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The use of citric acid to prolong the in vivo gastro-retention of a floating dosage form in the fasted state

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Abstract

Gastro-retentive dosage forms have the potential to improve local therapy and decrease the variation in bioavailability that is observed with a number of commercially available immediate and modified release preparations. In this study, a dosage form has been developed, utilising freeze-dried calcium alginate beads, designed to float on the surface of the stomach contents thus prolonging the retention time.

The aim of the study was to also assess the in vivo behaviour of the radio-labelled calcium alginate beads when they were administered under fasting conditions with either water or an aqueous solution of citric acid, a potential gut transit delaying substance. The study was performed in healthy male volunteers who swallowed the radio-labelled calcium alginate beads after a 10 h overnight fast. Gamma scintigraphy was selected as the method to monitor the movement of the calcium alginate beads. The volunteers consumed no further food or drink until gastric emptying of the calcium alginate beads was complete.

The results indicated that prolonged gastric retention was achieved when the dosage form was administered with the citric acid solution when compared to retention in the absence of citric acid. Citric acid, therefore, has the potential to delay the gastric emptying of the calcium alginate beads when administered to fasted volunteers.

Keywords: Alginate; Beads; Gastric retention; Scintigraphy; Citric acid; Floating dosage forms

1. Introduction

Oral dosage forms account for the largest proportion of administered pharmaceuticals. The administration of therapeutic agents in conventional immediate release and some modified release preparations results in a high variation in bioavailability due to inter-patient variables such as posture (Bennett et al., 1984), age, gender and disease state (Mojaverian et al., 1988). The largest contributor to the efficiency of drug absorption of substances from the small intestinal region of the gastrointestinal tract is whether the stomach is in the fed or fasted state. In the fed state, gastric emptying can extend to a period of hours (Whitehead et al., 1998), whereas in the fasted state gastric emptying may be complete in minutes (Washington et al., 2001). Dosage forms that can be retained in the stomach are, therefore, advantageous because drug delivery can be controlled and the ideal of the drug in the right place at the right time can be realised.

Many physiological or technological approaches have been made to develop dosage forms that can be retained in the stomach. These approaches include large, single dose units designed to be physically retained in the stomach and/or the use of bioadhesive systems that use polymers that enable adherence of the dosage form to the gastric mucosa (Lehr et al., 1992). Products designed to float on stomach contents include hydrodynamically balanced systems and gas generating systems (Hwang et al., 1998). An alternative approach showing particular promise is that of multi-particulate calcium alginate beads (Whitehead et al., 1998).

Alternatively, chemical methods have been suggested to delay gastric emptying with the use of fatty acid meals. Fatty acids and lipids are macronutrients that are insoluble in water

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(Peckenpaugh and Poleman, 1999) and digested mainly in the small intestine. They are emulsified by bile and bile salts (Peckenpaugh and Poleman, 1999) released into the liver by the gall bladder. Lipids, therefore, require longer periods of time (approximately 4 h), to be digested (Barasi, 1997), than other nutrients such as carbohydrates (1 h) and proteins (2 h) (Barasi, 1997).

Lipids are used clinically to detect *Helicobacter pylori*, the causative organism of chronic gastritis, which produces excess quantities of urease that in turn breaks down urea to carbon dioxide and ammonia. When radio-labelled urea is administered to a patient, the level of urease activity can be determined by detecting the amount of carbon dioxide exhaled. Test meals such as the fatty acid meals are given with the radio-labelled urea as they make for increased contact time with the bacterial urease (Graham et al., 1999). However, the disadvantage of fatty acid meals is that they are not palatable and are generally not welcomed by the patient.

A recent study proposed that citric acid solutions might be used as a viable alternative to fatty acid meals. For example, citric acid solutions have been used as test meals to diagnose for *H. pylori* (Dominguez-Munoz et al., 1997) and results using such solutions have compared well with those reported after using fatty acid meals (Graham et al., 1999). Citric acid is a naturally occurring product found in many fruit species including lemons, where the concentrations are in the region of 5–8% (w/w). Pharmaceutically, citric acid is used as a flavour enhancing agent in liquid preparations and with sodium bicarbonate in the preparation of effervescent granules and tablets.

The mechanism by which citric acid delays gastric emptying has been open to discussion. According to studies by Hunt and Knox (1962), the retardation of gastric emptying is achieved if sufficient oral intake of citric acid occurs to cause pH of the duodenal contents to fall below pH 6. It was proposed that a local negative feedback mechanism stimulates the release of bicarbonate and secretin in response to high levels of citric acid that neutralises the acidic environment and allows gastric emptying to re-commence (Hunt and Knox, 1962). The time for neutralisation of excess acid is responsible for the retardation of gastric emptying. In other studies Hunt and Knox also suggested that parameters such as acid volumes, molecular weights of acids (Hunt and Knox, 1969) and concentrations of acid salts (Hunt and Knox, 1973) may affect gastric emptying. These findings were substantiated by Leodolter et al. who also achieved delayed gastric times when using 0.1 M citric acid, resulting in a pH of the citric acid solution of 3.0–3.5 (Leodolter et al., 1999).

In this work, floating calcium alginate beads were made based on previous methods (Whitehead, 1998) and tested in an in vivo gamma scintigraphy study. The aim of the study was to assess the gastro-retention of placebo calcium alginate beads when they were administered under fasting conditions with aqueous vehicles. The first arm of the study investigated the behaviour of the calcium alginate beads when they were administered with 100 ml of water. In the second arm of the study the calcium alginate beads were administered with 100 ml of citric acid 1% (w/v) solution in order to determine whether citric acid influenced gastric emptying.

2. Materials and methods

Sodium alginate (ISP Alginates, Surrey, England), anhydrous citric acid (Thornton and Ross, Huddersfield, England), calcium chloride (BDH Chemicals, Poole, England) and stannous chloride (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. Choice of radio-label

Technitium-99m ($^{99m}TcO_4^-$) is the radioisotope of choice for nuclear medicine imaging studies. It has a short half-life of 6.03 h and is easy and inexpensive to produce. ^{99m}Tc is eluted as pertechnetate ($^{99m}TcO_4^-$), with sodium chloride 0.9% from a molybdenum-99 generator.

2.2. Preparation of the radio-labelled dosage form

Floating radio-labelled calcium alginate beads were prepared as follows. An amount of sodium alginate (sufficient to make a 2%, w/v, final solution), was weighed and incorporated into approximately three-quarters of the final volume of glass distilled water. The sodium alginate solution was left overnight to de-aerate. On the day of bead preparation, 1.25 ml of stannous chloride (0.1%, w/v), was removed from a stock solution and placed in a glass vial. The $^{99m}TcO_4$ eluate was added to the stannous chloride, the vial stoppered and the solution shaken to ensure sufficient mixing. The stannous chloride/$^{99m}TcO_4$ mix was added to the sodium alginate solution and stirred. The sodium alginate/stannous chloride/$^{99m}TcO_4$ solution was then weighed and made up to volume to give a final concentration of 2% (w/v) sodium alginate. The resulting solution was passed through a 21 G 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. Following curing for 30 min, the radio-labelled calcium alginate beads were removed using an Endecotts sieve of mesh size 10 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 (West Sussex, England), that maintained a temperature of −40 °C and a pressure of 80 Nm^{-2}.

2.3. Assessment of the efficiency of the radio-labelling process of the calcium alginate beads

The efficiency of the radio-labelling process of the calcium alginate beads was assessed on the day following completion of the freeze-drying process. A Packard Cobra II Auto Gamma Counter (Meriden, USA), was used to obtain the counts per minute for sample of calcium alginate beads ($n = 10$) and 1 ml of calcium chloride supernatant solution that had been used to cure the calcium alginate beads. The counts per minute for the calcium alginate beads and the supernatant were then compared and the amount of radioactivity that was associated with the calcium alginate beads was, therefore, determined.

2.4. Investigation of the release of the radio-label into the dosing vehicle and physiologically relevant media

The experimental method by which the calcium alginate beads were produced ensured that the initial sodium alginate solution and resulting calcium alginate beads were readily radio-labelled. In addition, an essential requirement of the radio-label was that it should also remain attached to the dosage form for the duration of the study. For calcium alginate beads administered under fed conditions, study periods were expected to be in excess of 5 h. However, when reviewing the gamma scintigraphic images obtained from the studies, areas of radioactivity were observed in the intestinal region within 30 min of swallowing the calcium alginate beads. Since volumes of non-nutrient liquids up to 1000 ml have a gastric emptying $t_{1/2}$ of 10 min (Bass, 1973), consideration was also given to the fact that a proportion of the radio-label may have been released from the dosage form into the vehicle used to administer the calcium alginate beads.

The release of the radio-label into selected media was assessed by comparing the radioactive counts of a sample of placebo calcium alginate beads ($n = 10$), with 3 ml of the selected media. The media selected were the administering vehicles (water and citric acid solution 1%, w/v) and 0.1 M HCl, pH 1.2, designed to reflect the environmental conditions of the stomach. For each media, three analyses were performed. From freshly prepared radio-labelled calcium alginate beads, a known quantity were removed and placed in a stoppered vial and the counts per minute obtained using a Packard Cobra II Auto Gamma Counter (Meriden, USA). An aliquot (3 ml), of selected media was then added to each vial and mixed for 10 s at maximum speed using a Hook and Tulcer Rotamixer Deluxe (Croyden, England). The calcium alginate beads were then separated from the media. The counts per minute were obtained for all calcium alginate beads and media and a percent release of the radio-label from the calcium alginate beads into the media was then calculated.

2.5. Characterisation of dry calcium alginate beads

The characterisation of placebo calcium alginate beads, produced by the method described, showed the production method to be robust and reproducible. Since technetium-99m has a short half-life, a full characterisation of the calcium alginate beads was not possible prior to the start of the study day and following production of the calcium alginate beads. Therefore, the calcium alginate beads were assessed by visual assessment, weight and for radio-labelling efficiency only on the day they were required.

2.6. In vivo study

Five healthy males with ranging ages (28–58), weights (63–79 kg) and heights (163–183 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 gastrointestinal disorders. Those volunteers who were smokers abstained during the study. Administration of Radioactive Substances Advisory Committee (ARSAC) and The University of Manchester Ethics Committee approved the study (reference number, 02144).

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 on a maximum of two occasions in a two way cross over design with a wash out period of at least 1 week between study days. The calcium alginate beads were placed loosely on the tongue and swallowed with 100 ml of water or 100 ml of citric acid solution 1.0% (w/v). 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 radio-labelled calcium alginate beads were administered. Successive images were taken at 10 min intervals until all the calcium alginate beads had left the stomach. No additional food or liquid was consumed until gastric emptying of the dosage form was complete.

2.7. 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 pixel × 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.

3. Results and discussion

Calcium alginate beads were found to take up in excess of 99% of the radio-label. They were, therefore, deemed suitable for the purposes of the study. Table 1 shows the results obtained for the uptake of the radio-label for a typical sample of calcium alginate beads. The results were representative of all batches of calcium alginate beads produced for the study days.

In practice, the calcium alginate beads may be presented to the patient enclosed in a gelatin capsule. The decision not to

Table 1
Results of radio-label uptake by calcium alginate beads

Sample (beads and supernatant)	Counts per minute for total number of beads and volume of supernatant	Total counts per minute per sample (number + volume)	Radio-label associated with calcium alginate beads (%)
Beads ($n = 10$)	3671444	3688695	99.5
Supernatant (volume = 3 ml)	17250		

enclose the beads in a gelatin capsule was made when considering previous studies that have shown an adherence of the capsule to the gastric mucosa (Hunter et al., 1980; Graham et al., 1990).

Consideration should be given to the fact that the administration of the calcium alginate beads loosely as opposed to within a capsule may have resulted in a delay of delivery of the beads to the stomach by approximately 30 min after swallowing the whole sample. This method of dosing may cause the calcium alginate beads to adhere to the oesophageal wall. Previous practical work using similar dosage forms has demonstrated such an occurrence (Hunter, 1980). Ultimately, it is unlikely that a significant delay in gastric emptying is achieved as a result of the mucoadhesion of the calcium alginate beads, as alginate has shown to be a poor mucoadhesive (Gaserod et al., 1998). Hence, flotation is the method by which gastro-retention is achieved for the study.

3.1. The release of radio-label into the dosing vehicle and physiologically relevant media

The release of the radio-label, technetium-99m, into different physiologically relevant media from the calcium alginate beads is shown in Table 2.

The greater affinity of technetium-99m for acidic media compared with water can be explained as a result of the radio-labelling process. The presence of stannous chloride (Sn^{2+}), is required to reduce TcO_4^- from an oxidation state of +7 to an oxidation state of +2 and ensure sufficient activity with which to label the sodium alginate solution. Alginate is anionic and carries a negative charge. HCl dissociates to H^+ and Cl^- and in a similar way the citrate ions of the citric acid solution will also carry a negative charge. Therefore, the presence of the HCl may be expected to exchange with some of the H^+ with the Tc^{2+} resulting in some de-labelling of the negatively charged alginate. Whitehead (1998) also noted that leaching of $^{99m}TcO_4$ occurred during similar experiments but in addition, it was also reported that approximately 60% of the radio-label was still associated with the calcium alginate beads 5 h after immersion in 0.1 M HCl.

Table 2
Radio-label release into physiologically relevant media when the calcium alginate beads were shaken in different aqueous solutions

Media and concentration	Release of radio-label into different media ($t = 10$ s) (%)
HCl (0.1 M)	13.0
Citric acid (1%, w/v)	13.0
Water	1.0

Therefore, for the purposes of both in vivo studies, the calcium alginate beads were considered to retain sufficient radioactivity to enable their detection and subsequent visualisation by the gamma scintigraphy equipment.

3.2. Characterisation of dry radio-labelled calcium alginate beads

All batches of calcium alginate beads produced for the study appeared spherical in shape when viewed with the naked eye. Weights of each batch of calcium alginate beads following the freeze-drying procedure showed that all batches had been dried to in excess of 97% of their wet weight.

3.3. In vivo study

Four of the volunteers took part in both arms of the study. An additional volunteer took part only in the initial stage of the study when the calcium alginate beads were swallowed with water.

Fig. 1 shows the series of gamma scintigraphic images that were obtained for volunteer 1 when the calcium alginate beads were swallowed with 100 ml of water. Within the series, the position of the beads in the stomach of the volunteer and the passage through to the intestine can clearly be seen. The red areas, indicating greater radioactivity, depict the greater masses of calcium alginate beads. Reduced masses of calcium alginate beads are show as coloured areas of orange and yellow. The images and anatomical visualisations shown in Fig. 1 were representative of all the volunteers.

Fig. 1a ($t = 30$ min) shows distinct areas of radioactivity with both stomach and intestinal areas highlighted. The appropriate identification of the stomach area is also confirmed by outlined images taken at other time-points. The area of radioactivity below the intestinal area in Fig. 1a can be attributed to the vehicle that was administered with the calcium alginate beads. As demonstrated, radio-labelled calcium alginate beads release some of the radio-label into non-nutrient liquids. Following consumption of non-nutrient liquids under fasting conditions, the MMC is interrupted and the rapid emptying of such liquids occurs.

Gastric emptying was deemed complete when either two successive images of minimal radioactivity were collected or by noting the last time that a percentage of beads were seen in the stomach and the next frame that clearly showed that all calcium alginate beads had left the stomach. The window of time in all cases between the two points was 10 min, and therefore, the measure of gastric emptying gastric emptying was complete within a time error of ±5 min. The gastric emptying times for volunteer 1 are reflected by Fig. 1e and f for the current study.

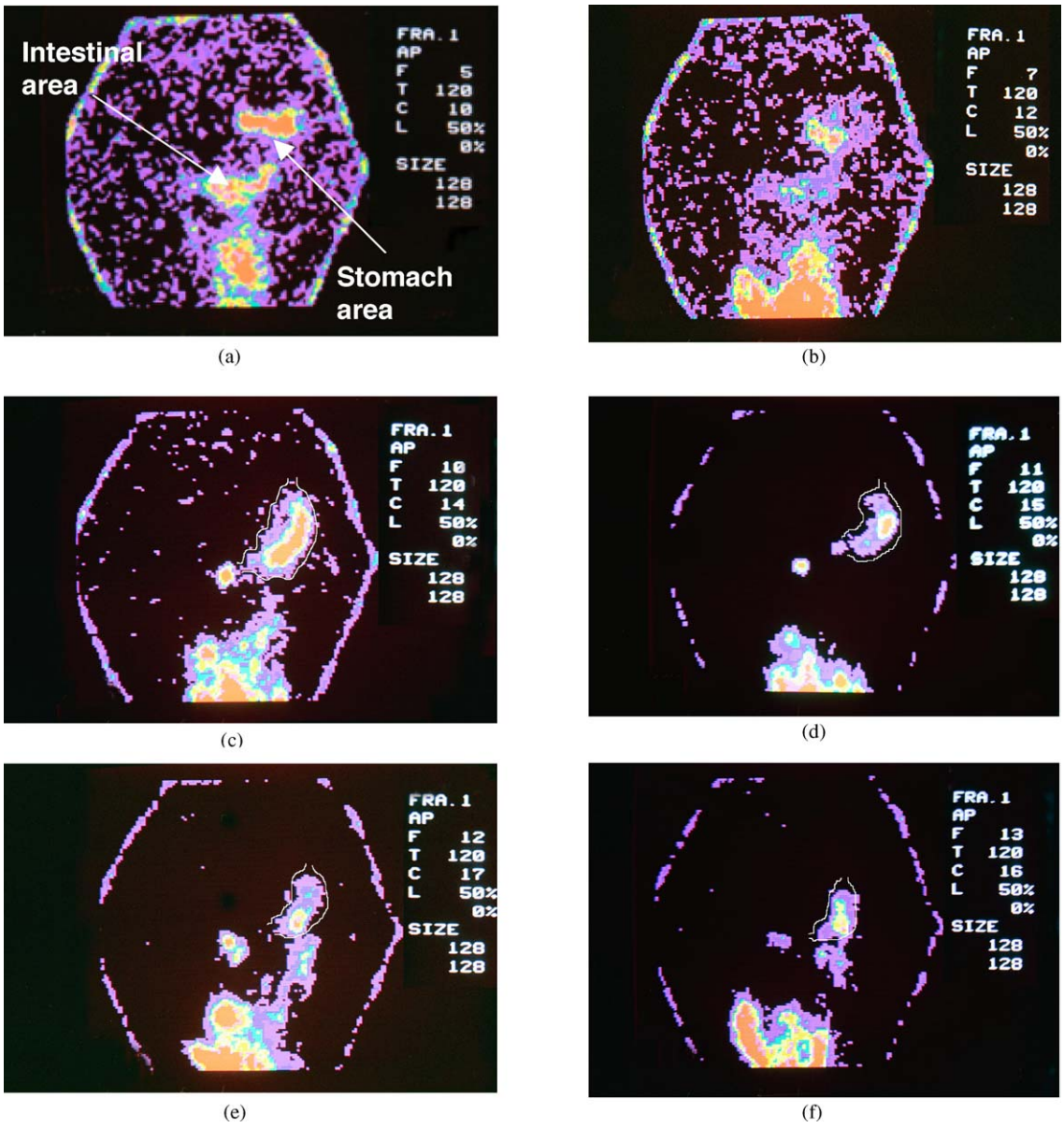

Fig. 1. Gamma scintigraphic images showing the movement of calcium alginate beads for volunteer 1 at selected time-points when the beads were administered with 100 ml of water: (a) $t = 30$ min; (b) $t = 50$ min; (c) $t = 80$ min; (d) $t = 90$ min; (e) $t = 100$ min; (f) $t = 110$ min.

The behaviour of the calcium alginate beads depended on whether they were in the stomach or the intestine. In all studies the calcium alginate beads remained as one or two distinct groups whilst in the stomach, but once in the intestine the calcium alginate beads appeared to split up into multiple groups. Such observations were expected, as calcium alginate is insoluble in the acidic stomach media but soluble in the more alkaline intestinal media.

A summary of the results showing the onset times to gastric emptying that were obtained for the four volunteers taking part in both arms of the study is shown in Fig. 2.

Overall, Fig. 2 shows that the calcium alginate beads used in the study can be retained in the stomach for extended periods when administered with a citric acid 1% (w/v) solution. The retention of the calcium alginate beads was observed in three out of the four volunteers that took part in both arms of the study. When compared with the time to onset of emptying of the calcium alginate beads swallowed with water only, the residence time equates to an increase of approximately 50%.

In addition to the times recorded for the onset of gastric emptying, the total time from swallowing the calcium alginate beads to completion of gastric emptying was also noted. The analysis of gamma scintigraphic images for the volunteers showed that a proportion of the floating calcium alginate beads were found to remain in the stomach for in excess of 1 h for seven out of nine of the individual tests. In a similar study using calcium alginate micro-balloons administered in the fasted state all the dosage units were found to have emptied from the stomach within 1 h (Bass, 1993).

The times for the completion of gastric emptying showed no apparent pattern (Table 3). The erratic completion of gastric emptying times and the decreased residence time of the beads

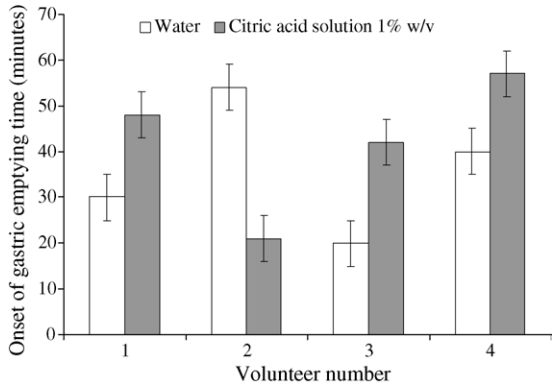

Fig. 2. Onset of gastric emptying times of calcium alginate beads when administered with and without citric acid.

Table 3
Gastric emptying times for volunteers from the in vivo study

Volunteer	Conditions	Time to onset of gastric emptying (min)	Time to completion of gastric emptying (min)
1	Fasted, water	30	55
1	Fasted, citric acid	48	15
2	Fasted, water	54	15
2	Fasted, citric acid	21	15
3	Fasted, water	20	5
3	Fasted, citric acid	42	35
4	Fasted, water	40	50
4	Fasted, citric acid	57	25
5	Fasted, water	82	5

displayed by volunteer 2 when the calcium alginate beads were swallowed with 100 ml of citric acid 1% (w/v) solution can be attributed directly to the migrating motor complex (MMC). In particular Phase III or the 'housekeeper wave' is the phase that consists of brief powerful contractions that results in an emptying from the stomach of any remaining undigested material or dosage forms. As Phase III will occur at different times for each volunteer, the gastric emptying times will vary considerably and also affect the total time to emptying.

The assessment of gastric emptying times for calcium alginate beads following administration in the fasted state was in direct contrast to a similar study when calcium alginate beads were administered in the fed state. In the fed state gastro-retention times in excess of 5 h were achieved for all volunteers (Whitehead et al., 1998). The studies have shown that the calcium alginate beads cannot be retained in the fasted state for the same time period as the fed state.

4. Conclusion

The administration of floating calcium alginate beads in the fasted state has been investigated, but a significant delay in gastro-retention has not been achieved when gastro-retention times are compared with those achieved in the fed state. However, in the fasted state, citric acid has been shown to markedly delay gastric emptying times when administered with floating calcium alginate placebo beads.

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