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In vitro dissolution testing of oral controlled release preparations in the presence of artificial foodstuffs. II. Probing drug/food interactions using microcalorimetry

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Summary

The dissolution of tetracycline hydrochloride from a commercial sustained release product (Organon-Tetrabid) and from a dispersion of the drug in a semi-solid matrix was studied. Two techniques were used to follow dissolution, these were the USP paddle method (with UV analysis) and microcalorimetry. The influence of calcium on the dissolution rate was studied using the USP method, and was found to have no effect. Dissolution experiments were undertaken in the calorimeter using hydrochloric acid alone or with the addition of: (i) calcium; (ii) milk; (iii) Ensure; and (iv) Intralipid. It was possible to observe the known interaction between tetracycline and calcium, milk and Ensure during the dissolution process in the microcalorimeter. The calorimetric output during dissolution (a composite response for dissolution of the drug and base, and any measurable interaction that occurs) of the products in acid and the non-interacting Intralipid, being distinctly different to the output obtained during the dissolution in interacting fluids. This was equally true for both products. The kinetics describing the release from the semi-solid matrix show that an apparent zero-order process occurs for about 150 min followed by an apparent first-order process. The data obtained from the microcalorimeter showed considerable differences in the first-order release process, depending upon the nature of the dissolution fluid; those with the highest content of lipid giving different results to the more aqueous fluids. With careful selection of experiments microcalorimetry is a useful technique for the in vitro study of drug/food interactions and the influence of food on drug release profiles.

Introduction

In the previous paper in this series, a study exploring the use of microcalorimetry to monitor the dissolution of oral controlled release dosage forms in complex media was detailed (Ashby et al., 1989).

The use and design of dissolution tests remains a contentious issue, with some workers holding the view that only simple official testing procedures should be considered, whilst others advocate a need for testing over and above the conventional, with a view to produce a more physiologically realistic test. These issues were addressed in a

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previous publication (Ashby et al., 1989). It is our view that there is a need for an in vitro test that can be used, during formulation research and development, to attempt predictions of dissolution rates in the presence and absence of foodstuffs, and to examine the existence of drug/food interactions. Such a test would be a suitable forerunner to animal experimentation. The existence of such unofficial tests should not be taken to imply that they would replace the official procedures which must remain as the norm to which reference is made.

It has been demonstrated (Ashby et al., 1989) that the presence of model foodstuffs will alter the dissolution rate of Phyllocontin continus (controlled release aminophylline) compared with the rate observed in buffer alone. In some circumstances it is possible to use a conventional USP method with such complex media (e.g. Ensure – a nasogastric foodstuff) as acidification and centrifugation allowed precipitation and removal of suspended matter and thus facilitated assay by ultraviolet spectrophotometry. With some model foodstuffs (e.g. Intralipid – a fatty emulsion) it was not easy to produce a clear solution suitable for UV assay, hence the need for an alternative analytical technique. Microcalorimetry was selected to monitor the dissolution of the product as it is not demanding with respect to the form of sample that is presented to the instrument; thus it is possible to monitor dissolution rate directly, using a flow-through system, without the need to remove suspended/coloured/interfering material(s) (Ashby et al., 1989).

As well as understanding the extent to which food will alter the rate of release of drug from a product, it would also be valuable to know whether any major interactions were observed between the product and contents of the gastrointestinal tract. It is clear that drug which has reacted/interacted with components of foodstuffs may no longer be available for systemic absorption.

A well-known case of reduced drug absorption due to gastric contents is that of tetracycline. Tetracycline is predominantly absorbed from the stomach and small intestine where the pH is low, but in the presence of milk, milk products, antacids, calcium salts and iron preparations (amongst others) the absorption is significantly lowered. This is a well-known interaction, due to chelation (Neuvonen et al., 1974; Barr et al., 1971).

This study involves the use of microcalorimetry to investigate the interaction between model foodstuffs, calcium and tetracycline.

One of the dosage forms that was used was a semi-solid matrix system designed to give sustained release. The role of semi-solid matrix technology in the preparation of controlled release preparations will be discussed.

Materials and Methods

The semi-solid matrix formulation

The product was prepared by incorporating sufficient tetracycline hydrochloride in a molten Gelucire base (Gattefosse, 50/02) to produce unit dosage forms containing 250 mg of drug. Total weight of dosage form was about 0.75 g, being prepared in a size '00' hard gelatin capsule.

Thermal history of the molten base was controlled in order to standardise the structure of the preparation. In order to avoid the need for stability studies, all products were freshly prepared prior to testing. Chemical assay for active ingredient demonstrated that no detectable loss of activity was apparent within one week of manufacture.

Other materials

The dissolution and calcium interaction of the sustained release product Tetrabid (Organon, capsule of tetracycline hydrochloride 250 mg) were also investigated.

A standard solution of calcium chloride was used such that an approximate ratio of 1 : 2 existed for tetracycline/calcium ion. Model foodstuffs containing calcium were used. These were: long life UHT milk (low fat) and Ensure (Abbott, an enteral foodstuff containing protein, fat, carbohydrate, minerals (several of which are known to interact with tetracycline) and vitamins). Intralipid (KabiVitrum, lipid emulsion with no ionic additives) was used to investigate the effect of supposedly non-interacting foodstuffs. In all cases the additive was diluted with a suitable quantity

of hydrochloric acid, so reference below to dissolution in, for example, Ensure should be taken to mean Ensure diluted with hydrochloric acid. Dilution proportions for Ensure and Intralipid were 100 ml of product made up to 1000 ml with 0.1 mol/dm^3 HCl.

USP dissolution testing

Experiments were carried out in a litre vessel at 37 °C stirring (paddle) at 100 rpm. To avoid the presence of metal ions buffers solutions were not used, so dissolution was undertaken in 0.1 mol/dm^3 hydrochloric acid (which is to a reasonable extent self-buffering).

The following experiments were undertaken using the USP apparatus: dissolution of the semi-solid matrix product in 0.1 mol/dm^3 HCl with and without calcium ions. Four replicate determinations were carried out. Assay was by ultraviolet spectrophotometry at a wavelength of 353 nm. A calibration curve for absorbance against concentration was obtained from a solution of tetracycline (Sigma). By adding calcium to these standard solutions it was demonstrated that no interference was observed in the assay procedure for tetracycline.

Microcalorimetric studies

Dissolution studies were undertaken with the product held in the cell of the calorimeter as described previously (Ashby et al., 1989); however, since the earlier work, a larger stainless steel cell has been engineered so that all dosage forms can be easily placed within the cell, whereas previously it was necessary to halve tablets. The microcalorimeter (LKB 10700) measures the heat changes that accompany any reaction or interaction, giving an output of power as a function of time. The instrument will record the total heat change associated with the sum of all processes that occur within the calorimeter cell (e.g. dissolution, interaction and other unwanted effects such as degradation of the tetracycline). As a result, blanking experiments must be performed to allow subtraction of unwanted contributions to the overall response, e.g. investigation of possible response for degradation of tetracycline solution. To investigate whether instability of the tetracycline would produce a significant component to the response, a solution of the drug in hydrochloric acid was monitored as it flowed through the calorimeter.

Studies to investigate drug release from semi-solid matrix systems containing tetracycline hydrochloride were undertaken in the presence of: (i) 0.1 mol/dm^3 HCl; (ii) calcium ions, viz. calcium chloride in hydrochloric acid; (iii) Ensure; (iv) milk; and (v) Intralipid. In all cases the microcalorimeter was operated with an amplifier setting of 300 μV.

To see if the findings could be related to more than one tetracycline product, dissolution of Tetrabid-Organon was investigated in hydrochloric acid, and in the presence of: (i) calcium chloride; and (ii) milk.

Results

USP dissolution studies

The semi-solid matrix released drug by a process which fitted zero-order kinetics for approximately 150 min, this being equivalent to about 80% drug release. The remaining 20% of drug release followed an apparent first-order process. Typical dissolution profiles are shown in Fig. 1. The apparent zero-order rate constants obtained from the graphs of concentration released as a function of time were 4.45×10^{-8} mol·l^{-1}·s^{-1} for dissolution in hydrochloric acid (average value

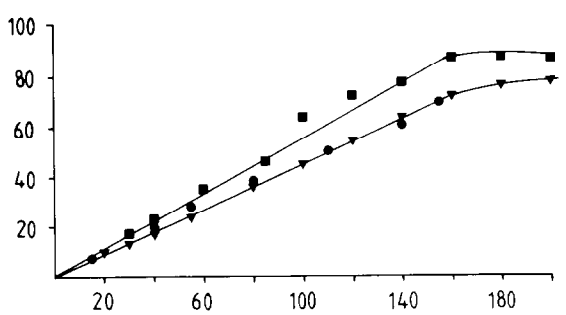

Fig. 1. Release profile obtained using the USP paddle apparatus, for dissolution of semi-solid matrix containing tetracycline hydrochloride, in interacting and non-interacting fluids. Key: ▼, calcium chloride; ● and ■, hydrochloric acid alone. *y* axis = % released; *x* axis = time (minutes).

quoted, range from 4.33 to 4.63×10^{-8}) and for dissolution in hydrochloric acid with dissolved calcium chloride, 4.48×10^{-8} mol · l^{-1} · s^{-1} (average value quoted, range from 4.35 to 4.62×10^{-8}).

This demonstrates that the presence of calcium did not alter the dissolution rate of the product.

Microcalorimetric studies on the tetracycline semi-solid matrix product

The first blanking experiment was to determine if a solution of tetracycline hydrochloride in hydrochloric acid produced a heat change due to instability of the solution. A sample of such a solution did not produce a deflection when passed through the calorimeter indicating that at this sensitivity any degradation of the solution would not contribute to the results. The results are expressed as displacement from the baseline as a function of time. The displacement from the baseline is a measure of the rate of change of heat with time (dq/dt) thus the results constitute a power–time (p–t) curve. The p–t curves include the response for the dissolution of the semi-solid matrix product in the presence of the various test fluids, together with a response for any interaction that occurs. Typical results are presented in Fig. 2. Fig. 3 shows that a plot of ln(displacement) as a function of time (for two representative systems) is non-linear during the period over which most of the drug is released, meaning that simple first-order kinetics cannot be applied to describe this section of the release profile. The second section of the release profile will be discussed later. If the response observed in the calorimeter was zero-order, the p–t curve would be parallel with, but displaced from, the baseline. The response seen in Fig. 2, that is two sequential straight lines for the p–t curve, is not easy to interpret. The calorimetric output represents a sum of the response for dissolution of the base, the dissolution of the drug and any interactions that take place. From the USP data it is clear that the release profile of the drug fits zero-order kinetics for the majority of the drug release process, the calorimetric response is, therefore, a sum of this zero-order response together with a further response for dissolution of the base. The dissolution of a placebo product resulted in a calorimetric output that was almost indistinguishable from that of the active semi-solid system in non-interacting fluids, i.e. the contribution due to dissolution of the drug is small. The response obtained for the placebo product was not altered by the addition of calcium ions, showing that calcium does not alter the dissolution process (this fact is supported by the USP data), and that calcium does not have a measurable interaction with the semi-solid base.

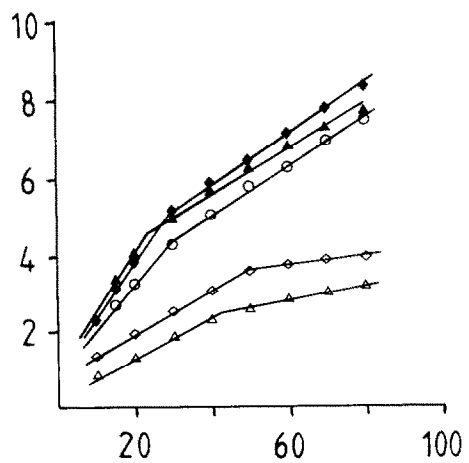

Fig. 2. Power–time curves obtained from the microcalorimeter for the dissolution of semi-solid matrix containing tetracycline hydrochloride in interacting and non-interacting media. Key: ◆, milk; ▲, calcium; ○, Ensure; ◇, HCl; △, Intralipid. y axis = dq/dt (arbitrary units); x axis = time (min).

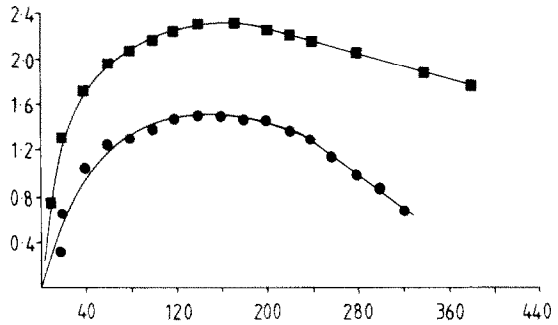

Fig. 3. Representative plots of ln(displacement) as a function of time for the calorimetric output during dissolution of the semi-solid matrix system. Key: ●, HCl; ■, milk. y axis = $\ln(dq/dt)$; x axis = time (min).

The gradients of the p–t curves obtained from the data presented in Fig. 2, are presented in Table 1. These values are descriptive of the pro-

TABLE 1

Mean apparent rate functions obtained from the gradient of the power–time output from the microcalorimeter, during the dissolution of semi-solid matrix systems containing tetracycline hydrochloride, in the presence of various interacting and non-interacting fluids

Dissolution fluid	Apparent rate functions	
	Stage 1 (arbitrary power units/s) ($\times 10^3$)	Stage 2 ($\times 10^3$)
Non-interacting		
0.1 mol/dm^3 HCl	1.00	0.26
Intralipid	0.81	0.32
Interacting		
Calcium chloride	2.46	1.08
UHT milk	2.63	1.12
Ensure	2.19	1.09

cess, and for want of a better term, will be described as apparent rate functions of complex nature. In Table 1 the notation of first and second stage refers to the process before and after 30 min for the interacting, and before and after 50 min for the non-interacting systems.

It is clear from Fig. 2 and Table 1 that the results fit into two discrete groups; the dissolution of the product in HCl and the dissolution in Intralipid (non-interacting model foodstuff) having apparent rate functions in the order of 1×10^{-3} arbitrary power units/s for stage 1 and 3×10^{-3} arbitrary power units/s for stage 2, this being distinct from the responses observed with calcium, milk and Ensure, which have apparent rate functions in the region of 2.5×10^{-3} arb. power units/s (stage 1) and 0.3×10^{-3} arb. power units/s (stage 2).

The results obtained for calcium chloride, milk and Ensure are indistinguishable within experimental error, this suggests that the calcium ion (and other interacting ions for Ensure) are present in excess concentrations in all the systems, thus the response is in each case limited by the release of the tetracycline from the product. As well as having different kinetics, the responses observed in the calorimeter in the presence and in the absence of interacting species have distinctly different peak heights. Table 2 shows the mean peak

TABLE 2

The mean peak height, and mean time taken to achieve peak height, of the power–time curve for dissolution of semi-solid matrix products in the presence of interacting and non-interacting fluids

Dissolution fluid	Peak height (arbitrary units)	Time to peak height (min)
Non-interacting		
0.1 mol/dm^3 HCl	4.78	155
Intralipid	3.50	160
Interacting		
Calcium chloride	8.95	165
UHT milk	10.65	160
Ensure	10.00	200

height response for the power/time output of the calorimeter under the defined experimental conditions. It can be seen that the response for the interacting samples was in the order of twice that observed for the non-interacting; however, there was (in general) no difference in the time required for this peak to be achieved.

Microcalorimetric dissolution of Tetrabid

To test the applicability of the results obtained, experiments were undertaken on the commercially available sustained release product Organon-Tetrabid. The microcalorimeter was used to monitor release/interaction in the presence of 0.1 mol/dm^3 hydrochloric acid alone, and with calcium chloride and UHT milk. The data were found

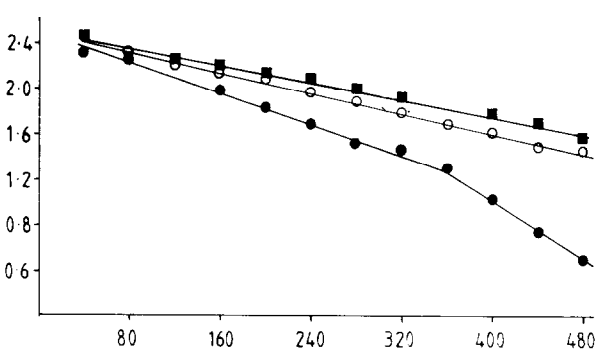

Fig. 4. $\ln(dq/dt)$ as a function of time for the dissolution of Organon-Tetrabid in 0.1 mol/dm^3 HCl, calcium chloride and milk. Key: ●, HCl; ○, calcium; ■, milk. y axis = $\ln(dq/dt)$; x axis = time (min).

to fit an apparent first-order profile for the interacting fluids, and two consecutive first-order profiles for the non-interacting fluid, as shown in Fig. 4. In all cases the dissolution of Tetrabid was endothermic, unlike the early stage of dissolution of the semi-solid matrix which was exothermic. The apparent first-order rate constants obtained for the dissolution of Tetrabid in the microcalorimeter are 3.4×10^{-5} s^{-1} with calcium present, 2.8×10^{-5} s^{-1} with milk, and with just hydrochloric acid 5.72×10^{-5} s^{-1} the first stage (0–360 min) and 1.12×10^{-4} s^{-1} for the second stage.

Discussion

The results described above provide a good deal of information. In a previous publication (Ashby et al., 1989), Intralipid was reported to produce a change in dissolution rate, in this work the change in dissolution rate (at least over the period in which most of the dissolution occurs) is minimal compared to hydrochloric acid alone.

The USP dissolution method demonstrates that the dissolution of the semi-solid matrix system is not influenced by the presence of calcium (i.e. the rate remains the same despite the existence of the known interaction). It is interesting to note that such a semi-solid matrix system delivers drug by an apparent zero-order mechanism, suggesting that it has considerable advantage as a controlled drug delivery system.

The differences in apparent rate constant observed between interacting and non-interacting fluids for the dissolution of the semi-solid matrix in the calorimeter are due to the response being a composite of the dissolution process and the interaction. It should be true that the presence of, for example, calcium ions, will not alter the actual release of tetracycline in the microcalorimeter cell if it does not alter release of tetracycline in the USP apparatus. However, the release constants obtained from the microcalorimeter should not be expected to be the same as those obtained from the USP method due to the different hydrodynamic regimes.

In each case the dissolution in the presence of interacting species produced a more exothermic reaction than in the presence of non-interacting species. The kinetics of the interaction were not allowed to dominate that particular response, because the presence of tetracycline was to some extent a rate-limiting step, thus the interaction process was linked to the dissolution kinetics.

Comparison of the results obtained for the dissolution of Tetrabid and the dissolution of the semi-solid matrix product shows that the dissolution of the excipients dominate the power–time response for at least one of the products. Tetrabid release is characterised by an endothermic response, whilst the semi-solid matrix release profile is initially exothermic. The interaction between the ions and tetracycline is exothermic as shown by an increased exothermic response in the presence of the semi-solid matrix system, and a decreased endothermic response with Tetrabid.

The kinetics of release from the semi-solid matrix changed from being zero-order after a period of about 150 min (USP data, Fig. 1). The change in output from a net endothermic to net exothermic response, observed using microcalorimetry, also occurred at 150 min (Fig. 3). It is interesting to note that over the period in which about 80% of the drug release from the semi-solid matrix occurs in the USP test, the microcalorimetric output is exothermic indicating that the process may be favourable (depending upon entropy) and thus perhaps explaining why the product has not produced a slower release profile. After this period of exothermic release (corresponding with the zero-order release section obtained from the USP ex-

TABLE 3

Apparent first-order rate constants for the endothermic portion of the dissolution of the semi-solid matrix product, obtained from the microcalorimeter (after 150 min)

Dissolution fluid	Apparent rate constant (s^{-1}) ($\times 10^5$)
0.1 mol/dm^3 HCl	14.8
Intralipid	6.2
Calcium chloride	3.7
UHT milk	4.4
Ensure	2.4

periments), the process changes and an endotherm is observed. The form of this endotherm (corresponding to the very slow release of the remaining few percent of drug, as observed using the USP test) is dependent upon the dissolution fluid used.

The endothermic sections observed during the dissolution of the semi-solid matrix product in each of the dissolution fluids, fit first-order kinetics. The results for the apparent first-order rate constants of the endotherms are presented in Table 3. The change in rate of release observed at about 150 min is probably due to hydration of the semi-solid polymer, producing a gel-like structure from which drug will be released by a slow diffusion process, resulting in a very extended first-order release profile (endothermic calorimetric response). The information in Table 3 demonstrates that two processes are being monitored, these are: (1) the presence of interacting species; and (2) the influence of the different fluids, causing an alteration to the release process; for example, Intralipid will probably produce less hydration of the polymer, thus explaining the comparatively small endothermic response. It follows that the microcalorimetric results obtained after about 150 min do not just provide information on the interaction between the drug and the calcium, but also show how the different fluids alter the dissolution process. It is clear that the fluids with high lipid content (Intralipid and milk) limit the extent of the endothermic process. To extract information on any one process that is occurring it would be necessary to undertake a range of control experiments.

Conclusions

It is possible to detect interaction between contents of foodstuffs and drug substances by use of microcalorimetry. The most effective way to use such a technique would be in combination with conventional dissolution data, and with systems where an in-line analysis is possible (i.e. flow-through UV spectrophotometer). To make full use of the data available it would be important to understand if the dissolution fluid is altering the drug release or interacting with the drug species. This can be studied by using simple solutions of, for example, metal ions and monitoring their effect on both dissolution rate (USP) and microcalorimetric output. Interactions between drugs and food components can be studied by flowing two solutions (one of drug, one of foodstuff) into the calorimeter, where they are mixed (thus removing the dissolution response).

It can be seen, therefore, that with careful experimentation it is possible to build a profile of which factors will alter dissolution rate, and which components of the gastrointestinal tract will interact with the drug (and/or the excipients). This information would be a valuable forerunner to animal experimentation, allowing for early adjustments of dosage form design and for an early indication of possible drug/food interactions and subsequent changes in bioavailability in the presence and absence of food.

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