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Utility of *in situ* sodium alginate/karaya gum gels to facilitate gastric retention in rodents

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ABSTRACT

Target validation or demonstration of efficacy requires adequate *in vivo* exposure of tool molecules to determine their activity in order to validate the model or show the potential usefulness of the pharmacophore. Early discovery work is often carried out with compounds which possess undesirable PK properties in small rodents where the discovery formulation scientist is often forced to dose 2–4 times per day. Gastric retentive formulations in small rodents (rats/mice) could enable increased duration of exposure for compounds with narrow absorption windows or increased residence time for compounds with targets located in the GI tract. The aim of this work is to establish an easily administered gastric retentive gel for rodents *in situ* using a mixture of sodium alginate and karaya gum. Feasibility studies were conducted in Sprague-Dawley rats using barium sulfate as a radio-opaque tracer. The results show that gastric retention of barium was achieved for rats dosed with the gel formulation relative to a barium suspension. The gastric residence time of the gel varied from 1 h to >8 h ($n = 3$). The data suggest that sodium alginate/karaya gum gels may be a useful tool to achieve gastric retention in rodent studies.

1. Introduction

Pharmaceutical discovery teams often utilize compounds with non-optimized PK properties to test hypotheses related to the target of interest early in the discovery process. It is imperative that adequate exposure is obtained in these studies to relate pharmacological activity to blood levels. This may require dosing a compound multiple times per day to maintain the desired exposure. A gastric retentive formulation that can be dosed to small rodents in a discovery setting may be useful in increasing the duration of exposure of compounds with a narrow absorption window at the upper part of the GI tract or provide prolonged exposure to compounds whose targets are in the GI tract. For a compound with a narrow absorption window, enhancing the gastric residence time may significantly improve the extent of its absorption by increasing the absorption time in the proximal part of the small intestine that often is the most permeable part of the GI tract (*i.e.* larger gaps between the tight junctions and dense active transporters) (Hoffman et al., 2004). Published studies show an increased gastric residence time and increased bioavailability of riboflavin when dosed as a gastric retained multi-layer film in beagle dogs (Klausner et al., 2002), in capsules containing swellable polymer films in dogs and humans (Ahmed and Ayres, 2007) and as floating calcium alginate beads in humans (Stops et al., 2006). Likewise, gastric retentive formulations have been used to increase the bioavailability of the narrow absorption window drug, metformin, relative to an immediate release formulation (Lalloo et al., 2012; Choudhury et al., 2008). In addition, the use of gastric retention formulation strategies have been used to successfully treat helicobacter pylori locally in the stomach of rats (Katayama et al., 1999; Tripathi et al., 2011).

Alginate systems have previously been employed to test the concepts of sustained release formulations (Kubo et al., 2003; Rastogi et al., 2007; Satapathy et al., 2010). In alginate gels, hydrogen bridges between carboxyl groups are organized in zones of fusion joining the adjacent polysaccharide chains. The chains aggregate due to the formation of multiple bonds with divalent cations such as Ca^{2+} (Khotimchenko et al., 2001). Alginate gels are physically stable in the acidic pH environment of the stomach and erode in the higher pH environment of the intestines (Ain et al., 2003). Gastric retention with an alginate gel requires expansion of the gel to a size large enough to be retained in the fasted stomach in a safe and reliable manner (Davis, 2005). Ideally, the gastroretentive

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system would also display controlled release properties so that the drug is released at an appropriate rate for optimal absorption within the absorption window. Furthermore, the system should have sufficient rigidity to remain intact in the stomach and to withstand the mechanical forces exerted on it over a short period of time. Ultimately, the gastric retentive formulation will need to decrease in size and transit through the intestines to be eliminated from the body (Davis, 2005).

Much of the work in the pharmaceutical industry involving gastric retentive dosage forms is focused on developing solid dosage forms of marketed drugs for humans where various technologies such as floating dosage forms and high density, bioadhesive, and modified shape/size systems can be employed to help achieve gastric retention (Stepensky et al., 2001; Jagdale et al., 2009; Uddin et al., 2011; Burke et al., 2007; Tripathi et al., 2011). The focus of many of these recent publications has been on development of a commercial formulation. Our aim is not to translate our work to a commercial application, but to utilize gastric retention as a way to achieve extended coverage of less than ideal compounds in early preclinical rodent studies. The purpose of this work was to design and evaluate a compound- and resource-sparing *in situ* gastric retentive strategy for use in the early preclinical space where rats and mice are typically used as *in vivo* models. Due to the nature of our work in the early discovery space, which often requires large doses of compounds with varying physicochemical properties, we utilized the size mechanism for gastric retention since this technology should be amenable to a wide range of compounds and accommodate large doses.

Some authors have previously reported using *in situ* alginate gels in preclinical species to compare release profiles with commercial formulations (Kubo et al., 2003; Miyazaki et al., 2000). For example, Kubo et al. dosed alginate formulations in rats and rabbits to achieve sustained delivery of paracetamol. In those studies, alginate was formulated with calcium ions and the calcium chelator sodium citrate was added to prevent gel formation *ex vivo*. Gel formation was accomplished *in situ* by the release of the calcium ions in the low pH environment of the stomach resulting in cross-linking of the alginate matrix. *In situ* gelation reported by Katayama et al. was achieved by the separate oral administration of a solution of a sodium alginate immediately followed by a calcium salt in rats to evaluate sustained release of ampicillin to treat helicobacter pylori (Katayama et al., 1999). The present work utilized dosing a barium sulfate/alginate/karaya gum formulation followed by a calcium chloride chaser to form gels *in situ*. Unlike previous reports utilizing *in situ* alginate gels in rodents, digital radiography was used in the current study to enable direct real time monitoring of gastrointestinal transit of the gel over time.

2. Materials and methods

2.1. Materials

Sodium alginate was purchased from Spectrum Chemical. Karaya gum, calcium chloride, and docusate sodium were purchased from Sigma–Aldrich. Barium sulfate was obtained from Mallinckrodt (particle size < 5 μm). Methylcellulose E4M was obtained from Colorcon.

2.2. Optimization of calcium cross-linker concentration

In vitro optimization to determine the required calcium cross-linking concentration was conducted by preparing calcium chloride at concentrations ranging from 0.001 M to 0.1 M. Aliquots of these solutions were added to a solution containing 1% sodium alginate/0.625% karaya gum to facilitate gel formation. The resulting gels were transferred to a pH 2 environment at 37 °C for overnight observations. A separate study was conducted to confirm that the gels erode in simulated fasted intestinal fluid (pH 6.5).

2.3. Measurement of gel strength using cone and plate rheology

2.3.1. Gel preparation

One gram of sodium alginate powder and 0.625 g of karaya gum were added to 100 mL milli-Q water while stirring. The mixture was stirred on a high shear overhead mixer (Kinematica Polytron PT3100) at 12,000 rpm for 10 min at room temperature. Fifty mL of the homogenous mixture was poured into a 100 mL straight-walled jar, to which either 50 mL of 0.01 M or of 0.1 M calcium chloride was added and allowed to quench for 1–2 h at room temperature. The residual calcium chloride was decanted from the gel just prior to testing to ensure that the gel remained wetted for analysis. Gel disks were carved from the bulk gel. The size was tailored to fit the selected geometry and gap width to form a disk that extended out to, but not further than, the edge of the plate. Each gel was run once.

2.3.2. Gel rheology method

The shear moduli and viscosity of the gels were measured using a TA Instruments AR 1000 Rheometer with a cross-hatched 20 mm diameter steel parallel plate geometry. The peltier plate temperature was 25 °C, and the gap width was 1000 μm. For the viscosity measurement, the shear rate was 0.1–100 s^{-1}. Twenty mL aqueous solution containing 1% sodium alginate:0.625% karaya gum was prepared. Twenty mL of 0.01 M or 0.1 M $CaCl_2$ aqueous solution was then added to quench and form gel. The gel was allowed to sit for several hours prior to rheological analysis. Prior to testing, all aqueous solution was decanted in order to ensure the gel remained wetted for analysis. Ideally, sample prep was done to form a cylinder under the plate which extended out to, but not further than, the edge of the plate. Each gel was run five times. For the shear moduli measurements, gels were scanned using an oscillatory frequency sweep from 1 to 10 Hz, holding the oscillatory stress constant at 1 Pa for the more lightly cross-linked gel (0.01 M calcium chloride), and at 10 Pa for the more highly cross-linked gel (0.1 M calcium chloride). These stresses were selected to be well within the constant shear modulus *versus* oscillatory stress region at 1 Hz. Gel conditioning time at each data collection point was 20 s, and data sampling time was 20 s.

2.4. Barium sulfate concentration optimization by digital radiography

A preliminary study was conducted to determine the appropriate amount of barium sulfate to be adequately visualized by digital radiography. Gels containing varying amounts (5–50 mg/mL) of barium sulfate were prepared by adding the barium sulfate to the alginate/karaya gum powder, adding milli-Q water and mixing using a Heidolph homogenizer at maximum speed (26000 rpm). Three mL of 0.1 M calcium chloride was then added to 3 mL of the alginate/karaya gum/barium mixture. The resulting gels were analyzed *via* digital radiography to ensure sufficient contrast.

2.5. In vivo gastric retention of barium sulfate in rats

2.5.1. Formulation preparation

One hundred mg of sodium alginate and 62.5 mg of karaya gum was added to a 20 mL scintillation vial. Just prior to dosing, 500 mg of barium sulfate was added to the alginate/karaya gum powder. Ten mL of milli-Q water was added to the powder and the resulting suspension was immediately vortexed and homogenized using a Heidolph homogenizer at maximum speed (26,000 rpm) for approximately 5–10 min to ensure mixing. The

resulting suspension quickly became very viscous. Dosing syringes were immediately filled after preparation to ensure that the formulations could be dosed (time between preparation and dosing <1 h). The barium suspension was prepared by adding 50 mg/mL barium sulfate to 0.5% methylcellulose E4M/0.02% docusate sodium and homogenizing to ensure uniformity.

2.5.2. *In vivo* rat study

All *in vivo* studies were approved by the Bristol-Myers Squibb Animal Care and Use Committee (Lawrenceville, NJ) and were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). Sprague-Dawley rats were fasted overnight and during the 8 h duration of the study. Rats were given free access to water. Syringes were filled for dosing at 1.4 mL to achieve a 5 mL/kg (250 mg/kg) dose of the barium formulation either in aqueous suspension or alginate/karaya gum suspension formulation for the rats which averaged 270–280 g. A separate set of syringes was filled with 1.4 mL of 0.1 M calcium chloride which was used as the cross-linking agent in this study. Rats were dosed with the barium formulation (aqueous suspension or alginate/karaya gum suspension formulation) followed immediately by the calcium chloride solution. The time between dosing the two fluids was <1 min. Digital radiographs were taken over an 8-h period after dosing to monitor barium transit. Necropsy was performed after the termination of the study to confirm data from radiographs.

2.5.3. Digital radiography

Digital radiographs were taken using an Eklin RapidStudy EDR6 clinical digital radiography system manufactured by Sound-Eklin. Rats were placed in restraint devices for each film. Dorsal ventral views of the abdomen were obtained of each rat during each time point. The films were reviewed for movement of barium out of the stomach into the intestines using an Eklin RapidView 3GS Diagnostic Workstation.

2.5.4. Post-mortem necropsy

Within 5 min of the last radiograph, the rats were euthanatized by carbon dioxide overdose. Necropsies were performed to verify the presence (or absence) of the gel in the rat stomach or intestine. The GI tract was removed from each rat and the stomach opened to recover gastric contents.

3. Results

3.1. Optimization of calcium cross-linker concentration

Rheometry was performed to determine the gel strength, which was quantified in terms of storage modulus, of the gels dosed *in vivo*. Typically, gels are defined as materials having a ratio storage to loss modulus (G'/G'') of >1, whereas materials for which $G'/G'' < 1$ are considered viscous solutions. As shown in Fig. 1, cross-linking the alginate/karaya gum solutions in either 0.01 M or 0.1 M calcium chloride solutions resulted in the formation of gels having a ratio of storage to loss modulus of 4.5 and 8.8, respectively. The data in Fig. 1 show that the gel strength, as determined by the storage modulus at 1 Hz, increases by a factor of approximately 18 as the concentration of the calcium chloride cross-linking agent increases ten-fold, from 0.01 M to 0.1 M. Fig. 2 shows that at low shear rates the viscosity of the gel made using 0.1 M calcium chloride was 5–6 times higher than that made with 0.01 M calcium chloride. Additional experiments showed that the gels are stable in a simulated gastric environment at 37 °C after overnight storage and start to erode within minutes of being exposed to simulated fasted

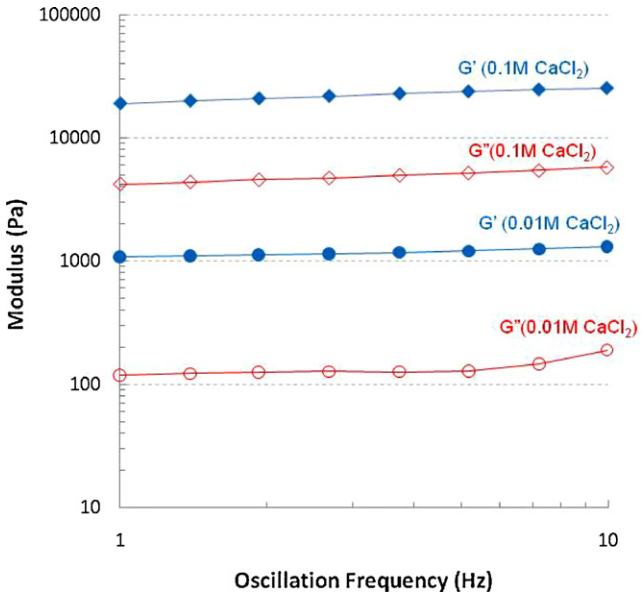

Fig. 1. Storage and loss modulus of 1% alginate/0.625% karaya gum gels cross-linked with 0.01 M or 0.1 M calcium chloride.

intestinal fluid (pH 6.5). Based on these results, a 0.1 M calcium chloride solution was used to crosslink the gels *in vivo*.

3.2. Optimization of barium sulfate in alginate/karaya gum matrix

The digital radiograph data show that 50 mg/mL (corresponding to a dose of 250 mg/kg) was adequate to visualize the barium on the digital radiograph and thus was chosen for the *in vivo* study (data not shown). These experiments also served to ensure that the alginate/karaya gum gel containing 50 mg/mL barium sulfate could be gavaged within 1 h after preparation since the barium ions, like calcium, can crosslink the alginate/karaya gum polymers through complexation with the carboxylic acids making the liquid too viscous to dose.

3.3. In vivo gastric retention study

A summary of the *in vivo* studies is shown in Table 1. Animals were dosed with an aqueous barium suspension. Digital

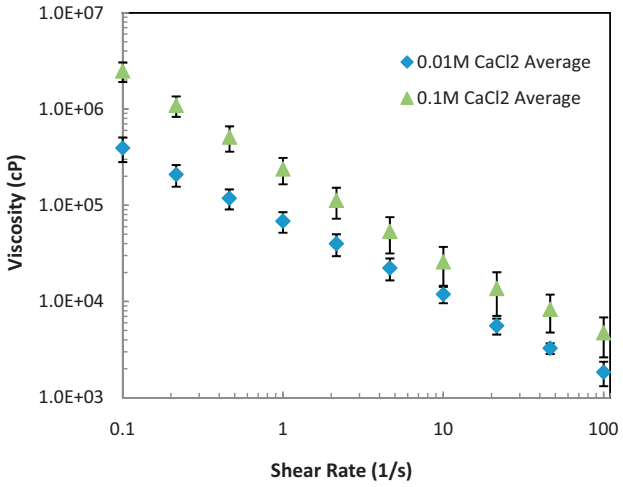

Fig. 2. Viscosity of 1% alginate/0.625% karaya gum gels cross linked with 0.01 M or 0.1 M calcium chloride.

Table 1
Summary of gastric residence time of 1% sodium alginate/0.625% karaya gum crosslinked with 0.1 M calcium chloride *in situ* in rats.

Rat identification	Time of last appearance of barium in stomach	Time of first appearance of barium in intestines
Rat 1 control barium suspension	3 h	30 min
Rat 2 control barium suspension	3 h	Initial timepoint
Rat 3 control barium suspension	3 h	30 min
Rat 1 alginate/karaya gum/barium gel	8 h (study duration)	Not observed
Rat 2 alginate/karaya gum/barium gel	5 h	5 h
Rat 3 alginate/karaya gum/barium gel	1 h	2 h

radiographs taken at the start of the experiment confirmed the presence of barium in the stomach of the rats (Fig. 3). Radiograph data for the aqueous barium suspension administration revealed that barium had transited from the stomach to the intestines within the first 30 min of the study (Table 1). The aqueous barium suspension appeared to completely evacuate the intestines of all rats by the 6 h time point (Table 1). This is in agreement with previously published data where the mean gastric emptying time of barium sulfate in the stomach of rat was 11 ± 4 min and the mean intestinal transit time was 5 h (± 0.75) (Perry et al., 1993) (Figs. 4a and 5a).

The rats dosed with the barium/alginate/karaya gum matrix formed gels *in situ*. The presence of intact gel in the stomach was apparent as a white cast (Fig. 3b). The barium sulfate containing gel was retained in the stomach for at least 1 h in all rats. Rat #3 had the most rapid gastric transit of the gel from the stomach by 2 h with all evidence of barium being depleted from the GI tract by 7 h (Table 1). Rat #2 appeared to lose the gel from the stomach between the 5 and 6 h time points but still showed the presence of barium in the intestines at the end of the study (Table 1). Rat #1

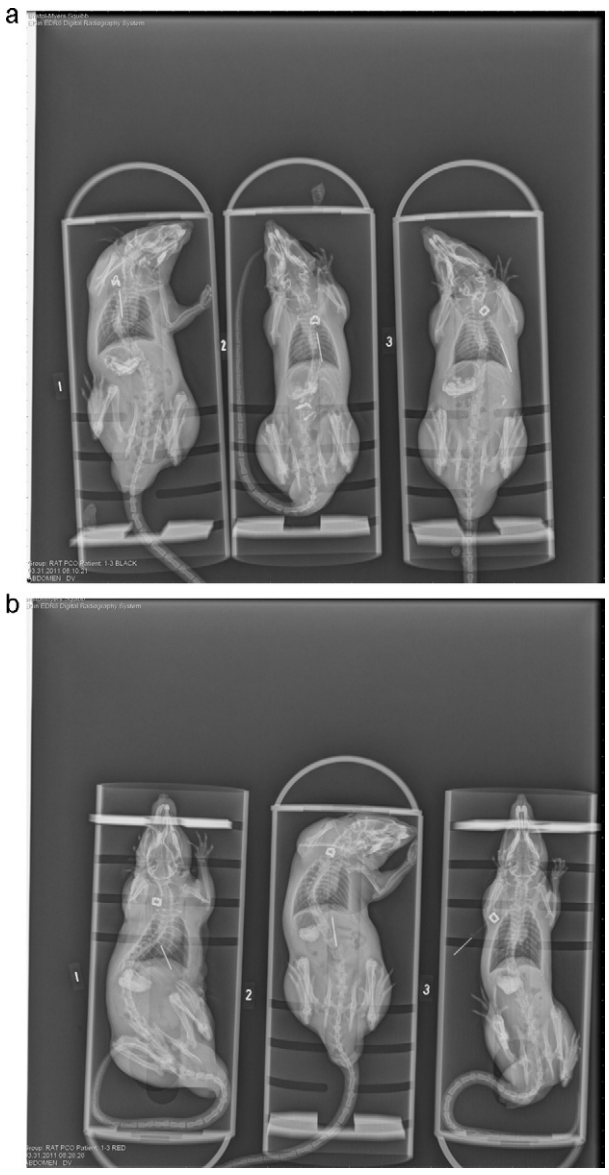

Fig. 3. Digital radiographs of rats dosed with barium aqueous suspension (a) and barium suspension/alginate/karaya gum gel (b) immediately after dosing.

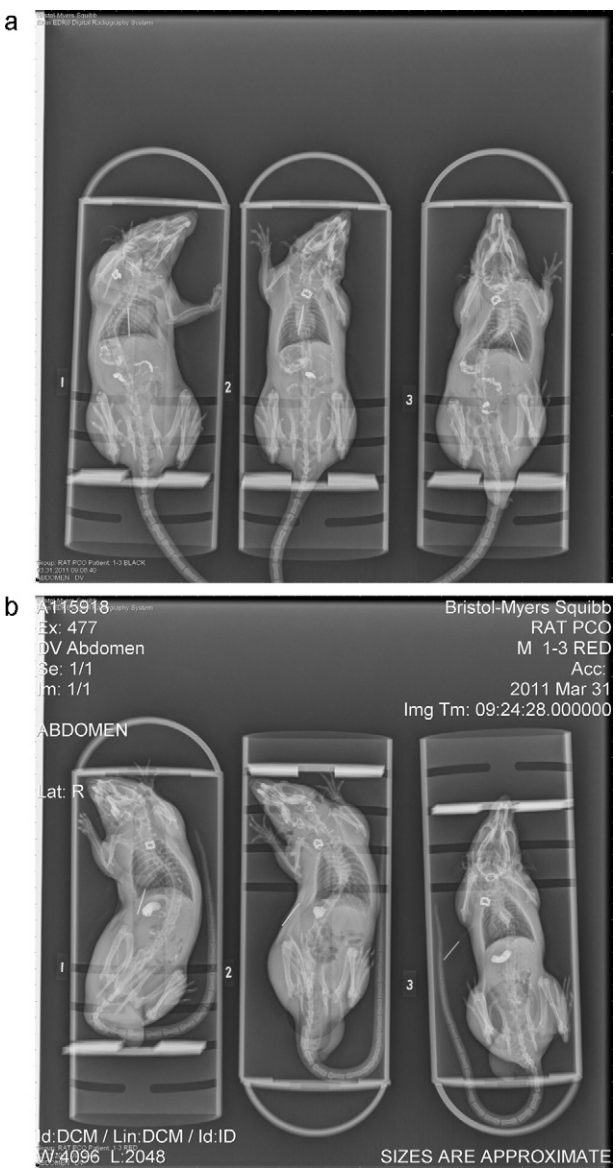

Fig. 4. Digital radiographs of rats dosed with barium aqueous suspension (a) and barium suspension/alginate/karaya gum gel (b) 1 h after dosing.

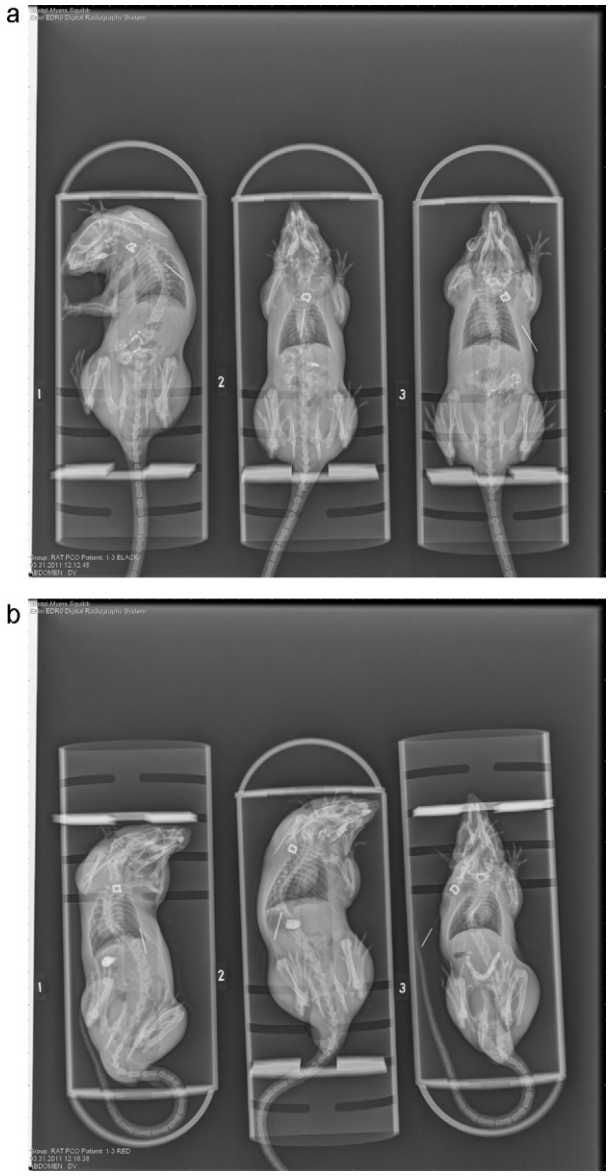

Fig. 5. Digital radiographs of rats dosed with barium aqueous suspension (a) and barium suspension/alginate/karaya gum gel (b) 4 h after dosing.

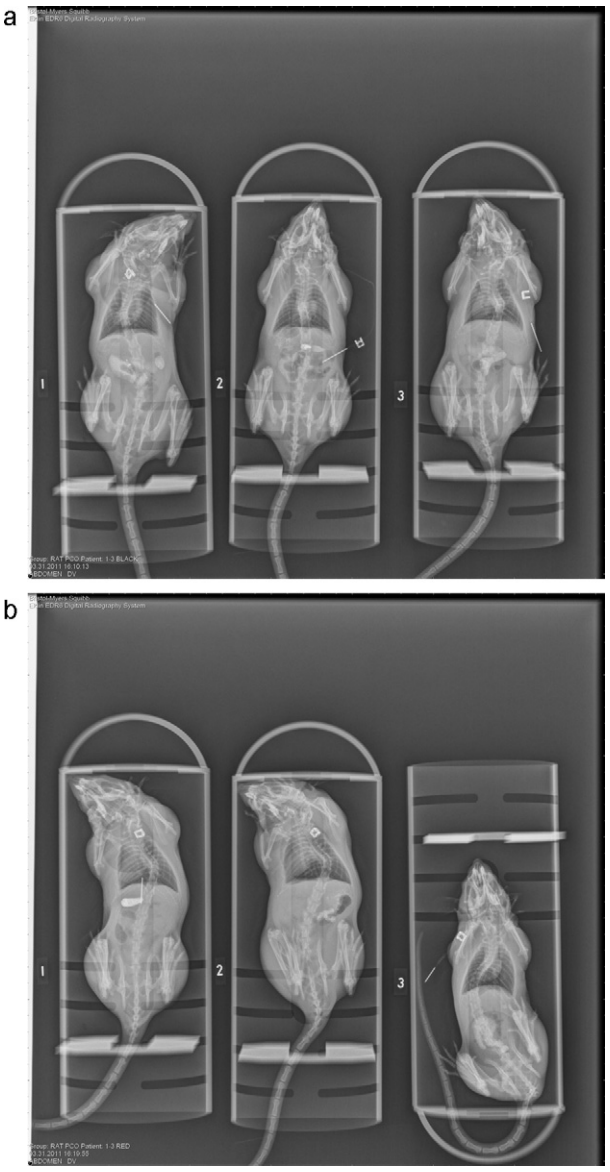

Fig. 6. Digital radiographs of rats dosed with barium aqueous suspension (a) and barium suspension/alginate/karaya gum gel (b) at 8 h.

kept the gel retained in the stomach for the entire 8 h duration of the study (Fig. 6b).

Post-study necropsy confirmed data obtained by digital radiography. The entire length of the intestines was opened at necropsy. Where gel had migrated into the small intestine, only small particles (1–2 mm) of material could be found. In no instance was an intact gel found in the small intestine. The intact gel can be seen in rat #1's stomach (Fig. 7). Necropsy of rat #2 post-study revealed pieces of the barium sulfate containing gel were observed in the jejunum which confirms what was observed by digital radiography (Fig. 8). No evidence of barium was observed in the GI tract of rat #3.

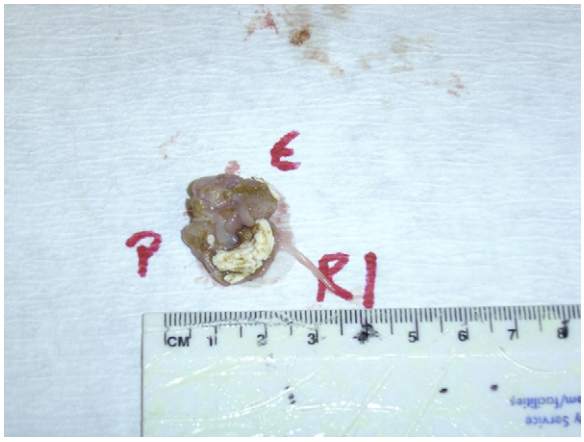

Fig. 7. Necropsy of stomach from rat #1 dosed with barium suspension/alginate/karaya gum gel post-study. The picture shows that the esophagus (E) and pylorus (P) orientation of rat #1's (R1) stomach. The gel measured ~1.5 cm in length and ~0.6 cm in width.

4. Discussion

This work employs a mixture of sodium alginate and karaya gum to form gels *in situ* using the divalent cation calcium dosed as a chaser immediately following administration of the polymer. To our knowledge, this is the first report using the alginate/karaya gum system as a model for gastric retention in rodents. Barium

Fig. 8. Necropsy of small intestine from rat #2 (R2) dosed with barium suspension/alginate/karaya gum gel post-study. The gel appears to have eroded in the small intestine into small fragments containing barium sulfate.

sulfate was used as a marker for gastric retention of alginate/karaya gum gels *in vivo*. Barium sulfate was chosen for its opacity in X-ray imaging and due to its low solubility ($ksp = 1.08 \times 10^{-10}$). Though barium, like calcium, is a divalent cation, we expected minimal cross-linking of barium relative to calcium due to the low solubility of barium sulfate relative to calcium chloride. The barium was expected to stay retained in the gel and not easily diffuse from the alginate/karaya gum matrix during transit in the GI tract due to its poor solubility which enabled the authors to get a visual read on gastric retention.

It can be difficult to achieve extensive retention of a dosage form in the stomach since the natural activity is to evacuate its contents into the small intestine. The basis for gastric retention of the currently described alginate system is presumed to be the size and strength of the gel formed *in situ* coupled with slow degradation of the gels in the low pH environment of the stomach prevents migration through the pylorus to the small intestine (Hoffman et al., 2004). Though the size of the pyloric sphincter in rats is not known, Saphier et al. investigated the gastric retention of various capsule sizes in rats and showed a correlation between capsule length and gastric retention (Saphier et al., 2010). The authors dosed fasted rats orally with 3 different lengths of enteric coated capsules (diameter of each capsule was 2.64 mm and lengths of 7.18, 4.8 or 3.5 mm) and found that the 7.18 mm capsules were gastric retained longer than the shorter capsules. When Saphier dosed the 4.8 and 3.5 mm length capsules to rats (both rats received 2 capsules), five out of the eight rats emptied both capsules intact from the stomach to the intestine within 3 h of dosing. However, when the same rats were given the 7.18 mm length capsules, none of the capsules had left the stomach within 5 h. The anatomy of the pylorus was examined in relation to the size of the capsule and no evidence was found to indicate physical hindrance was preventing migration of the capsule to the intestine (Saphier et al., 2010). Saphier's studies suggest that, in general; a non-eroding delivery system >7 mm in length would be gastric retained longer than one with smaller dimensions.

The present data indicate that the gastric residence time of the alginate/karaya gum gel was highly variable among the three rats dosed. Variability in the duration of gastric retention for dosage forms which do not erode in the stomach has also been noted by other authors. Saphier et al. noted high variability in gastric retention of barium capsules (4.8 mm × 2.64 mm) among different rats ($n = 20$) and days of experiments in their studies (Saphier et al., 2010). Albrecht et al. noted that 2.65 mm × 8.4 mm capsules were gastric retained in fasted rats ($n = 4$) between 2 and 8 h without the use of a prokaryotic agent (Albrecht et al., 2006). It is unclear why the alginate/karaya gum gel was retained in rat #1 for the duration of the study and not for the other two rats. Potential reasons for this may be variability in the size and the strength of the gel formed *in situ* due to variability in the mixing dynamic in the rat stomachs. Based on Fig. 3b, the initial gel size formed in rat #1 did appear to be slightly larger than the gels formed in rat #2 and rat #3. Necropsy data from the present study showed that the gel length for rat #1 was approximately 1.25 cm in length and 0.6 cm in diameter at the end of the study (Fig. 7). It is possible that increasing the 5 mL/kg dose volume of the alginate/karaya gum formulation may result in a larger gel and thus lead to more reproducible gastric retention *in vivo*. Gel strength may be another variable to investigate further. Though we have not made measurements on gels formed using a broader range of calcium concentrations, the results of the present study suggest that the concentration of divalent cross-linking agent can be varied to optimize the strength of the gel for particular gastroretentive applications. Other potential variables to investigate include: cross-linking density, mass/concentration of polymer dosed and mixing procedure for the polymer/cross-linker.

5. Conclusions

This initial *in vivo* work shows promise for gastric retention in rodents in a drug discovery setting. We were able to demonstrate that the alginate/karaya gum gels formed *in situ* and were retained in the stomach significantly longer than aqueous suspensions. Once the alginate/karaya gum gels left the stomach, they appeared to break down, presumably through an erosion mechanism, when entering the higher pH environment of the small intestine. The current work focused on the poorly soluble radio-opaque marker barium sulfate. In order to apply this technology to a Discovery program, it may be necessary to optimize the system for a particular compound of interest. One can envision that compounds with varying physicochemical properties (*i.e.* solubility) may behave differently in the alginate/karaya gum matrix and may have different release profiles from the gel. The *in vivo* absorption profiles of model compounds from the alginate/karaya gum gels will be the subject of future investigations.

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