorption of drug from these products is limited by the release rate. It is conceivable that the in vitro release studies with CR formulations are of extreme importance in regard to their in vivo availability.

This realization, growing over the years, prompted the Food and Drug Administration (FDA) to adopt the in vitro dissolution test as a tool for exploring and establishing in vitro/in vivo correlations with the CR formulations. However, it has been concluded recently (Skelly, 1986) that lack of consistent in vitro/in vivo correlations has been observed in the majority of cases.

In the field of theophylline CR formulations this inconsistency has been ascribed to the unexpected in vivo behaviour of several theophylline formulations when administered under non-fasting conditions. In a number of reports (Pedersen, 1981; Lagas and Jonkman, 1983; Osman et al., 1983; Hendeles et al., 1984; Pedersen and Moller-

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Pedersen, 1984; Karim et al., 1985; Hendeles et al., 1985; Karim, 1988), the influence of food on the absorption of theophylline CR products was found to be dependent upon the formulation and varied considerably (increased, decreased or unaltered bioavailability). These findings demonstrate that the predictability of the existing dissolution techniques is poor, mainly because of their incapability of simulating theophylline absorption under non-fasting conditions. In view of the single-dose, high-fat, food effect bioavailability study and the in vitro dissolution studies required by FDA for approval of a CR formulation, the demand for an in vitro dissolution test to mimic adequately the concomitant intake of the "high fat meal" is obvious.

A number of attempts (Jonkman et al., 1981; Wearley et al., 1985; Maturu et al., 1986) have been made in recent years to improve the predicting ability of the dissolution technique for the well-known food–CR theophylline formulations interaction. Two research groups (Jonkman et al., 1981; Wearley et al., 1985) suggested changes either in the pH or in both pH and composition of the dissolution medium. Wearley et al. (1985) studied the dissolution of CR theophylline formulations in buffers containing a combination of bile and fatty acids in order to mimic the GI fluids. Jonkman et al. (1981) concluded that only formulations exhibiting pH-independent profiles correlated well with the in vivo data. Wearley et al. (1985) gave indications for an association between the release rate of CR theophylline formulations dosed with a high-fat meal and the participation of the acids in the in vitro release of drug. Besides, the pH-independent character of theophylline dissolution from most of the CR formulations (Karim et al., 1985; Wearley et al., 1985) made ineffective (Maturu et al., 1986) the outstanding treatment (Skelly et al., 1986) of the in vitro percent dissolved as a function of time and pH in a 3-dimensional format. Based on this fact and attempting to explain the unexpected behaviour of several CR formulations when administered concurrently with a high-fat meal, Maturu et al. (1986) developed an in vitro dissolution test consisting of a pretreatment of CR theophylline formulations with peanut oil followed by the typical NF XIII in vitro dissolution test procedure for timed-release tablets and capsules. The authors (Maturu et al., 1986) established linear correlations between the in vitro data obtained with or without pretreatment with peanut oil and the in vivo data obtained under fed and fasting conditions, respectively.

The above studies demonstrate that the unanticipated in vivo behaviour of certain CR theophylline formulations cannot be explained on the basis of pH alterations in the dissolution medium. On the contrary, the presence of bile and fatty acids (Jonkman et al., 1981) in the dissolution medium or the pretreatment with peanut oil (Maturu et al., 1986) have been shown to improve the predictability of dissolution tests. Relying on these observations, we support the idea that a suitable in vitro model for exploring food effects on CR theophylline formulations should be capable of providing dissolution data as a function of the fat content of the dissolution medium. Thus, this investigation describes an in vitro model which utilizes the topographical dissolution characterization with time and fat content as independent variables to mimic and explore CR theophylline formulations–high-fat interactions. Milk has been utilized as a dissolution medium for the study of the dissolution of CR theophylline formulations since: (i) medicines are frequently swallowed with milk or milk-containing drinks; (ii) previous experience (Macheras et al., 1987) has shown that the dissolution profiles of CR theophylline formulations can be distinguished in milk; and (iii) its fat content can be both adjusted and varied.

Materials and Methods

Anhydrous theophylline and caffeine were kindly supplied by Lavipharm (Athens, Greece). Both drugs were found to be > 99% pure by high-performance liquid chromatography. Phosphate buffer 0.02 M of pH 6.5 was prepared using Na_2HPO_4 (Merck) and concentrated 18 M NaOH solution. All other chemicals used were of analytical grade, and used as received.

Skim milk and milk cream were obtained from FAGE (Metamorphosis, Greece). The milk fat content of each type of milk used, was adjusted by

mixing the appropriate volumes of skim milk and milk cream according to the Pearson's square rule. The final fat content of the milk used for the studies was estimated in undiluted samples by infrared absorption spectrophotometry (Multispec-N Infrared Milk Analyzer, York, U.K.). It was found to be always $\pm 0.1\%$ of the desired fat content, i.e. 2.0%, 5.0% and 7.5%. The fat content of the skim milk was measured and found to be 0.1%.

The CR formulations of theophylline used in this study were: (**A**) Theodur tablets (Key Pharmaceuticals, Miami, FL, U.S.A.) formulated by Lavipharm (Greece, Lot 60202); and (**B**) Theodur Sprinkle capsules (Key Pharmaceuticals, Miami, FL, U.S.A.) formulated by Lavipharm (Greece, Lot 51102).

Binding studies

The binding of theophylline to milk proteins and other milk components was determined by equilibrium dialysis of milk samples, against a phosphate buffer (0.2 M, pH 6.5) solution as described previously (Macheras et al., 1988). Skim milk and milk of 2.0% fat content were used in these studies. The equilibrium dialysis was carried out at 37 °C, and equilibrium was reached after 4 h. Samples were prepared as follows: theophylline was initially dissolved in 0.01 M NaOH; this stock solution was diluted using phosphate buffer (0.2 M, pH 6.5) and the final concentration in the samples ranged from 200 to 800 μg/ml.

The "free" concentrations of theophylline were determined by a modified high-performance liquid chromatography (HPLC) method (Shihabi, 1978). Each sample (1 ml) was vortexed with 0.2 ml of a caffeine solution (200 μg/ml), which was used as the internal standard; 20 μl of the vortexed solutions were injected in the chromatograph. The HPLC system consisted of a Waters Model U6K universal injector, a Model 590 solvent delivery system, a Model 481 LC variable wavelength UV detector set at 254 nm, and a Lichrosorb 10RP18 reversed-phase column (25 cm $\times$ 4.6 mm). The mobile phase consisted of 10% ACN in 0.01 M sodium acetate buffer with pH 4.0. The HPLC conditions were: flow rate 2.5 ml/min; pressure 900 psi; attenuation 0.1–0.2 AUFS.

The binding of theophylline to the membranes and cell was checked and found negligible.

Dissolution studies

The dissolution studies were performed in a DT 6 Erweka dissolution apparatus. The paddle method was used with a rotation speed 100 rpm. The dissolution of the two CR theophylline formulations were studied in milk with fat content: 0.1% (skim milk), 2.0%, 5.0% and 7.5% at 37 ± 0.5 °C. For comparative purposes the dissolution was also run in a phosphate buffer (0.02 M, pH 6.5).

Milk samples (4 ml) were diluted to 10 ml with phosphate buffer (0.02 M, pH 6.5) and dialysed together with standard solutions prepared in the same medium, using the automated flow-injection dynamic dialyzer described previously (Macheras et al., 1987). The determination of theophylline in the milk dialysates was performed by the HPLC method described above. However, the measurement of the absorbance of the samples at 271 nm (λ_{max} of theophylline at pH 6.5) gave identical results with the HPLC method and was used because of its simplicity in the later part of the study. All other conditions, not specified above, relevant to dissolution, sample collection, dialysis and validation of analytical technique were as described previously (Macheras et al., 1987).

Results and Discussion

The binding of theophylline to skim milk (0.1% in fat content) and milk of 2% fat was found to be too low in the range studied. The percent bound was 3.68 ± 3.38 and 4.92 ± 1.78 ($\pm$S.D., $n = 8$) for the milk of 0.1 and 2.0% fat, respectively. These estimates of binding indicate that theophylline in milk is almost totally unbound (> 95%). Binding studies with milk of 5.0% and 7.5% fat were not carried out since these milk types could produce problems to the membrane (Syversen and Ratkje, 1985). Nevertheless, the binding results obtained, coupled with the hydrophilicity of theophylline molecule (log $P = 0.02$) (Hansch and Leo, 1979), imply a similar extent of theophylline bind-

ing to milk types with higher fat content (Macheras et al., 1988; Antimisiaris, 1988).

Dissolution profiles in a 3-dimensional format in all media for the two formulations are shown in Figs. 1 and 2. For the sake of clarity, the standard deviations of the percent dissolved drug are not shown on the graphs. However, the standard deviation of the percent dissolved theophylline for the dissolution experiments in milk varied from $\pm 1.0\%$ to $\pm 9.1\%$ while the experiments in buffer showed a variation from $\pm 0.6\%$ to $\pm 6.1\%$ ($n = 6$). These results are indicative of the precision of the dissolution profiles in all media studied.

Inspection of Figs. 1 and 2 reveals a marked difference between the formulations with regard to the effect of medium (buffer vs milk) and milk fat content on the rate of dissolution of theophylline. The dissolution of product **A** in skim milk is faster than in buffer. However, by increasing the fat content of milk, product **A** exhibits a progressively similar pattern in buffer and milk. Thus, the

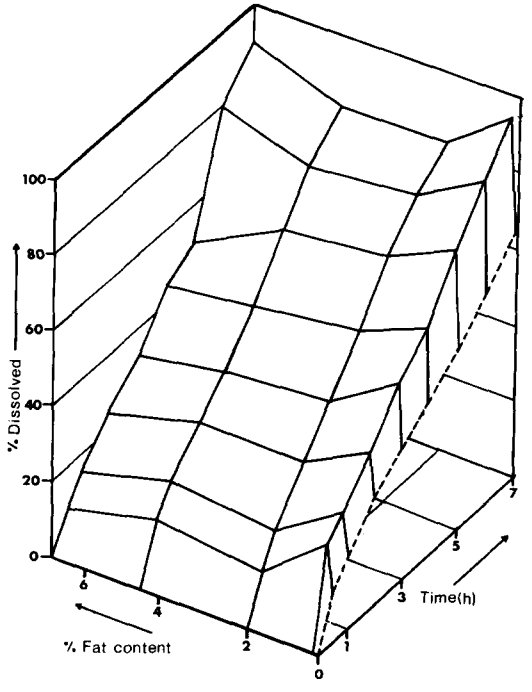

Fig. 1. Topographical dissolution characterization of Theodur tablet (200 mg) as a function of time and milk fat content. The dashed line corresponds to the dissolution in buffer pH 6.5. Each point is the mean of 6 experiments.

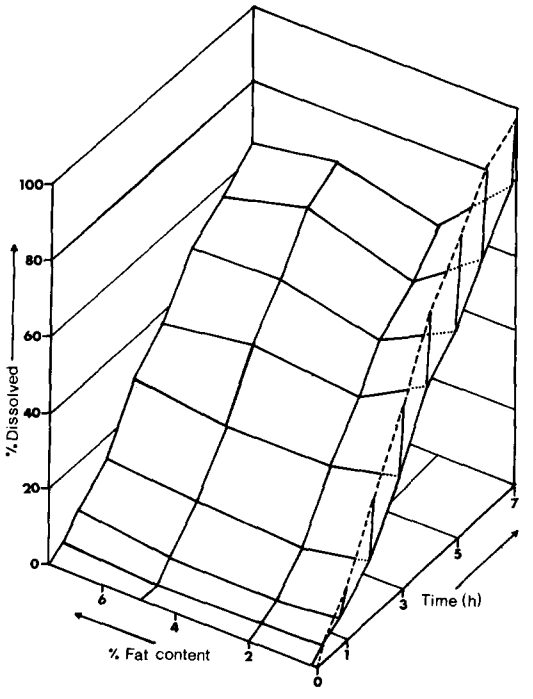

Fig. 2. Topographical dissolution characterization of Theodur Sprinkle capsule (200 mg) as a function of time and milk fat content. The dashed line corresponds to the dissolution in buffer pH 6.5. Each point is the mean of 6 experiments.

dissolution of product **A** in milk with 7.5% fat content shows an initial (0–2 h) low dissolution rate which becomes faster resulting in statistically higher percent dissolved theophylline only at the latest time points, 6 and 7 h (Table 1). In contrast, product **B** shows lower dissolution profiles in all types of milk utilized than in buffer (Fig. 2). The lowest dissolution profile for product **B** was noted at 2% fat content milk. An overall view of the statistical comparison of the dissolution profiles of the two formulations in the various media at all time points is presented in Table 1.

The conventional application of the in vitro topographical characterization is to delineate effectively the differences observed in vitro for bioinequivalent products. So far, pH, in addition to time, has been utilized as an independent variable to provide dissolution data for a multidimensional topographical image (Skelly, 1986). However, both products **A** and **B** exhibit pH-independent dissolution rates (Maturu et al., 1986). Accordingly, an in

TABLE 1

Comparison [a] *of dissolution profiles of CR theophylline products in buffer vs milk with varying fat contents*

Time (h)	Product A [b]				Product B [c,d]
	Milk fat content (%)				
	0.1	2	5	7.5	
0.5	*	N.S.	N.S.	* [d]	*
1.0	*	N.S.	N.S.	* [d]	*
2.0	*	N.S.	N.S.	* [d]	*
3.0	*	N.S.	N.S.	N.S.	*
4.0	*	*	N.S.	N.S.	*
5.0	*	*	*	N.S.	*
6.0	*	*	*	*	*
7.0	*	*	*	*	*

[a] Based on the percent dissolved theophylline ($\pm$S.D., $n = 6$) in the two media at the time point indicated utilizing t-test; theoretical (95%), 2.22.

[b] Asterisks denote a statistically significant difference at 0.05 level; N.S. denotes statistically insignificant difference at 0.05 level.

[c] For all milk types examined at all time points considered, the statistically significant difference was observed at 0.05 level.

[d] Lower percent dissolved theophylline in milk than in buffer.

vitro model was developed in the present study to mimic the in vivo availability of products **A** and **B** under "high-fat meal" conditions. Milk was utilized as the dissolution medium and the percent of theophylline dissolved in vitro was followed as a function of time and fat content of milk. Thus, if one assumes that the dissolution profiles in buffer correspond to the in vivo dissolution of theophylline under fasting conditions, then the bioavailability changes induced by food (Maturu et al., 1986) on products **A** and **B** can be qualitatively correlated with some of the dissolution profiles in milk presented in Figs. 1 and 2. For example, the initial retardation of the dissolution of product **A** in milk with 7.5% fat content adheres closely to the in vivo observations, i.e. the lower rate of absorption under non-fasting conditions. In addition, the dramatic decrease of the rate and extent of absorption of theophylline from product **B** under high-fat breakfast conditions (Maturu et al., 1986) is predicted from either of the lower dissolution profiles in the various types of milk (Fig. 2).

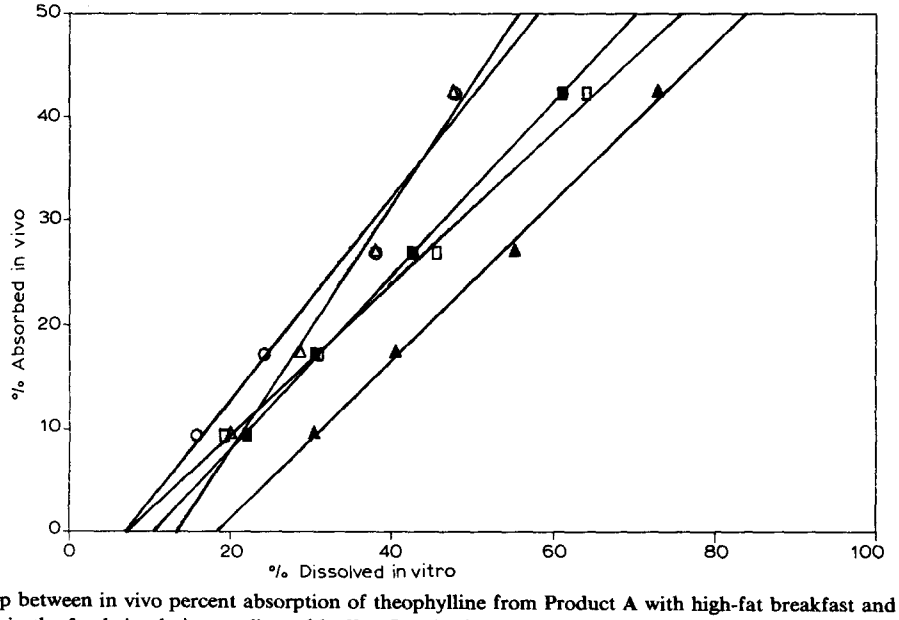

Fig. 3. Relationship between in vivo percent absorption of theophylline from Product A with high-fat breakfast and in vitro percent release of the drug in the food-simulating media and buffer. Standard deviations of the percent dissolved (not shown for the sake of clarity) ranged from 1.6% to 6.3% and from 1.0% to 9.0% for the dissolution in buffer and the various types of milk, respectively. *Key* (medium, % fat content): △, (buffer) ($r = 0.990$); ▲, (milk, 0.1) ($r = 0.998$); □, (milk, 2) ($r = 0.998$); ■, (milk, 5) ($r = 0.9997$); ○, (milk, 7.5) ($r = 0.985$).

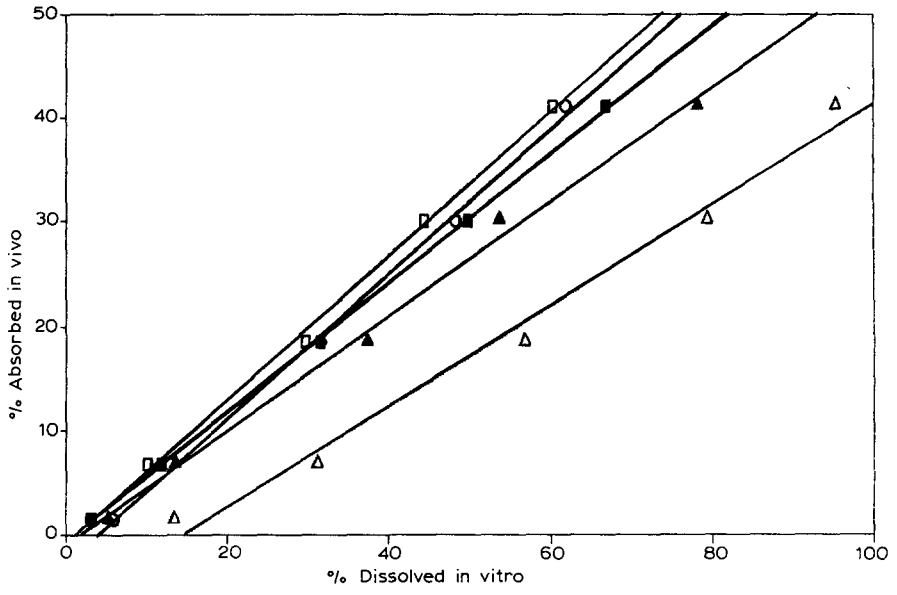

Fig. 4. Relationship between in vivo percent absorption of theophylline from Product **B** with high-fat breakfast and in vitro percent release of the drug in the food-simulating media and buffer. Standard deviations of the percent dissolved (not shown for the sake of clarity) ranged from 1.3% to 5.4% and from 0.6% to 6.3% for the dissolution in buffer and the various types of milk, respectively. *Key*: as in Fig. 3; the correlation coefficients, r, in the same order as in Fig. 3 were: 0.993, 0.997, 0.993, 0.999, 0.9991.

In order to evaluate further in a quantitative manner the predicting ability of the in vitro model proposed, in vitro/in vivo correlations were attempted. The in vivo data under the "high-fat meal" conditions as percent of theophylline absorbed at various times were taken from Maturu et al. (1986). Linear relationships were found between the percent of the in vitro dissolved drug and the percent of the in vivo absorbed drug at identical times for the two products in all cases examined (Figs. 3 and 4). Some interesting conclusions can be drawn from Figs. 3 and 4 if one assumes that the batch-to-batch variability of the formulations is negligible in comparison with the variability of the in vitro and in vivo data observed. Although the analysis resulted in linear correlations with high correlation coefficients, the linear relationships do not necessarily imply the equivalence of the predicting ability of the vitro media utilized. This can be concluded by considering the significant differences in the values of slope and intercept of the regression lines (Table 2). From the data available, however, the 7.5% fat content milk can be considered as the most akin, from the media examined, to the "high-fat meal" conditions for the following reasons: (i) the lower initial dissolution rate in comparison to this in buffer for product **A** (Fig. 1, Table 1) is in accordance with the lower absorption rate observed under non-fasting conditions (Maturu et al., 1986);

TABLE 2

Values of slope and x-intercept of linear correlations of Figs. 3 and 4

Product	Slope (S.D.)–x-intercept (S.D.)				
	Buffer	0.1% [a]	2% [a]	5% [a]	7.5% [a]
A	1.17(0.12)–13.11(3.7)	0.761(0.031)–18.3(2.3)	0.734(0.031)–7.0(1.9)	0.834(0.014)–10.28(0.73)	0.97(0.12)–6.9(4.2)
B	0.48(0.031)–14.56(4.2)	0.55(0.027)–1.86(1.7)	0.69(0.029)–1.11(0.80)	0.620(0.004)–1.06(0.29)	0.69(0.021)–3.83(0.92)

[a] Milk fat content.

the statistical comparisons of dissolution profiles in Table 1 (column 5) is in agreement with the equivalency of the extent of absorption of product **A** under fed and fasting conditions; (ii) the in vitro/in vivo correlation for product **A** was the best since it is accompanied by a slope nearly equal to one (0.97) and with the lowest x-intercept, (Table 2); and (iii) the lower bioavailability of product **B** anticipated under non-fasting conditions from Fig. 2, is adequately well described as is demonstrated by the increase of slope (0.48 in buffer, 0.69 in milk with 7.5% fat) and the reduction of the x-intercept (14.56 in buffer, 3.83 in milk with 7.5% fat) of the in vitro/in vivo correlation.

Another point which requires clarification is the relevance of binding to dissolution studies. It was shown above that theophylline is negligibly bound ($< 5\%$) to milk, and therefore the dissolution profiles represent free theophylline levels. Accordingly, the discussion and the analysis presented is valid since it is focused exclusively on the well-established (in the literature) interaction of CR theophylline formulations with food. The binding results of the present study, utilizing concentrations encountered during the in vitro and in vivo dissolution process, confirmed the lack of any specific interaction between fat and theophylline. However, it is conceivable that the model proposed can have a more general application. Other marketed products, e.g. procainamide CR, quinidine CR, phenytoin extended etc., could be also examined. In view of prospective studies and whether or not food effects are unrelated to the dosage form, binding studies are indispensable to elucidate the extent of drug–milk (of various fat contents) interaction. This will facilitate the proper interpretation of the in vitro data in relation to the in vivo observations in particular when the extent of binding is found to be dependent on the fat content of milk.

The findings of the present study show that the wetting of products **A** and **B** in the "high-fat meal" simulating medium, milk of 7.5% fat content, is retarded slightly and significantly, respectively. This resulted in dissolution profiles which were found to be in good agreement with the in vivo data. In comparison to other studies on this topic (Jonkman et al., 1981; Wearley et al., 1985; Maturu et al., 1986; Macheras et al., 1987), the present work seems to be the first providing an insight into the CR theophylline formulation–food interaction. The novel method proposed extends the classical use of topographical dissolution characterization since other variables of interest, e.g. protein content, miscellaneous media of varying composition, can be incorporated in a 3-dimensional image.

The model proposed would aid the development of an appropriate dissolution test capable of simulating "high-fat meal" conditions. This is certainly of value in view of the unsuccessful in vivo approach attempted recently (Shiu et al., 1988).

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