Available online at www.sciencedirect.com
SCIENCE DIRECT
International Journal of Pharmaceutics 308 (2006) 14–24
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Citric acid prolongs the gastro-retention of a floating dosage form and increases bioavailability of riboflavin in the fasted state

Frances Stops [a], John T. Fell [a,*], John H. Collett [a], Luigi G. Martini [b], Harbans L. Sharma [c], Anne-Marie Smith [c]

[a] *School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester M13 9PL, UK*
[b] *GlaxoSmithKline Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow CM19 5AW, UK*
[c] *Department of Imaging Science and Biomedical Engineering, University of Manchester, Manchester M13 9PL, UK*

Received 18 March 2005; accepted 30 September 2005
Available online 15 December 2005

Abstract

A floating dosage form based on calcium alginate beads has been developed. Riboflavin, was selected as the model drug and successfully incorporated into calcium alginate beads. The aims of the current study were to: (a) assess the influence of prolonged gastro-retention on the bioavailability of riboflavin from freeze dried calcium alginate beads administered under varying conditions of food intake and (b) to investigate the potential of citric acid to delay the gastric emptying of the calcium alginate beads. Gamma scintigraphy was selected as the method to monitor the movement of the calcium alginate beads in vivo. Riboflavin concentrations in the urine were analysed by HPLC.

Prolonged gastro-retention can be achieved, in the fasted state, when citric acid solution is used as an administering vehicle. However, prolonged gastro-retention is not achieved to the same extent when the gastric emptying times are compared to those obtained in the fed state.

The bioavailability of riboflavin improved when calcium alginate beads were administered in the fasted state with citric acid solution, compared to the bioavailability obtained when the calcium alginate beads were administered in the absence of citric acid.

Keywords: HPLC; Scinitgraphy; Bioavailability; Riboflavin; Citric acid; Gastric retention

1. Introduction

Prolonged gastro-retention can be achieved in the fasted state when citric acid as opposed to water, is used as a vehicle with which to administer the calcium alginate beads (Stops et al., 2006). For the current study, riboflavin has been selected as the model drug and has successfully been incorporated into the calcium alginate beads.

Riboflavin, also known as Vitamin B_2, is a water-soluble vitamin found in a variety of nutritional sources including yeast, milk, green leafy vegetables, heart, liver and kidney. Commercially, riboflavin is manufactured synthetically. The UK Reference Nutrient Intakes suggest that adult males and females require 1.4 and 1.2 mg per day respectively of riboflavin to prevent deficiency (Bates, 1997).

Riboflavin is ideal as a model drug to use in experiments to monitor gastro-retention as it has a specific absorption site within the proximal part of the gastro-intestinal tract. Several authors have also detailed the kinetics and usefulness of riboflavin as a model drug (Jusko and Levy, 1967).

Levy and Jusko have shown that when riboflavin is given with food there is a linear relationship between the dose of riboflavin administered and the amount of riboflavin recovered in the urine (Levy and Jusko, 1966). However, when given on an empty stomach the amount of riboflavin that was recovered decreased with increasing dose. From their data, they concluded that there was a limited capacity for riboflavin absorption. In later studies that compared the fasted and fed state, the process was shown to be saturable following the administration of large doses (Stripp, 1965). The saturation process was confirmed by figures that initially demonstrated a decrease in the excretion rate of

* Corresponding author. Tel.: +44 161 275 2365; fax: +44 161 275 2396.
E-mail addresses: john.fell@manchester.ac.uk, John.Fell@man.ac.uk (J.T. Fell).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.09.039

riboflavin when given on empty stomach, but following a meal the excretion rate increased. It was concluded that the absorption mechanism was located solely or mainly in the proximal region of the gastro-intestinal tract. However, the effect was not due to delayed gastric emptying but instead due to re-absorption in the small intestine that in turn was as a result of the presence of food stimulating bile flow (Jusko and Levy, 1967).

The ingestion of excess quantities of riboflavin results in its renal clearance from the body. Therefore urine collection will make for an ideal non-invasive method to collect biological samples and hence calculate the amount of riboflavin absorbed.

We have previously reported on the potential of citric acid to prolong the gastric retention of a dosage form (Stops et al., 2006). Therefore the use of citric acid to prolong gastro-retention has been extended to the current study.

2. Materials and methods

Sodium alginate (ISP Alginates, Surrey, England), riboflavin (Merck Darmstadt, Germany), anhydrous citric acid (Thornton and Ross, Huddersfield, England), calcium chloride (BDH Chemicals, Poole, England), ethanol 96% GPR grade (BDH Chemicals, Poole, England), stannous chloride (BDH Chemicals, Poole, England), potassium dihydrogen orthophosphate (BDH Chemicals, Poole, England) and methanol HPLC grade (BDH Chemicals, Poole, England) were used as received. Technetium-99m ($^{99m}TcO_4$), as pertechnetate in sodium chloride 0.9%, was obtained from The Manchester Royal Infirmary, Department of Nuclear Medicine (Manchester, England).

2.1. Preparation of the radiolabelled dosage form

The procedure for the preparation of radiolabelled calcium alginate beads has been described previously (Stops et al., 2004). For the current study, floating radiolabelled calcium alginate beads were prepared at an ambient temperature of 25 °C by incorporating $^{99m}TcO_4^-$ and stannous chloride (0.1% (w/v)), into sodium alginate solution and made up to three quarters of the final volume. Riboflavin was dispersed in 0.25 ml (w/v) of ethanol. The riboflavin/ethanol was then incorporated into the sodium alginate solution and made up to volume with glass distilled water to give final concentrations of 0.06% (w/v) riboflavin and 2% (w/v) sodium alginate. The resulting solution was extruded through a 21G needle from a height of 21 cm at a rate of 0.54 ml min^{-1} into a stirred solution of 0.02 M calcium chloride to precipitate the gel beads. Following curing for 30 min, the calcium alginate beads were recovered from the calcium chloride solution and 'snap frozen' with liquid nitrogen. The calcium alginate beads were then freeze-dried overnight using an Edwards Modulyo 4 freeze-dryer (Crawley, England), that maintained a temperature of −40 °C and a pressure of 80 N m^{-2}.

2.2. In vivo study

Five healthy males with ranging ages (28–51), weights (63–74 kg) and heights (163–175 cm) were selected and they provided written consent to take part in the study. No volunteers were taking any regular medication or had a history of gastro-intestinal disorders. Those volunteers who were smokers abstained during the study. ARSAC and The University of Manchester Ethics Committee approved the study, reference number 02144.

Table 1
Composition of breakfast consumed by volunteers taking part in the fed study

Breakfast item	Amount	Calorific value (Humphries, 2002)
Fried egg	One	102
Grilled bacon	Three rashers	375
Oven cooked sausage	One	230
Toast with butter	One slice	154
Orange juice or	100 ml	35
Tea	150 ml	7
Total calorie count		975 (orange juice) 947 (tea)

The study was designed so that each subject took the requisite number of beads after a 10 h overnight fast to give a dose of approximately 4 MBq of $^{99m}TcO_4^-$. Freshly prepared calcium alginate beads were administered on three occasions in a three-way cross over design with a wash out period of at least 1 week between study days. The calcium alginate beads were administered by placing them loosely on the tongue and swallowing with 100 ml water or 100 ml of citric acid 1.0% (w/v) solution. When the calcium alginate beads were administered under fed conditions, volunteers consumed a standard breakfast, Table 1, immediately prior to the start of the study.

When the calcium alginate beads were administered under fasting conditions, no additional food or liquid was consumed until gastric emptying of the dosage form was complete. When the calcium alginate beads were administered under fed conditions a soft drink was provided 2.5 h after the start of the study and lunch was provided 1 h later, Table 2. Volunteers were also instructed to keep a diary for any further food and/or drink consumed for up to 24 h from the start of the study.

Following administration, the volunteers were instructed to sit or remain standing for the duration of the study to avoid any possibility of posture affecting the gastric emptying of the calcium alginate beads. When taking gamma images, measures were also taken to ensure that volunteers stood in the same position for each image. In addition, providing an adjustable platform on which to stand corrected any major differences in height of the subjects, ensuring that all images were taken with the gamma camera in the same position. Failure to provide such a platform would have necessitated the constant movement of the camera head to allow for height differences of the volunteers and thereby introducing a possible source of error for the results obtained. An initial gamma scintigraphic image ($t = 0$), of the stomach was taken immediately after the radiolabelled calcium alginate beads were administered. Successive images were taken at 10 min intervals until all the calcium alginate beads had left the stomach.

Volunteers provided a sample of urine prior to the start of each study day that acted as a control for the study samples. Once

Table 2
Details of lunch consumed by the volunteers when calcium alginate beads were administered under fed conditions

Volunteer number	Lunch item	Amount	Calorific value (Humphries, 2002)	Total lunch calories
1	Beef/cheese sandwich	1	1150	1875
	Crisps	1 Large bag	530	
	Soft drink	500 ml	195	
2	Tuna roll	1	256	981
	Crisps	1 Large bag	530	
	Soft drink	500 ml	195	
3	Chicken roll	1	433	963
	Crisps	1 Large bag	530	
	Water	500 ml	0	
4	Ham salad roll	1	227	952
	Crisps	1 Large bag	530	
	Soft drink	500 ml	195	
5	Beef/cheese sandwich	1	1150	1680
	Crisps	1 Large bag	530	
	Soft drink	500 ml	0	

the study day had begun, the volunteers were then instructed to collect the total volume of voided urine over the 24 h, noting the time each sample was collected. Following collection of the urine samples, all samples were frozen at −40 °C until required for analysis. Riboflavin concentrations in the urine were analysed by HPLC.

2.3. The collection and treatment of gastric emptying data

A Ohio Nuclear Sigma 410 single headed gamma camera (Packard Instrument Company, Meriden, USA) that was fitted with a 40 cm parallel hole collimator designed to detect 140 keV gamma radiation with a 20% energy window was used to image the areas of interest for all the volunteers. The data were recorded using MAPS 2000 software and stored as 128 × 128 pixel images.

The gamma scintigraphic images were assessed by visual examination. Using an acetate sheet, a master outline of the stomach was drawn and placed over subsequent images. The time to the onset of gastric emptying was determined as the time that showed hotspots of radioactivity leaving the stomach and entering the small intestine. When the hotspots depicting the mass of beads no longer appeared in the outline, the calcium alginate beads were deemed to have left the stomach and hence gastric emptying was complete.

2.4. HPLC

Reverse phase HPLC is the method of choice for the analysis of urine samples because it allows for the separation and identification of small amounts of analyte in a provided sample.

2.5. Method development

An initial method using two mobile phases of different concentrations of buffered water/methanol running over a gradient, was found to be unworkable.

Samples of urine were prepared with an amount of riboflavin in glass distilled water added to the urine sample to give a known concentration of riboflavin. Each sample was analysed three times. The method was deemed to be unworkable as, when reviewing the chromatograms for the analysed samples, it was found that regardless of riboflavin concentration, the identification of the peaks was not always conclusive. The identification was not possible because the riboflavin peaks of some samples occurred so close to peaks of other compounds so as to make accurate isolation of one peak impossible. For the samples that did produce adequate peak separation, the actual riboflavin concentration of the urine samples with an added known amount of riboflavin corresponded well with the expected theoretical riboflavin concentrations. Hence, although the method mentioned was not used, it was shown that for those peaks that were identifiable, riboflavin concentrations were not affected when mixed with urine.

An alternative method based on a gradient system using potassium dihydrogen orthophosphate was found to be workable (Supelco, 2001). Once modified, the gradient profile, shown in Fig. 1 was produced. The system demonstrated that the riboflavin peak could be separated from the other compounds in the urine samples using a run time of 20 min per sample. Subsequently,

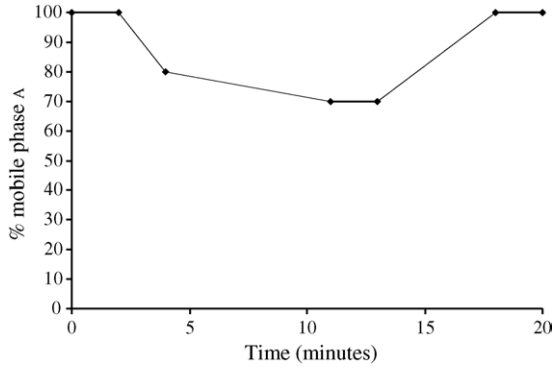

Fig. 1. HPLC gradient profile for mobile phase A.

it was also noted that a similar method has previously been used effectively to determine riboflavin levels in urine by HPLC (Smith, 1980).

A ThermoSpectronic Unicam UV 300 spectrophotometer (Hampshire, England) with bandwidth 2 nm and analysis by Vision 32 software, version 1.25 was used to assess all mobile phases, urine samples taken prior to the start of study days and eluent produced as a result of the HPLC analysis. The examination of all the aforementioned solutions was necessary to determine whether there were any components present within the solutions that would absorb at 267 nm, the absorption wavelength of riboflavin.

2.6. Apparatus

A Hewlett Packard Series II 1090 LC (Waldbronn, Germany), with a diode-array detector (DAD), was fitted with a Luna C18(5μ) column of dimensions 150 mm × 4.6 mm (Phenomenex®, Cheshire, England) for all chromatographic separations.

2.7. Mobile phase systems

Mobile phase A consisted of potassium dihydrogen orthophosphate dissolved in glass distilled water to give a final concentration of 50 mM. Mobile phase B consisted of 100% methanol, HPLC grade.

2.8. Operating conditions

Both mobile phases were degassed prior to use. The ratios for mobile phases A and B were not fixed during the analysis, but run over a gradient, as shown in Fig. 1.

The column was conditioned to mobile phase A 15 min prior to use and for 2 min between samples. The flow rate was 1 ml min^{-1}. The injection volume for each sample was 50 μl and UV detection was performed at 267 nm (4 nm bandwidth). All analysis were performed at room temperature.

2.9. Sample preparation

Samples of urine were removed from the freezer and allowed to defrost overnight before being used for analysis. The urine samples were prepared for HPLC analysis by centrifuging 1.5 ml of each sample at 30,000 rpm for 15 min using a Denver Instruments Company 13,000 × *g* microcentrifuge (Colorado, USA). No further preparation of the urine samples was required before analysis by HPLC (Kamberi et al., 1998).

The precision of the analytical procedure was assessed by examining the ability of the method and apparatus to repeat the results of each sample over a short period of time. For the current method, the precision was determined by analysing the results from repeated sampling of known dilutions of riboflavin in glass distilled lab water ($n = 8$). The dilutions were run ahead of the urine samples provided by the volunteers.

The detection limit (i.e. the lowest amount of analyte in a sample that can be reliably detected) was determined. Solutions of riboflavin in glass distilled water with concentrations of 15, 12.5, 10, 7.5, 5, 2.5, 1.2, and 0.6 mcg ml^{-1} were assessed. Each sample was analysed and the analysis repeated twice.

In order to confirm any change in retention times of the riboflavin peaks and to identify the riboflavin standard peak, between the samples for each volunteer for a particular study, a standard solution of riboflavin in glass distilled water of the minimum detectable concentration was analysed.

2.10. Analysis of chromatograms

HPLC chromatograms and all peak areas were obtained for all samples. Identification of the riboflavin peak in the urine samples was made considering the retention times of the riboflavin peak from the standard solutions at the start of the analysis and matching them with corresponding peaks from the urine samples.

3. Results and discussion

The results for the study are discussed below. The gastric emptying times have been discussed initially followed by the bioavailability results.

3.1. Gamma scintigraphy and gastric emptying results

Selected gamma scintigraphic images demonstrating the movement of the calcium alginate beads that were obtained for volunteer 3 when the calcium alginate beads were swallowed with 100 ml of water in the fasted state and with 100 ml of water in the fed state are shown in Figs. 2 and 3, respectively.

The series of images in Fig. 2 shows the behaviour of the beads from the start of the study at $t = 0$ min to the end of the study at $t = 40$ min. At $t = 0$ min, the beads had been swallowed immediately before the image was taken and the progression of the calcium alginate beads down the oesophagus to the stomach can be seen. At $t = 30$ min, the calcium alginate beads have started emptying from the stomach and at $t = 40$ min there are no beads remaining in the stomach as the hotspots of radioactivity depicting the calcium alginate beads appear in the small intestine.

The behaviour of the calcium alginate beads depended on whether they were in the stomach or the intestine. For all the volunteers, the calcium alginate beads remained as one or two distinct groups whilst in the environment of the stomach, since calcium alginate is insoluble in acidic pH (Stops et al., 2006).

The image at $t = 0$ min (Fig. 3) was taken immediately after the beads were swallowed and shows the beads as a single mass in the stomach. At $t = 120$ min, the beads can be seen to start emptying into the small intestine. However, the bulk of the beads remain in the stomach. At $t = 350$ min, the main mass of the beads are emptying into the small intestine, indicating that gastric emptying is nearing completion. For all the volunteers, the calcium alginate beads remained in one or two distinct groups throughout the study when the calcium alginate beads were administered in the fed state. However, it was expected that multiple groups of beads might have been seen. As discussed, after administration

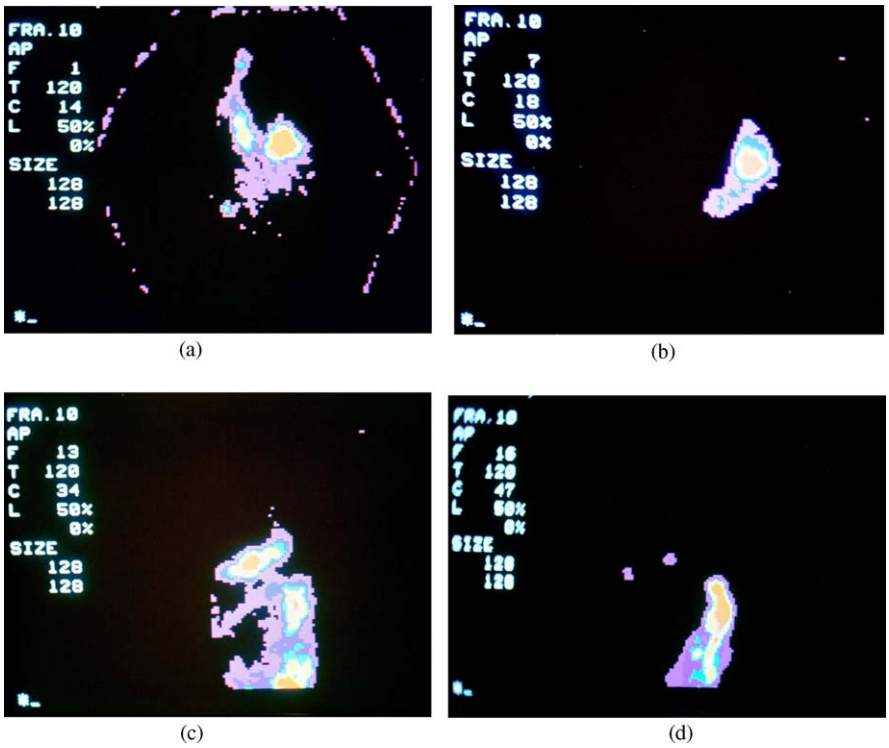

Fig. 2. Gamma scintigraphic images showing the movement of calcium alginate beads for volunteer 3 at selected time-points when the beads were swallowed with 100 ml of water in the fasted state: (a) $t = 0$ min, (b) $t = 20$ min, (c) $t = 30$ min and (d) $t = 40$ min.

in the fasted state, the beads remained in one or two groups in the stomach possibly as a result of the insolubility of calcium alginate in acidic pH. The calcium alginate beads administered in the fed state and considered in Fig. 3 remained in the stomach for the duration of the study period but the presence of food increases the pH of the stomach contents to approximately pH 6, thereby making the environment more favourable to permit dissolution of the calcium alginate beads. Hence the presence of multiple groups of beads may have been visualised.

All volunteers for the study were approximately the same height (155–175 cm, mean height 163.6 cm), and the same procedures as for study 1 were taken with regard to positioning of the volunteers in front of the gamma camera (Stops et al., 2006). However, when calcium alginate beads were administered under fasting conditions compared to the administration of calcium alginate beads under fed conditions, it was noted that in order to obtain images where the masses of calcium alginate beads occurred in the centre of the image, the gamma camera was required to be adjusted upwards. The observation would therefore indicate that the calcium alginate beads are floating on the surface of the stomach contents as opposed to being submerged by the mass of food or adhered to the mass of food.

Data obtained for the study is displayed graphically in Fig. 4. Fig. 4 shows the gastric emptying times obtained from the gamma camera images when the calcium alginate beads were administered under different conditions of food intake.

From the data obtained, considering the total time from swallowing of the calcium alginate beads to completion of gastric emptying, mean gastro-retention times of 80.4 ± 5 min and of 100 ± 5 min were obtained when the calcium alginate beads were administered with 100 ml of water and 100 ml of citric acid 1% (w/v) solution, respectively. In all cases the calcium alginate beads emptied from the stomach faster when administered under fasting conditions compared to administration in the fed state regardless of administrative vehicle. The presence of either nutrient or non-nutrient liquid in the stomach interrupts the migrating myoelectric complex and initiates the pattern of digestive motor activity so that the stomach is emptied of existing contents and prepared for subsequent food and/or drink intake. Therefore, the results shown in Fig. 4 were expected.

3.2. The influence of citric acid on gastro-retention

The influence and investigation of citric acid as a pharmaceutical agent to prolong gastro-retention has been discussed previously (Stops et al., 2004). The administration of calcium alginate beads with a citric acid 1% (w/v) solution in the current study showed that a proportion of the calcium alginate beads were retained in the stomach for up to 100 min. The figures for the onset of gastric emptying compare well to those previously obtained for the previous study (Stops et al., 2006), and confirm that citric acid has the ability to prolong gastro-retention of the dosage form.

3.3. The gastric emptying of calcium alginate beads when administered under fed conditions and the influence of a high calorie/high fat diet

The foods regimens used in the study were designed to reflect the volunteers' normal eating habits.

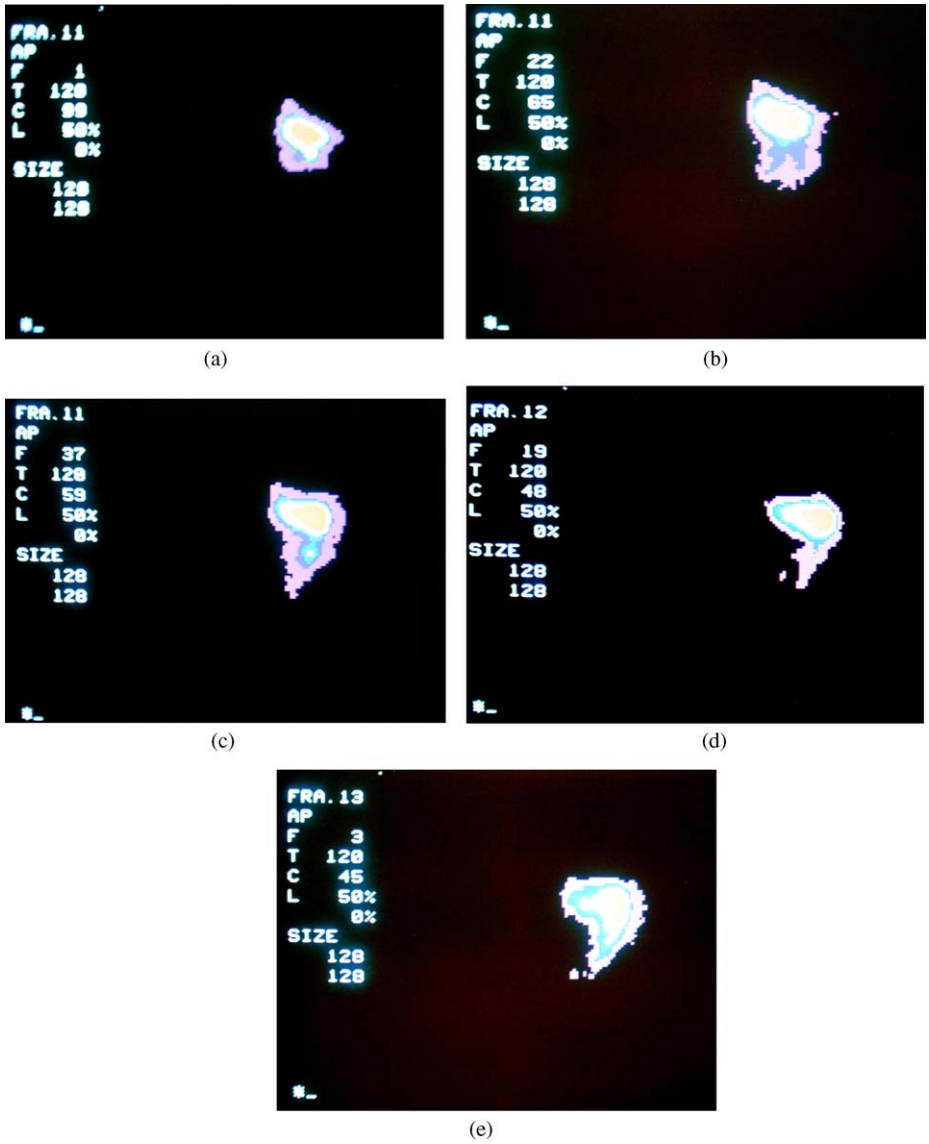

Fig. 3. Gamma scintigraphic images showing the movement of calcium alginate beads for volunteer 3 at selected time-points when the beads were swallowed with 100 ml of water in the fed state: (a) $t = 0$ min, (b) $t = 70$ min, (c) = 120 min, (d) $t = 240$ min and (e) $t = 350$ min.

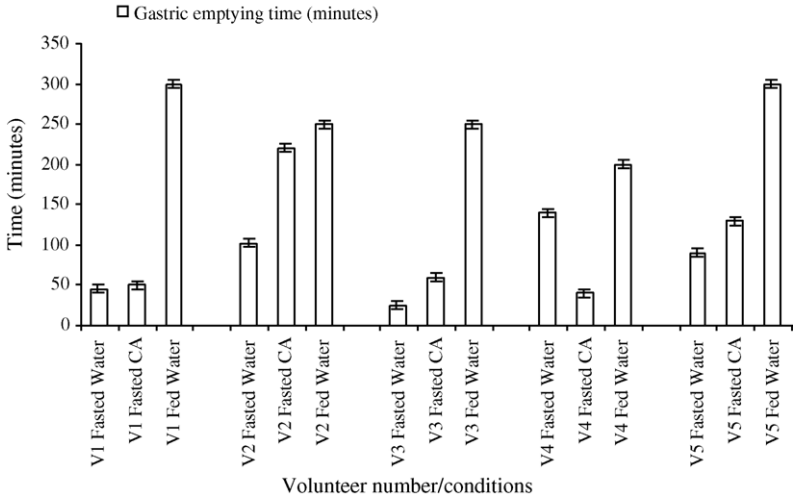

Fig. 4. Gastric emptying times for volunteers from time of swallowing to time of completion of gastric emptying under different conditions of food intake.

When reviewing the breakfast and lunch meals consumed by the volunteers, the following observations can be made. An adult male consumes approximately 2500 calories per day (Humphries, 2002). Therefore, in order to maintain his weight, he should consume at least that amount in a day. The foods consumed for breakfast and lunch provided minimum and maximum calories of 1899 and 2850, respectively, mean 2374 calories, Fig. 2. Since only two meals had been consumed, it is likely that when the rest of the food consumed for the day is considered, a total count of 2500 calories will be exceeded, suggesting that the diet is high calorie.

As the breakfast was consumed after an overnight fast, the consumption of a standard breakfast for all the volunteers for the fed state study was necessary to eliminate the possibility of any changes in gastro-retention times being attributed directly to the initial meal of the day. In addition the standard breakfast resembled that used previously (Whitehead, 1998). Hence similar gastric emptying times were expected.

The breakfast test meal contained a high proportion of fat. Fats and lipids are macronutrients, the composition of which makes for the greatest variability in drug absorption and alteration of gastric emptying. Consequently, high fat meals, such as the one used for the study, are the test meals of choice of the FDA when investigating the food effect for bioavailability studies (FDA, 2002).

When considering the test meal employed in the study, it should be noted that the fat content can be considered as two separate entities; namely the fat introduced into the meal as part of the cooking process and the fat component of the meal itself, e.g. an additional source of fat has been used to fry the eggs but eggs themselves contain a high proportion of fat.

Delays in gastric emptying have been reported following the addition of fat to a meal (Washington et al., 2001). Should a meal contain a high proportion of fat both from the food source and the method of preparation, then an increase in gastric pH results and a further delay in gastric emptying is achieved (Washington et al., 2001).

However, some studies show that high fat meals do not affect gastric emptying or bioavailability. No changes in lower oesophageal sphincter activity were found when comparing standard and high fat meals (Penagini and Bianchi, 1998). The drug methylphenidate, used to treat attention deficit hyperactive disorder in children, has also shown no change in bioavailability when administered with a high fat breakfast (Lee et al., 2003).

In addition to a high fat breakfast meal, the lunch meal contained foods such as cheese and crisps that are also high in fat content and therefore increase the amount of total fat within the food regimen. Overall, the food regimens can be considered not only to be high in calories but also high in fat content. Considering the combination of a high calorie and high fat meal, the longer period of time required to digest consumed fats and the frequent feeding regimen, the calcium alginate beads displayed extended gastro-retentive times. For the current study, in all cases when the calcium alginate beads were administered under fed conditions with water, gastro-retention times were in excess of 200 min, Fig. 4, and the values obtained corresponded well to those from a similar study (Whitehead et al., 1998). None of the calcium alginate beads administered under fasting conditions, either with water or citric acid 1% (w/v) solution, demonstrated gastro-retention times that corresponded with gastro-retention times that were obtained when calcium alginate beads were administered under fed conditions.

The presence of food in the stomach is acknowledged to delay gastric emptying (Washington et al., 2001) and thereby influence the amount of drug absorbed and the rate at which it is absorbed. In addition, dosage forms designed to float have been shown to maintain their floating abilities when administered after food (Moës, 1993). Frequent eating patterns, typical of the current study and of a Western diet, have also been shown to maintain floatation of the dosage form.

The results of the current study indicate that maintaining the stomach in the fed state enables the calcium alginate beads to be retained in the stomach, and therefore the beads are emptied more rapidly in the fasted state than in the fed state. However, administration of the beads in the fasted state with citric acid solution rather than water increases the retention time by a maximum of 58%.

3.4. HPLC results and determination of riboflavin bioavailability

HPLC has proved to be a useful analytical tool for the calculation of the amount of riboflavin within a sample, and, ultimately in determining the bioavailability of riboflavin from floating radio-labelled calcium alginate beads.

3.5. Validation of HPLC method to analyse urine samples

UV scans of methanol, potassium dihydrogen orhophosphate and glass distilled lab water, showed no absorption at 267 nm, indicating that none of the solutions would interfere with the HPLC analysis. The eluent showed a small amount of absorption at 267 nm that was expected. The absorbance is due to the small amounts of riboflavin that were present in the eluent after passing through the HPLC system. Samples of urine without riboflavin also showed absorbance at 267 nm. Many impurities are present within a urine sample, and it is likely that some impurities will show absorbance in the UV spectrum. As a result, the potential existed for the riboflavin peak of interest to be masked by other impurities of the urine that absorbed at the same wavelength. Therefore, it was necessary to adjust the mobile phase gradient profile to that shown in Fig. 1 in order that the peak of interest was separated from other interfering peaks.

Dilutions of riboflavin in glass distilled lab water were made and analysed by HPLC. The accepted criteria state that the correlation coefficient of an HPLC assay should be $\geq$0.990. The correlation coefficient value (R^2), from the calibration curve was 0.999, demonstrating that a high linearity had been achieved. The minimum detectable concentration of riboflavin in glass distilled water was determined as 1.2 mcg ml^{-1}.

The concentration of 1.2 mcg ml^{-1} riboflavin in glass distilled water was selected as the concentration of riboflavin in glass distilled water to be analysed between the samples for each volunteer for a particular study day. Fig. 5 shows the results

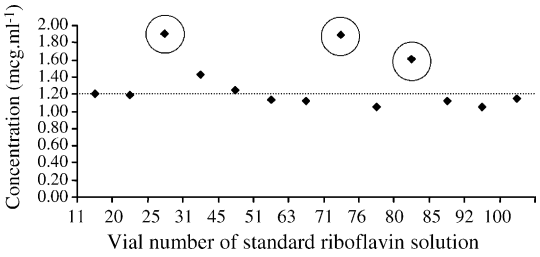

Fig. 5. Concentration of standard samples of riboflavin in glass distilled water included in the HPLC analysis.

obtained when standard samples of riboflavin dissolved in glass distilled water at a concentration of 1.2 mcg ml^{-1} were included at selected sample points throughout the analysis.

Fig. 5 shows the samples and respective concentrations of the standard solutions of riboflavin in glass distilled water that were included throughout the HPLC analysis of the volunteers urine samples. The samples of riboflavin in glass distilled water were prepared at a concentration of 1.2 mcg ml^{-1}. Following the construction of a calibration curve, actual concentrations of the analysed riboflavin solutions were calculated. The concentration of all but the three circled samples in Fig. 5 is 1.2 ± 0.2 mcg ml^{-1}. The three samples occurring outside the range, circled in Fig. 5, result in concentrations higher than the intended concentration of 1.2 mcg ml^{-1}. However, this is not significant since the three exceptional values do not form part of a trend of the riboflavin concentrations away from the required concentration of 1.2 mcg ml^{-1} and subsequent values are close to the required concentrations. The differences may be attributed to the build up of impurities on the HPLC column that are consequently cleared by the riboflavin in glass distilled water, a solution that is relatively free of impurities.

The accuracy of the HPLC method is displayed graphically in Fig. 6. The curve has been produced after analysing samples of known concentrations of riboflavin in glass distilled water and repeating the analysis twice.

With regard to determining the amount of riboflavin in the urine samples, calculations were performed as follows. The weight of the riboflavin loaded calcium alginate beads administered to each volunteer on each occasion was noted. As a result of the drug loading experiments performed, the actual amount of riboflavin contained within the sample was calculated. The total

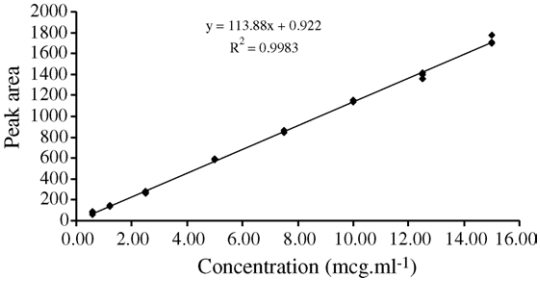

Fig. 6. Calibration curve of known concentrations of riboflavin in glass distilled lab water demonstrating the accuracy of the method by performing the analysis of the sample in triplicate. (concentrations used: 15, 12.5, 10, 7.5, 5, 2.5, 1.2, and 0.6 mcg ml^{-1}). The equation $y = 113.88x + 0.922$ and the R^2 value are applicable to every analysis at each concentration.

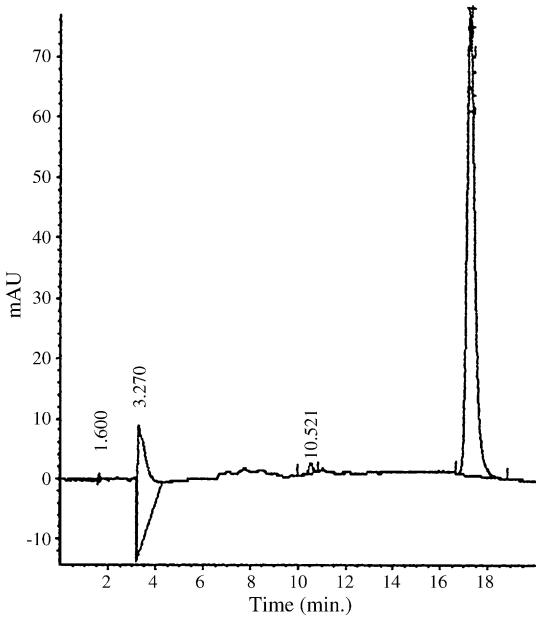

Fig. 7. Representative HPLC chromatogram of riboflavin in glass distilled lab water (retention time, 17.244 min; peak area, 1775; concentration, 15 mcg ml^{-1}; linearity result from three analysis 15.17 mcg ml^{-1}).

volumes of urine voided by each volunteer on each study day were recorded. The results of the calibration curve, were then used to calculate the amount of riboflavin in the urine samples and hence the total amount of riboflavin absorbed.

Figs. 7–9 show the chromatograms of riboflavin in glass distilled water, in urine with an added amount of riboflavin to give a known concentration of riboflavin and a representative sample of urine obtained from a volunteer for the current study.

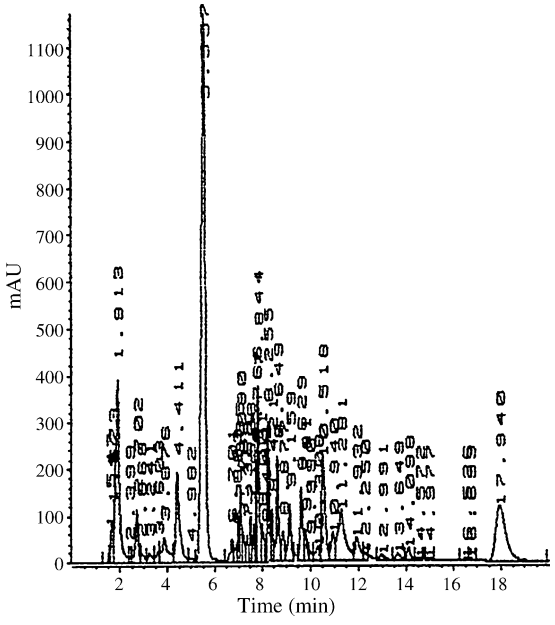

Fig. 8. Urine sample with a known amount of riboflavin added to give a final known amount of riboflavin (retention time of riboflavin peak, 17.940 min; peak area, 3540; theoretical concentration, 33.3 mcg/ml; calculated concentration, 31.1 mcg/ml).

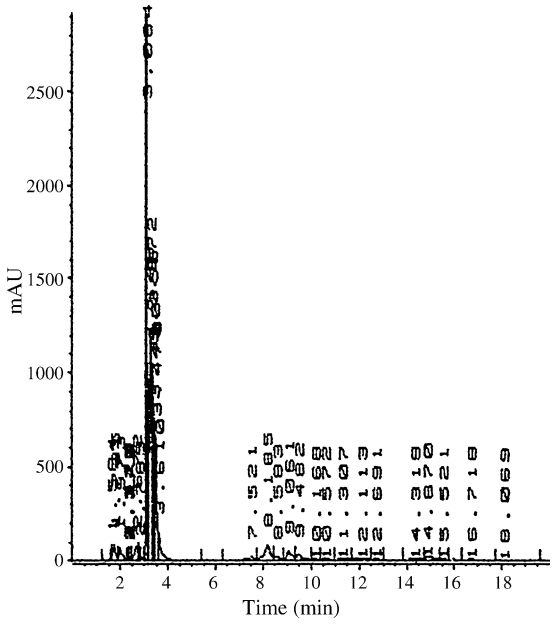

Fig. 9. Representative HPLC chromatogram of riboflavin in urine (retention time, 16.718; peak area, 472.13; calculated concentration, 2.71 mcg ml^{-1}).

Fig. 7 shows a typical HPLC chromatogram of riboflavin dissolved in glass distilled lab water. The peak is sharp, clearly identifiable and separate to any other peaks.

Fig. 8 shows a urine sample with a known amount of riboflavin solution added, that resulted in a final theoretical concentration of 33.3 mcg ml^{-1} (w/v) riboflavin. The retention time of the riboflavin peak is shown at 17.940 min. The peak can clearly be defined as riboflavin as both the retention time and peak area correspond well to that obtained for the standard riboflavin solution shown in Fig. 7. In practice, riboflavin concentrations from the urine samples of the order of 33.3 mcg ml^{-1} were not anticipated. Consequently the development of a method that allowed for the isolation of the riboflavin peak was important. Fig. 8 also demonstrates the importance of the run time of 20 min per sample. Prior to obtaining a riboflavin peak at 17–18 min, peaks of other compounds within the urine sample have been isolated. Such peaks would have caused the possible masking of the required riboflavin peak or made its identification impossible.

With reference to Fig. 9, the riboflavin peak can be identified as occurring at a retention time of 16.718 min. Such identification is possible as when reviewing all the standards that were run initially before all the urine samples, all the riboflavin peaks were observed to have retention times in the range 16.72–17.27 min.

3.6. Comparison of the amount of riboflavin absorbed in the fed state with the amount of riboflavin absorbed in the fasted state

Fig. 10 shows the amount of riboflavin absorbed during the study when the calcium alginate beads were administered under the varying conditions of food intake.

With regards to the administration of oral dosage forms, the presence or absence of food in the stomach is a major factor in determining the amount of drug absorbed and also the rate at which it is absorbed. Therefore, the bioavailability of riboflavin was expected to be maximal when the calcium alginate beads were administered under fed conditions. The results shown in Fig. 10, and, overall the results show this to be true.

As shown, Fig. 4, administration of the calcium alginate beads under fasted conditions with citric acid 1% (w/v) solution compared with water increases the retention time of the calcium alginate beads by a maximum of 58%. The increased residence time of the calcium alginate beads in the stomach allows for an extended amount of time for riboflavin to be released from the calcium alginate beads in the stomach. Therefore increased absorption of riboflavin in the small intestine occurs. Fig. 10 shows that administering calcium alginate beads under fasting conditions with a citric acid solution 1% (w/v) compared with water results in an enhancement in the bioavailability of riboflavin by a maximum of 88%, giving an overall bioavailability of riboflavin near to or better than that obtained in the fed state.

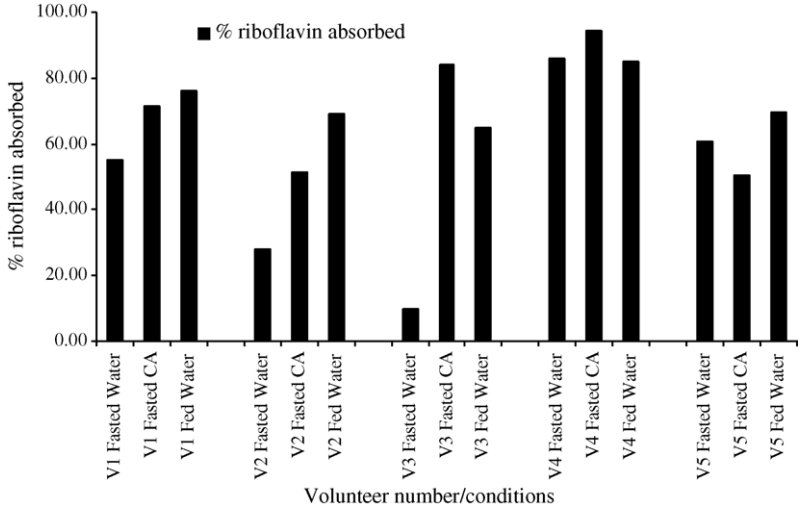

Fig. 10. Percentage of riboflavin absorbed under different conditions of food intake.

When considering the gastric emptying and bioavailability results overall, the figures correspond well. However, in the fasted state the comparatively short gastro-retention times that have been obtained, coupled with maximum riboflavin bioavailability indicate that the riboflavin is released from the beads immediately on contact with aqueous media. The incorporation of a polymer within the formulation may enable the riboflavin release to be retarded but such modification was beyond the scope of the current work.

When the calcium alginate beads were administered in the fasted state to volunteer 4, a further explanation of the results is warranted. In direct contrast to the gastric emptying times of the rest of the volunteers, the gastric emptying time increases by 71.4% when the calcium alginate beads were administered with citric acid 1% (w/v) solution compared with water. Furthermore an increase of 10.05% in riboflavin bioavailability has been recorded for volunteer 4 when the calcium alginate beads were administered with citric acid solution compared with water. A similar pattern was noted when reviewing the behaviour of the calcium alginate beads administered in the fasted state for volunteer 5. When the gastric emptying times of calcium alginate beads administered with citric acid solution 1% (w/v) are compared with the administration of the calcium alginate beads with water, an increase of 24% in gastric residence time is noted. However, the corresponding bioavailability decreases by 17.3%. The reason for the differences in riboflavin bioavailability can be attributed to the immediate release of riboflavin from the calcium alginate beads, as stated previously.

With specific regard to volunteer 4, when administered under fasting conditions, with the citric acid 1% (w/v) solution compared with water, the calcium alginate beads emptied faster from the stomach. The reverse is true for all other volunteers within the study; calcium alginate beads emptied faster when administered under fasting conditions with water compared to citric acid solution 1% (w/v). The difference in the gastric emptying profile of volunteer 4 compared to the other volunteers can be credited to inter-patient variation. More specifically it is possible that the MMC was displaying Phase III activity for volunteer 4, whereas for the remaining volunteers, Phase III may not have been commenced.

4. Conclusion

Floating radio-labelled calcium alginate beads have been produced and used in two in vivo studies. Formula modifications allowed for the inclusion of riboflavin as a model drug for the gamma scintigraphy and bioavailability study.

For both of the current studies, when the calcium alginate beads were administered under fasting conditions, gastric retention times in excess of 1 h were recorded. When calcium alginate beads were administered under fed conditions, gastric retention times were in excess 3.5 h. The figures compare well with a similar study using microballoons containing riboflavin. The study using the microballoons also showed that the excretion half life for the drug was longer using when floating microballoons were administered, compared with non-floating forms. Therefore a longer gastro-retention time for the floating microballoons was observed, albeit irrespective of whether the microballoons were administered under fed or fasting conditions (Kawashima et al., 2000). However, the structure of the microballoon is such that it contains one spherical cavity of air. In comparison, the calcium alginate beads have multiple cavities. Should the outer surface of the microballoon be compromised then buoyancy will be lost and it will pass into the small intestine, behaving as a normal single unit dosage form. Conversely, if the outer surface of the calcium alginate bead becomes damaged many more cavities are still available to maintain flotation. Hence, the calcium alginate beads are not emptied in an ‘all or nothing’ nature.

The in vivo studies investigated the potential use of citric acid as an excipient to delay gastric emptying. Although citric acid has not been used previously to delay the gastric emptying of a dosage form or to improve the bioavailability of a drug, the use of citric acid to delay gastric emptying for the purposes of the diagnosis of *Helicobacter pylori* is well documented (Dominguez-Muñoz et al., 1997; Graham et al., 1999). The in vivo studies completed for the current project demonstrate that administering floating calcium alginate beads in the fasted state with citric acid prolongs gastro-retention and improves bioavailability. However, the gastric emptying of the dosage form when administered in the fasted state has not been prolonged when compared to gastric emptying figures for the fed state. Therefore, when using floating calcium alginate beads of the formulation described to deliver drugs, maintaining the fed state is a primary requirement to prolong the gastric emptying of the dosage form. In summary, calcium algianet beads administered under fasting conditions with a citric acid solution showed greater gastro-retention times than in the absence of citric acid but was less than when calcium alginate beads were administered with food.

References

Bates, C.J., 1997. Bioavailability of riboflavin. Eur. J. Clin. Nutr. 51, S38–S42.

Dominguez-Muñoz, J.E., Leodolter, A., Sauerbruch, T., Malfartheriner, P., 1997. A citric acid solution is an optimal test drink in the 13C-urea breath test for the diagnosis of *Helicobacter pylori* infection. Gut 40, 459–462.

Graham, D.Y., Runke, D., Anderson, S.-Y., Malaty, H.M., Klein, P.D., 1999. Critic acid as the test meal for the ^{13}C-urea breath test. Am. J. Gastroenterol. 94, 1214–1217.

Humphries, C., 2002. The Hugely Better Calorie Counter. Foulsham, London.

Jusko, W.J., Levy, G., 1967. Absorption, metabolism and excretion of riboflavin 5′-phosphate in man. J. Pharm. Sci. 56, 58–62.

Kamberi, M., Tsutsumi, K., Kotegawa, T., Nakamura, K., Kakano, S., 1998. Determination of ciprofloxacin in plasma and urine by HPLC with ultraviolet detection. Clin. Chem. 44, 1251–1255.

Kawashima, Y., Takeuchi, Yamamoto, H., 2000. A gastroretentive microparticulate system to improve oral drug delivery. In: Wise, D.L. (Ed.), Handbook of Pharmaceutical Controlled Release Technology. Marcel Dekker, New York, pp. 505–511.

Lee, L., Kepple, J., Wang, Y., Freestone, S., Bakhtiar, R., Wang, Y., Hossain, M., 2003. Bioavailability of modified-release methylphenidate: influence of high fat breakfast when administered intact and when capsule content sprinkled on applesauce. Biopharm. Drug Dispos. 24, 233–243.

Levy, G., Jusko, W.J., 1966. Factors affecting the absorption of riboflavin in man. J. Pharm. Sci. 55, 285–289.

Moës, A.J., 1993. Gastro-retentive dosage forms. Crit. Rev. Therap. Drug Carrier Syst. 10, 143–195.

Penagini, R., Bianchi, P.A., 1998. Effect of increasing the fat content but not the energy load of a meal on gastro-oesophageal reflux and lower oesophageal sphincter motor function. Gut 42, 330–333.

Smith, D.M., 1980. Rapid method for the determination of riboflavin in urine by high performance liquid chromatography. J. Chromatogr. 182, 285–291 (Biomedical Applications 8).

Stops, F., Fell, J.T., Collett, J.H., Martini, L.G., Sharma, H.L., Smith, A-M., 2004. Floating dosage forms to prolong gastro-retention: an in vivo study in the fasted state. J. Pharm. Pharmacol. 56, S-67.

Stops, F., Fell, J.T., Collett, J.H., Martini, L.G., Sharma, H.L., Smith, A-M., 2006. The use of citric acid to prolong the in vivo gastro-retention of a floating dosage from in the fasted state. Int. J. Pharm. 308, 8–13.

Stripp, B., 1965. Intestinal absorption of riboflavin by man. Acta Pharmacol. Toxicol. 22, 353–362.

Supelco, 2001. Chromatography—Products for Analysis and Purification. Sigma-Aldrich Company Limited, p. 543.

US Department of Health and Human Services, FDA, CDER. 2002. Guidance for Industry on Food-Effect Bioavailability and Bioequivalence Studies, December.

Washington, N., Washington, C., Wilson, C.G., 2001. Physiological Pharmaceutics, Barriers to Drug Absorption, 2nd ed. Taylor & Francis.

Whitehead, L., 1998. An Investigation into a Gastroretentive Dosage Form. PhD Thesis. University of Manchester.

Whitehead, L., Fell, J.T., Collett, J.H., Sharma, H.L., Smith, A.-M., 1998. Floating dosage forms: an in vivo study demonstrating prolonged gastric retention. J. Control. Rel. 55, 3–12.