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Floating dosage forms to prolong gastro-retention—The characterisation of calcium alginate beads

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Abstract

Floating calcium alginate beads, designed to improve drug bioavailability from oral preparations compared with that from many commercially available and modified release products, have been investigated as a possible gastro-retentive dosage form. A model drug, riboflavin, was also incorporated into the formula.

The aims of the current work were (a) to obtain information regarding the structure, floating ability and changes that occurred when the dosage form was placed in aqueous media, (b) to investigate riboflavin release from the calcium alginate beads in physiologically relevant media prior to *in vivo* investigations.

Physical properties of the calcium alginate beads were investigated. Using SEM and ESEM, externally the calcium alginate beads were spherical in shape, and internally, air filled cavities were present thereby enabling floatation of the beads. The calcium alginate beads remained buoyant for times in excess of 13 h, and the density of the calcium alginate beads was $<1.000\,g\,cm^{-3}$. Riboflavin release from the calcium alginate beads showed that riboflavin release was slow in acidic media, whilst in more alkali media, riboflavin release was more rapid.

The characterisation studies showed that the calcium alginate beads could be considered as a potential gastro-retentive dosage form.

Keywords: Floating dosage forms; Riboflavin; Citric acid; Gastro-retentive dosage form; *In vitro* release; Dissolution release profile; Zero order

1. Introduction

Floating dosage forms that are designed to be retained in the stomach for extended periods of time have been developed as a drug delivery system. Floating dosage forms have the advantage of allowing local delivery of a drug to the stomach, for example in the treatment of *Helicobacter pylori* (*H. Pylori)*, and reducing the variability in bioavailability that occurs with some currently available immediate and modified release systems. For the current work, the calcium alginate beads are prepared by extruding sodium alginate solution dropwise into a calcium chloride solution. The precipitated gel beads are then separated by filtration and freeze dried. Since, the density of the calcium alginate beads is less than that of gastrointestinal fluids, they therefore float. Formulae were modified to allow for the inclusion of a model drug, riboflavin and, citric acid, an agent shown to retard gastric emptying *in vivo* (Stops et al., 2006a,b). The presence of citric acid allowed for an extended amount of time for riboflavin to be released from the beads.

Since the dosage form is expected to remain buoyant on the stomach contents for the duration of the dosing period, a full characterisation of the calcium alginate beads was necessary.

2. Materials and experimental

2.1. Materials

Sodium alginate (M/G ratio 1.56; Kelco International, 1987; ISP, Surrey, England), anhydrous citric acid (Thornton & Ross, Huddersfield, England), calcium chloride (BDH Chemicals, Poole, England), riboflavin (Merck, Darmstadt, Germany), riboflavin-5′-phosphate (Sigma–Aldrich, UK), ethanol 96% GPR grade (BDH Chemicals, Poole, England), and liquid nitrogen (BOC, Manchester, England) were used as received.

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2.2. Experimental

2.2.1. Preparation of the floating calcium alginate beads

Placebo calcium alginate beads were prepared as follows. Sodium alginate was dissolved in water to give a final concentration of 2% (w/v) sodium alginate. The solution was passed through a 21G needle at a height of 21 cm and a rate of 0.54 ml min^{-1} into a stirred solution of 0.02 M calcium chloride. Following curing for 30 min, the calcium alginate beads were recovered 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 N m^{-2}.

Calcium alginate beads containing riboflavin were prepared as follows. The required amount of riboflavin was dispersed in 0.25 ml ethanol 96% GPR grade. 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 suspension of riboflavin in ethanol was then added to the sodium alginate solution and mixed for 2 min before making up to volume. Calcium alginate beads were prepared as described above.

Citric acid was incorporated into the calcium alginate beads in the following way. The citric acid was weighed and dissolved into three quarters of the final volume of water. Riboflavin and sodium alginate were then incorporated into the solution as described above.

The final formulae of the calcium alginate beads used for the current work were:

A: 0.06% (w/v) riboflavin, 0% (w/v) citric acid;
B: 0.06% (w/v) riboflavin, 0.5% (w/w) citric acid;
C: 0.06% (w/v) riboflavin, 0.75% (w/v) citric acid.

Throughout the current work, the letters A, B and C have been used to denote the different formulae.

2.2.2. Determination of diameter

The diameter of a sample of calcium alginate beads was determined. Measurements for each sample were repeated twice. Values obtained were measured to 0.001 g. Mean diameters and standard deviations were recorded.

2.2.3. Determination of weight

The weight of a sample of calcium alginate beads was determined. Measurements for each sample were repeated twice. Values obtained were measured to 0.001 g. Mean weights and standard deviations were recorded.

2.2.4. Determination of density

The densities of the calcium alginate beads were derived mathematically and experimentally.

Mathematically, the densities of the calcium alginate beads were calculated using the weight and diameter of the beads. It was assumed that the beads were spherical.

Experimental determinations of density were determined using the AccuPyc® 1330 helium pycnometer (Norcross, USA), with a 1 cm^3 sample cup.

2.2.5. Determination of buoyancy properties of calcium alginate beads using resultant weight apparatus

The buoyancy properties of the placebo and riboflavin loaded calcium alginate beads were determined using the method of resultant weight. A full description of the method and theoretical background is given by Timmermans and Moës (1989, 1990a,b) and Timmermans (1991).

On the day of the experiment a volume of 1200 ml of media was allowed to equilibrate to room temperature and added to the test vessel. The media used to assess the resultant weight of the calcium alginate beads was freshly prepared and consisted of 0.1 M HCl/0.05% (w/w) Tween 80 adjusted to pH 1.2. A mass of calcium alginate beads (approximately 100 mg) was taken from the bulk sample to be analysed and placed in a mesh cage. Both the cage and calcium alginate bead sample were weighed and the measurement recorded. Following immersion in the media, successive resultant weight measurements were taken at 1-min intervals throughout the test period and subtracted from the initial resultant value. Values were then normalised to give resultant weight results as g 100 mg^{-1} of the test material. Measurements were repeated twice.

2.2.6. Scanning electron microscopy (SEM)

The external and internal morphology of the freeze-dried calcium alginate beads were studied by scanning electron microscopy, using a Cambridge 360 SEM (Cambridge, England).

2.2.7. Environmental scanning electron microscopy (ESEM)

Dry and wet calcium alginate beads were visualised using a Philips XL30 ESEM-FEG (Eindhoven, The Netherlands). Dry calcium alginate beads were prepared as follows. Calcium alginate beads were attached to a platform with Tissue tac®. The sample was frozen by dipping it into liquid nitrogen slush under vacuum and drawn into the microscope chamber to prevent any condensation of the water vapour. The bead was sliced *in situ* and the sample heated to −60 °C. The sliced surface was then re-frozen in the cryo-transfer stage and sputter coated with gold to a thickness of 50 Å.

Calcium alginate beads prepared in the wet state were subject to the following additional preparation prior to attachment to the platform with Tissue tac®. A Caleva USP XXII dissolution apparatus (Heusenstamm, Germany) was used to circulate a sample of placebo calcium alginate beads in Sørensen's citrate buffer (pH 3.0), for 45 min. Samples for imaging by ESEM were then prepared as described above.

2.2.8. Digital photography

A Caleva USP XXII dissolution apparatus, was used to circulate a sample ($n = 10$), of placebo calcium alginate beads in singly distilled lab water prior to observation by digital photography. Paddles were rotated at 50 rpm, the volume of the media

was 900 ml and the temperature was maintained at 37 ± 1 °C. A Fuji S1 digital camera (Tokyo, Japan), with Meiji light microscope (Tokyo, Japan), attachment and 10× focus, was then used to view and photograph the calcium alginate beads.

2.2.9. Confocal laser scanning microscopy-flourescence recovery after photobleaching (CLSM-FRAP)

The translational diffusion of riboflavin from the riboflavin loaded calcium alginate beads has been measured using CLSM-FRAP from methods derived by Axelrod et al. and Kubitscheck et al. The apparatus used was a confocal laser microscope (MRC-1000, Bio-Rad, Hemel Hempstead, Herts, UK) with an upright epifluorescence microscope (Optiphot 2, Nikon, Tokyo, Japan).

A calcium alginate bead loaded with riboflavin (excitation 480 nm, emission 565 nm) was placed in a cavity microscope slide and 2–3 drops of singly distilled lab water was added to the well. The slide was then placed under a confocal microscope and diffusion images taken at 5 s intervals until 25 images were acquired. The data were than analysed by calculating the translational diffusion from the calcium alginate beads using methods previously determined (Axelrod et al., 1976; Kubitscheck et al., 1994).

2.2.10. In vitro drug release

The *in vitro* release of riboflavin from the calcium alginate beads was assessed using a standard Erweka Caleva USP XXII device (Heusenstamm, Germany), that had been modified by placing a ring and mesh assembly in the apparatus. The only purpose of the mesh was to ensure that the beads were totally immersed in the dissolution media.

In vitro release studies were performed for each sample of beads. The paddles were rotated at 50 rpm, the volume of the media used was 900 ml and the temperature was maintained at 37 °C ± 1 °C. The studies used media of different pH in an attempt to simulate the various pH values found throughout the gastro-intestinal tract.

A Cecil 1020 UV spectrophotometer (Cambridge, England), operating at 267 nm and using 10 mm quartz cells was used to assay the dissolution media.

2.2.11. Analysis of the in vitro release of riboflavin from the calcium alginate beads

The release of riboflavin from the calcium alginate beads was assessed graphically and mathematically. Mathematically, the release profiles were compared using the f_2 metric; a method approved by the FDA (US Department of Health and Human Sciences, 1997) that can be used where major changes in dissolution profile may be expected.

The f_2 metric is limited in that only two release profiles may be compared at any one time. Therefore, each release profile was compared in the following way:

- Dissolution profile (A) was compared with dissolution profile (B).
- Dissolution profile (B) was compared with dissolution profile (C).
- Dissolution profile (A) was compared with dissolution profile (C).

(A = 0.06%, w/v, riboflavin, 0%, w/v, citric acid; B = 0.06%, w/v, riboflavin, 0.5%, w/v, citric acid; C = 0.06%, w/v, riboflavin, 0.75%, w/v, citric acid).

3. Results and discussion

3.1. Diameter, weight and density

The mean diameter, weight and density values for the calcium alginate beads of the different formulations are shown in Table 1.

3.1.1. Diameter

The diameters varied according to the formulation. The results show that calcium alginate beads containing riboflavin were 16.3% larger than placebo calcium alginate beads.

Several authors have observed that process parameters can influence the size of the calcium alginate beads (Whitehead, 1998; Klokk and Melvik, 2002; Østberg and Graffner, 1992). As the process parameters were kept constant, the added material was responsible for the change in size of the calcium alginate beads. Within the studies, the standard deviations indicate that individual variability is low.

3.1.2. Weight

The weight of the calcium alginate beads depended on the formulation. Calcium alginate beads containing riboflavin had a mass 3.2% greater than the placebo calcium alginate beads. Since the process parameters can affect diameter, the weights of the calcium alginate beads may also be affected for the same reasons.

3.1.3. Density

The densities of the calcium alginate beads were determined by calculation and experimentally.

Table 1
Mean bead diameters ($n = 10$), mean weights of a sample of beads ($n = 30$), and mean experimentally determined densities of beads containing riboflavin and placebo beads

Calcium alginate bead sample	Mean diameter ($n = 10$) (mm)	Mean weight ($n = 30$) (g) ($\times 10^{-2}$)	Mean density (using 1 cm^3 sample cup) ($g\,cm^{-3}$)
Placebo	0.250 (S.D. 0.01)	0.0135 (S.D. 0.000351)	0.2305 (S.D. 0.026)
Riboflvain loaded	0.258 (S.D. 0.06)	0.0157 (S.D. 0.000153)	0.1915 (S.D. 0.0059)

n = number of beads sampled, S.D. = standard deviation.

3.1.3.1. Mathematical determination. The results obtained for the diameter and weight of the calcium alginate beads were used to calculate their density. The mathematically calculated densities of the samples of calcium alginate beads were similar and less than 1 g cm^{-3}. The results therefore suggest that the calcium alginate beads should float when placed in aqueous media (Singh and Kim, 2000). Experimentally, the resultant weight results confirmed the mathematical calculation and theory since the calcium alginate beads floated.

3.1.3.2. Experimental determination. Table 1 shows the density measurements for three samples of calcium alginate beads as measured by the pycnometer.

Density results obtained using the pycnometer for the placebo calcium alginate beads are approximately 0.2 g cm^{-3}. As calcium alginate beads have not been previously prepared with riboflavin no comparable figures are available. However, similar dosage forms such as hollow microspheres that are also designed to float on stomach contents have showed comparable density values (El-Gibaly, 2002). Table 1 also shows that variability occurs between and within samples of calcium alginate beads when measuring the density using the pycnometer. However, various points, noted as follows, should be considered when using a pycnometer to measure the densities of the calcium alginate beads.

- When the calcium alginate beads are placed in the sample cup, as a result of the spherical shape and relatively large size of the calcium alginate beads compared to the diameter of the 1 cm^3 sample cup, void volumes presented within the sample cup. Such volumes will have a direct effect on the results obtained as the equipment calculates the density measurements based on pressure changes in a standard volume.
- When passing a gas under pressure into a closed chamber containing fragile calcium alginate beads, the calcium alginate beads have the potential to become damaged easily. Hence damage to the outer surfaces of the calcium alginate beads will affect the pressure changes within the standard volume, thereby making results inaccurate.
- Since the calcium alginate beads are porous, it is it is possible that the helium used to make the measurements penetrates into the calcium alginate beads, thereby affecting the measurement.

Considering the results obtained and possible sources of error, the method of measuring densities of calcium alginate beads using a pycnometer has demonstrated that the results may only be considered as approximate values.

3.2. Resultant weight

Initial buoyancy tests were performed by placing 20 beads in a flask to which 25 ml of media was added. The flasks were then stoppered and shaken for 5 min using a bottle shaker. Observations of the number of beads that remained floating were made at specific time intervals, and overall they were left for 24 h. Although the method verifies that the calcium alginate beads are buoyant, the test is inadequate as such a system provides no information about the kinetics of the floating dosage form, or, how the behaviour of the dosage form changes once immersed in different media.

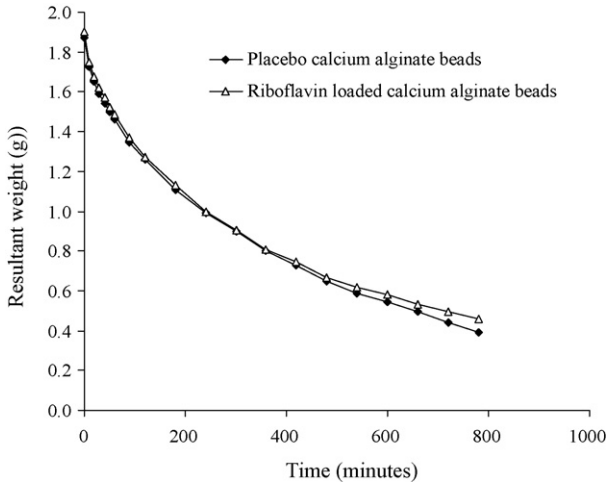

Fig. 1. Mean resultant weight values for placebo and riboflavin loaded calcium alginate beads.

Using the resultant weight technique developed by Timmermans and Möes, Fig. 1 shows the mean resultant weight values for both the placebo and riboflavin loaded calcium alginate beads.

The results for placebo and riboflavin loaded calcium alginate beads were similar and showed a positive resultant weight value over the study period and indicated that the calcium alginate beads continued to float for the specified time period. Statistically (Standard deviation, $p = <0.05$), there was no inter or intra variation between the sets of measurements taken demonstrating that consistency has been achieved throughout the experiment. Hence the buoyancy or floating ability of the beads has not been affected by the addition of riboflavin.

3.3. Scanning electron microscopy (SEM)

The SEM's of a whole and half calcium alginate beads are shown in Fig. 2a and b.

Overall, the shape of the calcium alginate beads that were produced was spherical, regardless of formulation. The outer surface of the beads are textured and contoured whilst the internal morphology of the calcium alginate beads clearly shows numerous cavities that form as a result of the freeze-drying process. The cavities are unique to the floating calcium alginate beads and enable flotation. The calcium alginate beads also had a spongy texture that was expected from the freeze-drying process.

3.4. Environmental scanning electron microscopy (ESEM)

Fig. 3a shows a cross section of a dry calcium alginate placebo bead as viewed by ESEM.

The comparison of images obtained by ESEM and SEM is sometimes difficult, since the sample preparation and equipment used to view the samples for both techniques are different. For

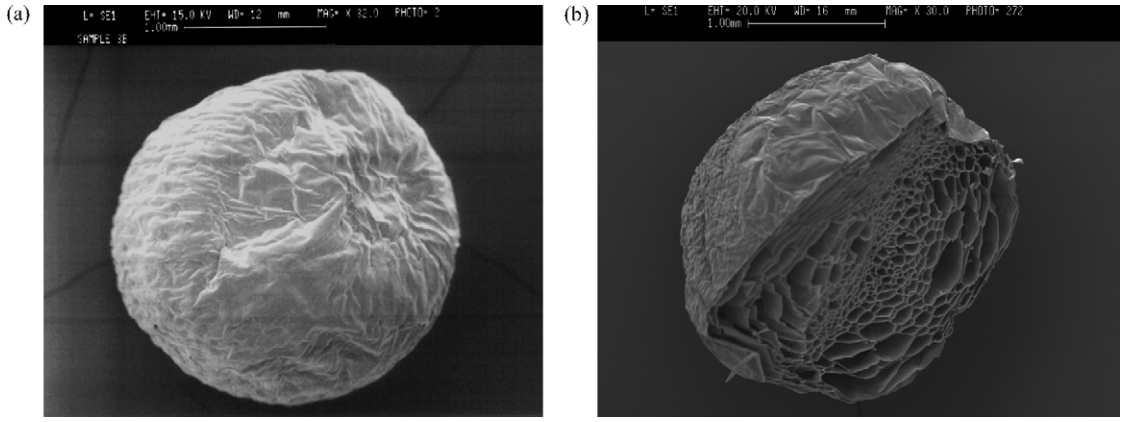

Fig. 2. (a) SEM of whole calcium alginate bead containing riboflavin showing external morphology (32.0×). (b) SEM of cross section of calcium alginate bead containing riboflavin showing internal morphology (30.0×).

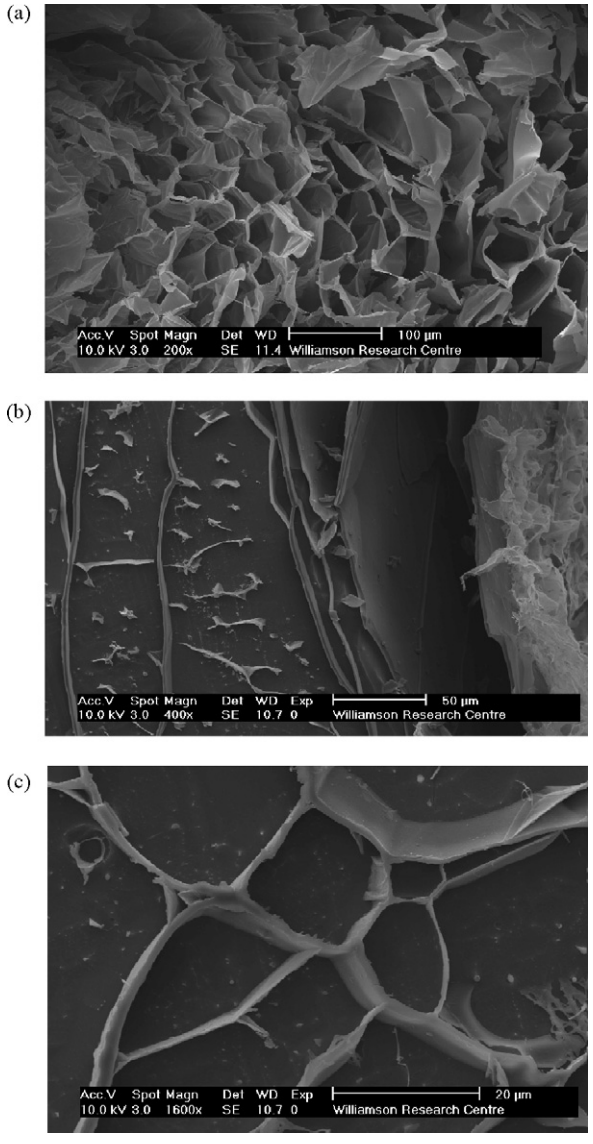

Fig. 3. (a) Cross section of a dry placebo calcium alginate bead as viewed by ESEM (200×), (b) ESEM image of placebo calcium alginate bead after immersion in aqueous media (400×), (c) ESEM cross-section image of placebo calcium alginate bead after immersion in aqueous media (1600×).

the current work, the ESEM image clearly shows the cavities within the calcium alginate beads, and, such cavities compare well to those viewed by SEM. Fig. 3b and c shows ESEM images detailing the morphology of a calcium alginate bead after immersion in Sørensen's Citrate Buffer (pH 3.0).

The outer surface of the calcium alginate bead is detailed on the right of the image in Fig. 3b. However, in contrast to the outer surfaces viewed by SEM, the surface is not contoured, but has a textured appearance. The textured appearance may occur as a result of immersion in aqueous media and subsequent swelling or erosion of the surface of the calcium alginate bead. Conversely, the appearance may be an artefact of ice crystals and occurs as a result of the cryo or freezing process used during preparation of the calcium alginate beads for ESEM imaging.

Fig. 3b may also provide some detail as to the processes that occur when the calcium alginate beads are immersed in aqueous media. Adjacent to the outer surface of the calcium alginate bead, two cavities are visible, both of which appear to be air filled. Moving towards the centre of the calcium alginate bead, the cavities appear to contain some aqueous media. Fig. 3c also confirms such a finding.

Fig. 3c shows at a higher magnification the cavities within the calcium alginate beads, and confirms that a change has occurred to the internal morphology on immersion of the calcium alginate beads in aqueous media. Although Fig. 3c shows the cavities to be partially filled with fluid, further evaluation by ESEM of calcium alginate beads after circulation in selected media is required. Additional assessment would confirm whether the calcium alginate beads fill with media and the subsequent changes that occur in the physico-chemical properties of the calcium alginate beads.

3.5. Digital photography

Fig. 4 shows a digital camera image of a placebo calcium alginate bead after circulation in aqueous media.

Freeze-dried calcium alginate beads consist of many air filled cavities that are formed as part of the freeze-drying process. Such cavities are shown in Fig. 4 as dark areas. The image shows the

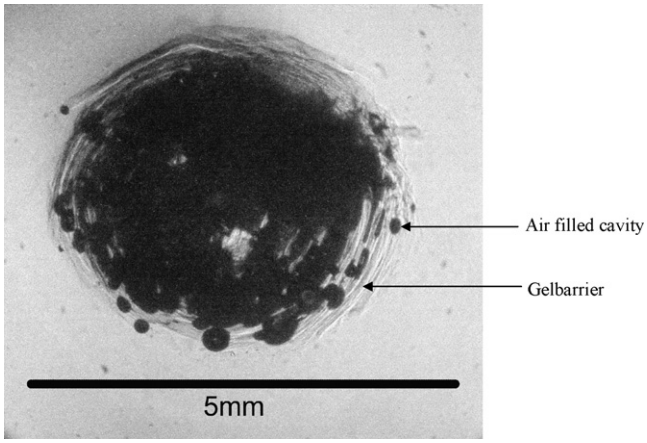

Fig. 4. Digital camera image of placebo calcium alginate bead after immersion in aqueous media.

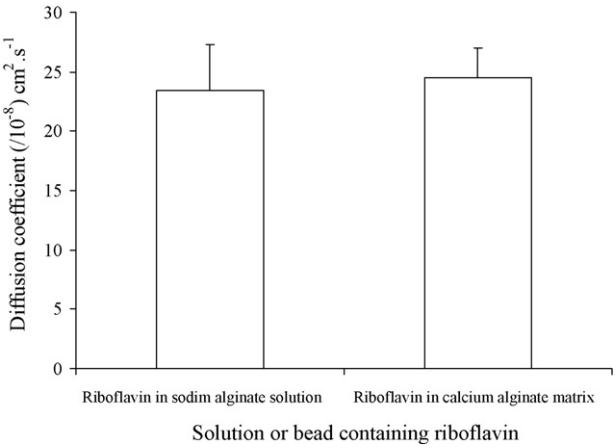

Fig. 5. Diffusion coefficients of riboflavin through a sodium alginate solution or through a calcium alginate bead matrix.

change in the outer surface of the calcium alginate bead and may be attributed to the development of a gel barrier around the calcium alginate bead, the formation of which is due to the hydration of the calcium alginate bead following immersion in glass distilled water. The hydration process was confirmed by the resultant weight results that showed an immediate decrease in resultant weight due to the uptake of liquid when the samples are initially placed in the media.

When considering the average diameter of calcium alginate beads in the dry state, a subsequent increase in the size of the calcium alginate bead by approximately 80% is evident when the calcium alginate beads were placed in aqueous media.

Evaluation of the swelling or erosion characteristics of calcium alginate beads as not been previously investigated. However, the hydration (Bussemer et al., 2003), swelling (Colombo, 1993), and formation of gas bubbles (Melia et al., 1993), within polymer matrices such as hydroxypropylmethylcellulose (HPMC) have been well documented. HPMC, therefore, serves as an ideal model polymer for which to initially consider the assessment and resultant method of drug release from the calcium alginate beads.

As confirmed by the resultant weight results, hydration of the calcium alginate beads occurs when the calcium alginate beads are placed in aqueous media. Digital camera images have also showed the formation of a gel barrier following immersion in aqueous media. Both aforementioned processes will have a direct effect on the rate of drug release from the calcium alginate beads. The calcium alginate beads are essentially hydrogel matrices throughout which the rate of drug release is complex and it is likely that any drug release will occur by more than one method.

The following mechanisms of drug release can be identified from a hydrogel matrix. The eroding or matrix–solvent front allows direct release of drug into the solvent. The diffusion front allows the drug to diffuse through the matrix for subsequent release into the solvent and the swelling front forms a glassy-rubbery region at the centre of the matrix. One or more of the mechanisms may occur at any one time, but a combination of all three mechanisms result in the linear release of drug from the matrix. However, the thickness of the gel layer that varies over time as a result of the aforementioned mechanisms must also be considered.

Additional studies surrounding the gel barrier with specific regard to formation, degradation over time and drug passage across diffusion, swelling and eroding fronts are required to provide more information regarding the physico-chemical aspects of the calcium alginate beads.

3.6. Confocal laser scanning microscopy-FRAP (CLSM-FRAP)

CLSM-FRAP was used to measure the diffusion of riboflavin from the calcium alginate bead to the aqueous media and also within the calcium alginate matrix. The results are shown in Fig. 5.

From Fig. 5, the diffusion coefficients of riboflavin from the calcium alginate bead into the aqueous media was $23.416 \pm 3.84/10^{-8}\,cm^2\,s^{-1}$ and the diffusion of riboflavin molecules within the matrix $24.471 \pm 2.50/10^{-8}\,cm^2\,s^{-1}$. Hence the results were similar and indicate that when whole calcium alginate beads are placed in aqueous media the movement of riboflavin molecules out of the matrix occurs rapidly. The rapid movement of molecules from the dosage form means that the drug is unlikely to remain in the dosage form for long enough to demonstrate prolonged gastro-retention.

Although further evaluation of the diffusion of riboflavin from calcium alginate beads was beyond the scope of this work, additional investigations are required to determine the reason for the rapid movement of riboflavin from the calcium alginate beads. Initial investigations may centre on the respective molecular sizes of riboflavin and calcium alginate, based on the results from the CLSM-FRAP. If riboflavin presents with a molecular size that is much smaller than that of pores of the calcium alginate beads, then rapid diffusion of the riboflavin through the matrix into solution occurs. In order to prevent the rapid diffusion of riboflavin into solution, it may be necessary to consider the incorporation of a polymer to retard riboflavin release from the matrix.

The technique of CLSM-FRAP has confirmed the rapid release of the model drug from the calcium alginate matrix, but further investigations are required to determine if the same occurs in other aqueous media.

3.7. *In vitro release of riboflavin from selected media*

Dissolution testing is a frequently used quality control method for assessing drug release from oral dosage forms and is essential to assess how the release of the model drug would occur *in vivo*.

The dissolution of a drug from a dosage form is affected by agitation intensity, pH, the type of medium, amount of aeration of the medium and the area of exposure of the drug delivery system to the medium. Traditionally baskets have been used to enclose multi-particulate formulations, but using such a method to assess drug release from the dosage from results in a reduced exposure of some of the calcium alginate beads to the medium, due to the volume of the basket being taken up by a comparatively large volume of beads.

Performing dissolution studies using the paddle method only is equally unacceptable for floating dosage forms because only a proportion of the dosage form is exposed to the media. In addition, the placement of the beads is not consistent and hence results are not reproducible (Fassihi, 1995).

The presence of the mesh, as used for the current work, ensured that the full surface area of the beads was exposed to the dissolution medium (Pillay and Fassihi, 1998) and the paddle ensured sufficient agitation of the medium for drug dispersion. Previous studies have shown that the mesh and paddle method provides more reproducible and reliable dissolution profiles compared to other conventional methods (Fassihi, 1995).

The *in vitro* release profiles (Fig. 6a–d,f) show the amount of riboflavin remaining in the calcium alginate beads at specific time-points.

The *in vitro* release of riboflavin from the calcium alginate beads in media of pH 1.2 (Fig. 6a) showed a steady release

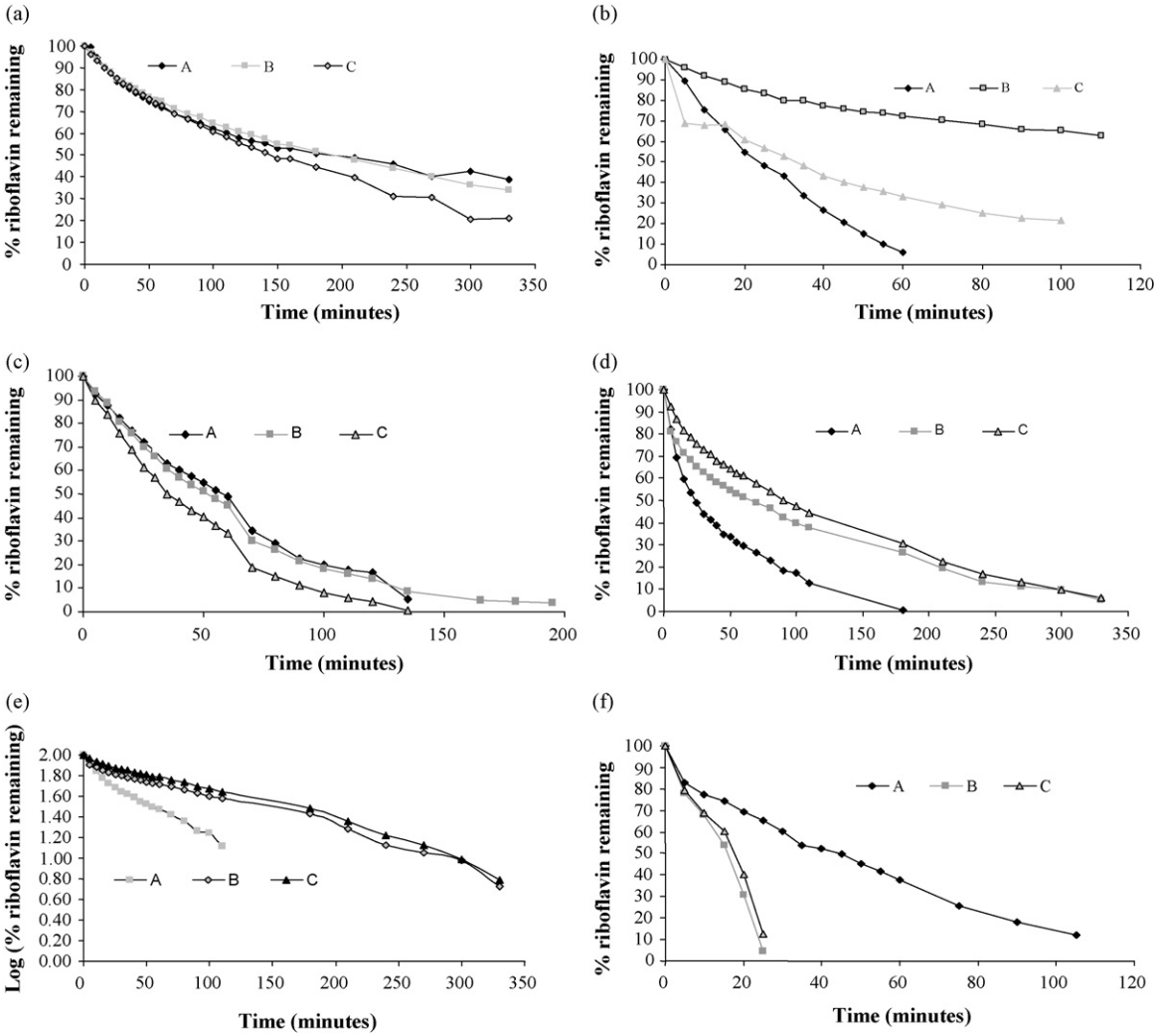

Fig. 6. Percentage of riboflavin remaining as a function of time for calcium alginate bead samples A–C in (a) 0.1 M HCl, pH 1.2 (British Pharmacopoeia, 2004); (b) citrate buffer, pH 3.0 (British Pharmacopoeia, 2004); (c) acetate buffer, pH 5.0 (Chambers and Rickwood, 1993); (d) glass distilled water, pH 6.7; (f) phosphate buffer, pH 7.4 (British Pharmacopoeia, 2004). (e) First order plot of riboflavin release from calcium alginate beads samples A–C in glass distilled water, pH 6.7 (A = 0.06%, w/v, riboflavin, 0%, w/v, citric acid; B = 0.06%, w/v, riboflavin, 0.5%, w/v, citric acid; C = 0.06%, w/v, riboflavin, 0.75%, w/v, citric acid).

of riboflavin over the time period of the experiment. In addition the calcium alginate bead samples prepared with citric acid showed an increased release of riboflavin from the calcium alginate beads when compared with calcium alginate beads that did not contain citric acid.

The reason for the increased release of riboflavin in the presence of citric acid may be attributed to the properties of citric acid. Citric acid is water-soluble and may dissolve from the calcium alginate beads (Nykänen et al., 2001) leaving pores. Therefore, a change in the structure of the calcium alginate beads can occur, and riboflavin is able to diffuse through the pores to the solution.

The steady release rate of riboflavin from calcium alginate beads in media of pH 1.2 over the study period may be as a result of the formation of alginic acid from the reaction of alginate with acid. Alginic acid is insoluble in media of pH 1.2 and swelling of the calcium alginate beads does not occur (Skaugrud et al., 1999). The release of riboflavin can therefore occur by erosion only, compared with a combination of erosion and swelling. Evidence for the insolubility of calcium alginate in acidic media was confirmed when, at the end of the dissolution tests, it was observed that only calcium alginate beads that had been placed in acidic media remained as individual calcium alginate beads in the dissolution vessel. For the *in vitro* tests of calcium alginate beads in acidic media, the ends of the tests were of times not exceeding 350 min.

The release of riboflavin from the calcium alginate beads in a media of pH 3.0 (Fig. 6b) and pH 5.0 (Fig. 6c) was complete in the initial 200 min of the experiment for all formulations.

The release of riboflavin from calcium alginate beads in media of pH 5.0 is in contrast to the release of riboflavin from calcium alginate beads in 0.1 M HCl. The results also showed that the release of riboflavin from the riboflavin loaded beads containing citric acid was slower when compared to riboflavin loaded calcium alginate beads containing no citric acid.

The *in vitro* dissolution tests of calcium alginate beads in media of pH 5.0 (Fig. 6c) also showed the calcium alginate beads to form a single mass unit, and, calcium alginate beads studied in media of higher pH were noted to have all dissolved within 350 min.

During the dissolution tests in media of pH 6.7 (Fig. 6d) in common with observations made when calcium alginate beads were placed in media of pH 5.0, it was noted that a swelling of the calcium alginate beads occurred.

In media of pH 5.0, it was noted that the riboflavin loaded beads appeared to swell. The swelling is due to the solubility of calcium alginate in media of pH 5.0 and above and its subsequent hydration. The swelling of the alginate is characterised by the formation of a gel layer on the outer surface of the calcium alginate bead that is in contact with the media. The release of riboflavin occurs by diffusion through the swelling/gel layer.

In glass distilled water, pH 6.7, all riboflavin was released from the calcium alginate beads within 350 min. Over the period of the experiment, riboflavin showed a release rate from the calcium alginate beads typical of first order kinetics. The first order kinetic release profile was confirmed when the data was presented as shown in Fig. 6e.

Although the formulae of the calcium alginate beads was modified to allow for the inclusion of citric acid, the results of the *in vitro* tests in media of pH 6.7 showed that the effect of citric acid neither enhanced nor retarded the release of riboflavin from the calcium alginate beads.

In phosphate buffer, pH 7.4 (Fig. 6f), all of the riboflavin was released in less than 200 min.

The presence of citric acid increased the rate of release of riboflavin from the beads in media of pH 7.4. In common with the studies performed at pH 5.0 and 6.7, swelling of the calcium alginate beads occurred.

Overall, riboflavin release from the calcium alginate beads was slowest in acidic media (pH 1.2) and quickest in more alkaline media (pH 3.0–7.4). The *in vitro* release times can be considered with subsequent *in vivo* gastric emptying times of the calcium alginate beads. In the fasted and fed state, calcium alginate beads demonstrated maximum gastric residence times of approximately 82 and 300 min respectively (Stops et al., 2006a,b). Therefore, considering the *in vitro* results for the current work and riboflavin release in acidic media, it is likely that *in vivo* the calcium alginate beads will have emptied from the stomach before all the riboflavin can be released. Conversely, in the fed state, considering *in vivo* and *in vitro* results, all the riboflavin will have diffused from the calcium alginate beads before they are emptied from the stomach or dissolved in the alkaline contents.

Ideally, when considering a gastro-retentive dosage form, it would be advantageous for the calcium alginate beads to be administered under fasting conditions when the environmental conditions of the stomach are acidic. It would also be an advantage for the calcium alginate beads to be resident in the stomach for an extended period of time. Under such conditions, the dosage form would be required to release the drug slowly over a period of time, thereby demonstrating zero order release kinetics. Calcium alginate beads of the current formulation do not show zero order kinetics in all media tested. If the dosage form is to be used for local therapy, such as *H. pylori*, an initial burst of drug from the dosage form is advantageous in order to reach therapeutic concentrations. However, the continued fast release rate of the drug in media of pH 5.0 and above, typical of the pH of the stomach contents under fed conditions is not desirable as complete release of the drug from the dosage form before the end of the dosing period may result. It would therefore be necessary to administer another dose in order to maintain effective plasma concentrations of the drug.

When considering the *in vitro* results, the release of riboflavin from the calcium alginate beads tests may be affected by parameters such as day to day variations in equipment set up, laboratory differences, water bath differences and the routine production of the riboflavin loaded calcium alginate beads. Such variations may make for incorrect conclusions to be drawn regarding the similarity or dissimilarity of the dissolution profiles. Therefore, it is necessary to account for the variations using analytical or statistical methods. The f_2 metric is not a statistical method, but is based on experimental design that allows for variation in analytical testing and production variations.

Table 2
Table of f_2 values for *in vitro* dissolution profiles for calcium alginate beads of different formulae

Media	0.1 M HCl (pH 1.2)	Citrate buffer (pH 3.0)	Acetate buffer (pH 5.0)	Glass distilled water (pH 6.7)	Phosphate buffer (pH 7.4)
In vitro dissolution profiles					
A–B	85.2	54.4	83.1	60.6	58.5
B–C	81.6	56.8	68.4	70.4	74.9
A–C	98.0	71.2	66.4	57.1	60.9

A = 0.06%, w/v, riboflavin, 0%, w/v, citric acid; B = 0.06%, w/v, riboflavin, 0.5%, w/v, citric acid; C = 0.06%, w/v, riboflavin, 0.75%, w/v, citric acid.

All calculated f_2 figures were in excess of 50%, Table 2, thereby demonstrating similarity with regard to the average percentage of drug dissolved.

When modifying the calcium alginate bead formula to include citric acid, it would be reasonable to conclude that the presence of citric acid would reduce the pH of the dissolution media. The release of riboflavin from the calcium alginate beads would be retarded due to the insolubility of alginate in acidic media. However, citric acid is a weak acid, and the quantities of calcium alginate beads used in the *in vitro* dissolution studies contained insufficient amounts of citric acid to affect the pH of the media.

3.7.1. Assigning release kinetics or riboflavin from riboflavin loaded calcium alginate beads

Consideration has been given to applying a kinetic model to the release of the riboflavin from the calcium alginate beads. Ideally, for a once daily orally administered dosage form, zero order kinetics are usually required in order to produce a constant plasma concentration profile and reduce the possibility of the occurrence of side effects. However, when considering a multi-particulate dosage form such as calcium alginate beads, the release rate of the drug may not display strict zero order kinetics and it may not be possible to define an exact model or mechanism for the drug release from the calcium alginate beads. Instead, it may be possible to consider that drug release occurs as a combination of mechanisms. The reason for this is as follows. Calcium alginate beads are essentially a polymeric drug delivery system. In common with most polymeric drug delivery systems, drug release occurs by the process of diffusion. However, diffusion of the drug in media of pH 1.2 (reflective of the stomach in the fasted state), as shown by the dissolution results, is slow; the release of the drug being determined by the alginate matrix. On contact with acidic media, the outer surfaces of the calcium alginate beads form insoluble alginic acid. However, when the calcium alginate beads were placed in media of pH 5.0–7.0, reflective of the stomach environment in the fed state or of the small intestine, the drug release rate from the calcium alginate beads increased when compared to the drug release into media of pH 1.2. First order kinetics are then observed. Overall, the calcium alginate beads do not display zero order kinetics that are essential for a gastro-retentive dosage form. The absence of additional information regarding the movement of drug into the media did not allow for further determination of the kinetic profile. The inclusion of a pH independent polymer may result in drug release profiles that demonstrate near zero order kinetics. Central to achieving such a profile is a thorough understanding of the gel barrier. The formation, thickness and subsequent erosion will all affect the drug release from the calcium alginate bead. Therefore, further investigations to determine the properties of the gel barrier are required. Such knowledge will also allow for the calcium alginate bead formula to be modified in order that zero order kinetics can be achieved.

Most models of drug delivery, whether swelling or membrane systems, rely on the fact that the model drug is contained in a matrix within the dosage form. For example, swellable tablets are usually produced with the drug enclosed in a pH independent polymer such as HPMC. Calcium alginate beads do not contain a separate matrix, rather that the drug is dispersed throughout the calcium alginate bead. Nor are they simply a swelling system, since erosion and swelling of the calcium alginate beads gives rise to the release of the drug. Therefore, none of the traditional mechanisms can be applied to the calcium alginate beads when considering drug release rate and mechanism.

Latter studies have attempted to determine the mechanism of drug release from swelling systems where both swelling and eroding fronts occur. Puttipipatkhachorn et al. (2001) reported that Hofenberg studied the erosion of a dosage form as the mechanism of drug release, but did not consider the combination of erosion and diffusion as a method of drug release. Other findings suggest that the drug release is affected by the type of polymer (Streubel et al., 2003), additional excipients and drug substances (Efentakis et al., 1997) used in the diffusing front, as well as the position of the swelling and eroding fronts. The release of drug from such a system has demonstrated that initially the eroding front follows non-linear kinetics but subsequent dissolution follows linear kinetics (Conte et al., 1998). Therefore, applying the findings to the current work, in order to apply an exact mechanism of drug release to the calcium alginate beads, further knowledge is required regarding the specific polymer, i.e. calcium alginate. A model of drug release from calcium alginate beads can only be suggested when investigations of the swelling and relaxation of the polymer in different media are completed. A study of the gel layer, formed when the calcium alginate beads are placed in media, is critical to such investigations since the thickness of the layer and the drug concentration within it will determine drug release rates and mechanisms.

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4. Conclusion

A thorough characterisation of calcium alginate beads with different formulations has been investigated and has resulted in obtaining an understanding of the physical properties of the dosage form. Additionally, initial *in vitro* assessments of the calcium alginate beads in different media designed to reflect changes that occur along the gastrointestinal tract have been completed. In summary, the release of riboflavin from the calcium alginate beads was complete within 350 min, despite extending most studies to 24-h. Therefore, although the physical property results suggest that the dosage form may be considered as a floating controlled drug delivery system, the *in vitro* studies indicate the current formula is not suitable for once daily administration and further formula modifications are required to alter the rate of drug release from the calcium alginate beads.

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References

Axelrod, D., Koppel, D.E., Schlessinger, J., Elson, E., Webb, W.W., 1976. Mobility measurement by analysis of fluorescence photobleaching recovery kinetics. Biophys. J. 16, 1055–1069.

British Pharmacopoeia, 2004, vol. IV. The Stationary Office, London (Appendix I).

Bussemer, T., Peppas, N.E., Bodmeier, R., 2003. Evaluation of the swelling, hydration and rupturing properties of the swelling layer of a rupturable pulsatile drug delivery system. Eur. J. Pharm. Biopharm. 56, 261–270.

Chambers, J.A.A., Rickwood, D., 1993. Biochemistry Labfax. Blackwell Scientific Publications, pp. 1–35.

Colombo, P., 1993. Swelling-controlled release in hydrogel matrices for oral route. Adv. Drug Deliv. Rev. 11, 37–57.

Conte, U., Colombo, P., Gazzaniga, A., Sangalli, M.E., La Manna, A., 1998. Swelling-activated drug delivery systems. Biomaterials 9, 489–493.

Efentakis, M., Vlachou, M., Choulis, N.H., 1997. Effects of excipients on swelling and drug release from compressed matrices. Drug Dev. Ind. Pharm. 23, 107–112.

El-Gibaly, I., 2002. Development and *in vitro* evaluation of novel floating chitosan microspheres for oral use: comparison with non-floating chitosan microspheres. Int. J. Pharm. 249, 7–21.

Fassihi, R., 1995. A novel device in conjunction with paddle method to replace the application of wire helix sinker to floating dosage form. Pharm. Res. 12, 298.

Kelco International Limited, 1987. Alginate Properties for Scientific Water Control, third ed. Kelco International Limited, San Diego, pp. 1–9.

Klokk, T., Melvik, J.E., 2002. Controlling the size of alginate gel beads by use of a high electrostatic potential. J. Microencapsul. 19, 415–424.

Kubitscheck, U., Wedekind, P., Peters, R., 1994. Lateral diffusion measurement at high spatial resolution by scanning microphotolysis in a confocal microscope. Biophys. J. 67, 948–956.

Melia, C.D., Rajabi-Siambhoomi, A.R., Hodson, A.C., Adler, J., Mitchell, J.R., 1993. Structure and behaviour of hydrophilic matrix sustained release dosage forms: 1. The origin and mechanism of formation of gas bubbles in the hydrated surface layer. Int. J. Pharm. 100, 263–269.

Nykänen, P., Lempää, S., Aaltonen, M.-L., Jurjenson, H., 2001. Citric acid as an excipient in multiple unit enteric coated tablets for targeting drugs on the colon. Int. J. Pharm. 229, 155–162.

Østberg, T., Graffner, C., 1992. Calcium alginate matrices for oral multiple unit administration. I: Pilot investigations of production method. Acta Pharm. Nord. 4, 201–208.

Pillay, V., Fassihi, R., 1998. Evaluation and comparison of dissolution data derived from different modified release dosage forms: an alternative method. J. Control. Release 55, 45–55.

Puttipipatkhachorn, S., Nunthanid, J., Yamamoto, K., Peck, G.E., 2001. Drug physical state and drug-polymer interaction on drug release rate from chitosan matrix films. J. Control. Release 75, 143–153.

Singh, B.N., Kim, K.H., 2000. Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention. J. Control. Release 63, 235–259.

Skaugrud, Ø., Hage, A., Borgerson, B., Dornish, M., 1999. Biomedical and pharmaceutical applications of alginate and chitosan. Biotechnol. Genet. Eng. Rev. 16, 23–40.

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

Stops, F., Fell, J.T., Collett, J.H., Martini, L.G., Sharma, H., Smith, A.-M., 2006b. Citric acid prolongs the gastro-retention of a floating dosage form and increases bioavailability of riboflavin in the fasted state. Int. J. Pharm. 308, 14–24.

Streubel, A., Siepmann, J., Bodmeier, R., 2003. Floating matrix tablets based on low density foam powder: effects of formulation and processing parameters on drug release. Eur. J. Pharm. Sci. 18, 37–45.

Timmermans, J., 1991. Floating hydrophilic matrix dosage forms for oral use. Factors controlling their buoyancy and gastric residence capabilities. Ph.D. Thesis. Free University of Brussels, Belgium.

Timmermans, J., Moës, A.J., 1989. Determining in vitro the resultant-force acting on a pharmaceutical form immersed in a fluid, an apparatus and a method. Proceedings of the Fifth APGI International Conference on Pharmaceutical Technology, Part 2, pp. 294–303.

Timmermans, J., Moës, A.J., 1990a. Measuring the resultant-weight of an immersed test material: I. Validation of an apparatus and a method dedicated to pharmaceutical applications. Acta Pharm. Technol. 36, 171–175.

Timmermans, J., Moës, A.J., 1990b. How well do floating dosage forms float. Int. J. Pharm. 62, 207–216.

US Department of Health and Human Services FDA, CDER, August 1997. Guidance for Industry. Dissolution testing of immediate release solid oral dosage forms.

Whitehead, L., 1998. An investigation into a gastroretentive dosage form. PhD Thesis. University of Manchester.