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Morphology and Buoyancy of Oil-entrapped Calcium Pectinate Gel Beads

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ABSTRACT

A new emulsion-gelation method to prepare oil-entrapped calcium pectinate gel (CaPG) beads capable of floating in the gastric condition was designed and tested. The gel beads containing edible oil were prepared by either being gently mixed or homogenized an oil phase and a water phase containing pectin, and then extruded into calcium chloride solution with gentle agitation at room temperature. The gel beads formed were then separated, washed with distilled water, and dried at 37°C for 12 hours. A model of the emulsion-gelation process to illustrate the formation of oil-entrapped CaPG beads was proposed. The effect of selected factors, such as type of oil, percentage of oil, and type of pectin on morphology and floating properties was investigated. The oil-entrapped calcium pectinate gel beads floated if a sufficient amount of oil was used. Scanning electron photomicrographs demonstrated very small pores, ranging between 5 and 40 μm, dispersed all over the beads. The type and percentage of oil play an important role in controlling the floating of oil-entrapped CaPG beads. The results suggested that oil-entrapped CaPG beads were promising as a carrier for intragastric floating drug delivery.

KEYWORDS: calcium pectinate, pectin, oil, emulsion, gel beads, floating, gastro-retentive.

INTRODUCTION

Gastro-retentive dosage forms (ie, those designed to exhibit a prolonged gastric residence time) have been a topic of interest in terms of their potential for controlled drug delivery.[1-2] These dosage forms are particularly appropriate for drugs (1) that are locally active to the gastric mucosa in the stomach, for example, antibiotic administration for *Helicobacter pylori* eradication in the treatment of peptic ulcer disease[3]; (2) with an absorption window in the stomach or in the upper small intestine; (3) that are unstable in the intestine or colonic environment; and (4) with low solubility at high pH values.[4] It is widely accepted that gastric emptying of conventional dosage forms in humans is affected by numerous factors and the time taken shows wide inter- and intrasubject variation. This variability, in turn, can lead to unpredictable times to achieving peak plasma drug levels and bioavailability, since many drugs absorb to the greatest extent in the upper part of the small intestine.[5] A drug that is released from a dosage form in a controlled manner in the stomach will empty together with fluids and will have the whole surface area of the small intestine available for absorption. Several approaches of gastro-retentive dosage forms have been proposed and investigated, such as bioadhesive delivery systems in which bioadhesive polymers adhere to the mucin-epithelial surfaces.[6] Another approach is density-controlled delivery systems or floating dosage forms, which remain buoyant on gastric contents because they have a lower density than gastric fluids.[7]

Floating dosage forms can be made by a gelling process of hydrocolloid materials or by incorporating a vacuum or gas-filled flotation chamber.[8] The most commonly used excipients are gel-forming or highly swellable cellulose-type hydrocolloids, polysaccharides, and matrix-forming polymers such as polycarbonate, polyacrylate, polymethacrylate, and polystyrene.[2] Highly porous carriers for intragastric floating drug delivery (eg, hollow polycarbonate microspheres,[7,9] hydrophobic polypropylene foam powder with low density,[4] coated calcium alginate beads containing air compartment[10]) have been developed. Floating properties of dosage form can also be fabricated using oils, for example, theophylline tablets are composed of mineral oil-entrapped agar for controlled drug release.[11]

The polysaccharide pectin is an inexpensive, nontoxic product extracted from citrus peels or apple pomaces and has been used as a food additive, a thickening agent, and a gelling agent.[12] In addition, pectin can reduce interfacial tension between an oil phase and a water phase and is efficient for the preparation of emulsion.[13] Pectin has a very complex structure that depends on both its source and the extraction process. Numerous studies contributed to elucidate the structure of pectin. Basically, it is a polymer of α-D-galacturonic acid with 1→4 linkages.[12] This chain is regularly interrupted by some rhamnogalacturonan segments that combine galacturonic acid residues and α-L–rhamnopyranose by a 1→2 linkage .[14] The galacturonic acid of the backbone is partially methyl-esterified. Low-methoxy pectin with degree of esterification less than 50% can form rigid gels by the action of

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calcium ions or multivalent cations, which cross-link the galacturonic acid chains. Calcium pectinate hydrogels are stable in low pH solution and are being investigated as a carrier material for different controlled release systems. In recent years, gel beads of calcium pectinate have been developed as a unique vehicle for drug delivery. The gel beads have been used in various ways in the gastrointestinal tract, for example, for sustained release of drugs[15-16] or for targeting drugs to the colon.[17] However, the floating properties of calcium pectinate gel (CaPG) beads and their potential as a gastro-retentive system has not yet been tested. The aims of this study were to develop the oil-entrapped CaPG beads using selected oils for a floating drug delivery system and to investigate their morphology and floating behavior. The effect of selected factors, such as type of oil, percentage of oil, and type of pectin on bead size, morphology, and floating properties was also investigated.

Materials and Methods

Materials

Low-methoxy pectin with degree of esterification (DE) of 36% (GENUpectin type LM-101 AS) and one with DE of 28% (GENUpectin type LM-104 AS-FS) were the generous gift of CP Kelco (Lille Skensved, Denmark) and are referred to as LM-101 and LM-104, respectively. Light mineral oil, olive oil, corn oil, soybean oil, rice oil, sesame oil, peppermint oil, and sunflower oil were standard pharmaceutical grade, and all chemical reagents used were analytical grade.

Methods

Preparation of Conventional Calcium Pectinate Gel Beads

Conventional CaPG beads were prepared by ionotropic gelation method, which was previously described.[15-16] In brief, 5 g each of LM-101 and LM-104 was dissolved in water with agitation to make 100-g solutions. The solutions were extruded using a nozzle of 0.80-mm inner diameter into a 0.34 M calcium chloride solution with gentle agitation at room temperature. The distance from the nozzle to the calcium chloride solution was 5 cm. The gel beads formed were allowed to stand in the solution for 20 minutes, and then were separated and washed with distilled water. The beads were dried at 37°C for 12 hours.

Preparation of Oil-entrapped CaPG Beads by Emulsion-Gelation Method

The oil-entrapped CaPG beads were prepared by emulsion-gelation method. Either LM-101 or LM-104 (5 g) was dissolved in water with agitation. Different amounts (ie, 5-40 g) of selected oils were added to the solution to make a 100-g mixture as shown in Table 1. Either homogenized or nonhomogenized mixture was extruded using a nozzle of 0.80-mm inner diameter into a 0.34 M calcium chloride solution with gentle agitation at room temperature. The oil-entrapped CaPG beads formed were treated in the same manner as conventional CaPG beads.

Study of Particle Size and Morphology of Gel Beads

The mean diameter of 50 dried beads was determined by optical microscopy (BH-2, Olympus, Beijing, China). The microscope eyepiece was fitted with a micrometer by which the size of the beads could be determined. Analysis of variance (ANOVA) and Levene's test for homogeneity of variance were performed using SPSS Version 10.0 for Windows (SPSS Inc, Chicago, IL). Post hoc testing ($P < .05$) of the multiple comparisons was performed by either the Scheffé or Games-Howell test depending on whether Levene's test was insignificant or significant, respectively.

Morphological examination of the surface and internal structure of the dried beads was performed using a scanning electron microscope (SEM) (model Maxim-2000, CamScan Analytical, Cambridgeshire, UK) equipped with back-scattered electron detector at an accelerating voltage of 25 keV. The samples were not coated with gold, so that the difference in atomic number of elements (ie, the difference between calcium and carbon) could be detected by back-scattered electron detector.[18] For examination of the internal structure of the beads, they were cut in half with a steel blade.

Buoyancy of Gel Beads

Specific gravity of the test solution (distilled water, USP simulated gastric fluid without pepsin (SGF) and normal saline solution, ie, 0.9% NaCl) previously measured using a standard pycnometer was 1.007, 1.013, and 1.014, respectively. The gel bead samples (n = 20) were steeped in 50 mL of each test solution, and their buoyancy (sink or float) was observed visually. The preparation was considered to have buoyancy in the test solution only when all of the gel beads floated in it.[3]

Results and Discussion

Preparation of Gel Beads

Pectin with low DE can form gel by ionotropic gelation with divalent calcium ions. When either aqueous solution of pectin or emulsion of selected oils containing pectin was dropped into calcium chloride solutions, spherical gel beads were then formed instantaneously in which intermolecular cross-links were formed between the divalent calcium ions and the negatively charged carboxyl groups of the pectin molecules. The gel beads were easily manufactured without any sophisticated equipment.

Table 1. Mean Diameter and Buoyancy of the Conventional and Oil-entrapped Calcium Pectinate Gel Beads Made of Pectin (LM-104) and Various Types of Oil in Different Test Solutions*

	Mean Diameter, mm ± SD (n = 50)	Buoyancy Behavior (n = 20)		
		Distilled Water	Normal Saline	SGF
No oil (conventional)	1.27 ± 0.08	S	S	S
Mineral oil (relative density† = 0.84)				
5%	1.65 ± 0.06	S	S	S
10%	1.78 ± 0.04	F	F	F
20%	1.92 ± 0.04	F	F	F
30%	2.08 ± 0.06	F	F	F
40%	2.22 ± 0.11	F	F	F
Olive oil (relative density = 0.91)				
5%	1.46 ± 0.04	S	S	S
10%	1.60 ± 0.02	S	S	S
20%	1.73 ± 0.03	F	F	F
30%	1.84 ± 0.05	F	F	F
40%	2.04 ± 0.03	F	F	F
Sunflower oil (relative density = 0.94)				
5%	1.46 ± 0.03	S	S	S
10%	1.52 ± 0.04	S	S	S
20%	1.75 ± 0.02	S	S	S
30%	1.85 ± 0.03	F	F	F
40%	1.95 ± 0.06	F	F	F
Soybean oil (relative density = 0.92)				
10%	1.65 ± 0.05	S	S	S
20%	1.82 ± 0.03	F	F	F
30%	1.91 ± 0.03	F	F	F
Corn oil (relative density = 0.92)				
10%	1.62 ± 0.05	S	S	S
20%	1.81 ± 0.07	F	F	F
30%	2.01 ± 0.04	F	F	F
Rice oil (relative density = 0.91)				
10%	1.62 ± 0.04	S	S	S
20%	1.84 ± 0.04	F	F	F
30%	1.93 ± 0.03	F	F	F
Sesame oil (relative density = 0.91)				
10%	1.60 ± 0.06	S	S	S
20%	1.73 ± 0.05	F	F	F
30%	1.85 ± 0.03	F	F	F
Peppermint oil (relative density = 0.90)				
10%	1.41 ± 0.07	S	S	S
20%	1.51 ± 0.04	F	F	F
30%	1.60 ± 0.03	F	F	F

*SGF indicates *USP* simulated gastric fluid without pepsin; S, sink; and F, float (immediately, and still afloat for at least 6 hours)

†Relative density was reported according to the manufacturer's specification.

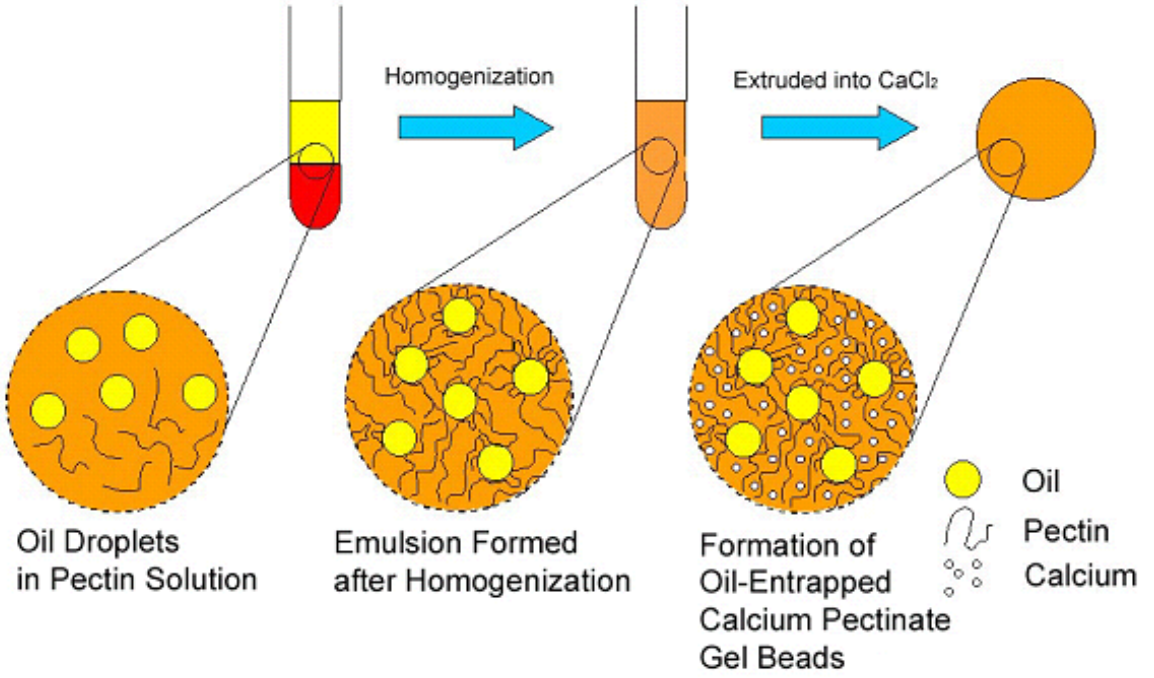

Figure 1. Diagram to illustrate the proposed model of emulsion-gelation process by which the oil-entrapped calcium pectinate gel beads are formed.

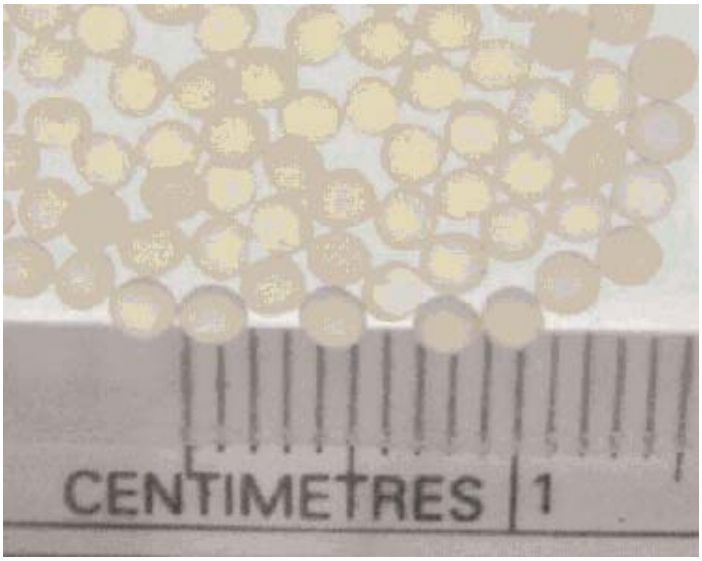

Figure 2. Photograph showing the appearance of oil-entrapped calcium pectinate gel beads containing light mineral oil (10%).

Without homogenization, the oil separated from the pectin solution despite being mixed by stirrer. The homogenized mixture gave the homogeneous texture of the combination because of the emulsifying property of pectin.[13] However, the emulsifying property was limited when the oil concentration was increased. As a consequence, oil began to leak from the beads at 40% wt/wt even when a homogenized mixture was achieved.

Pectin helped to emulsify the mixture of water and oil phases during the homogenization process. Although there is no clear explanation about the origin of the emulsifying function of pectin, its emulsion stabilization could be explained by its surface active ability to reduce the interfacial tension between an oil phase and a water phase.[13] Another explanation is that the emulsion stabilization is probably owing to steric and mechanical stabilization mechanisms, similar to other polysaccharides (eg, cellulose, guar gum, locust bean gum).[19] After the emulsion was formed, it was extruded into calcium chloride solution, and the gel formed by the action of calcium cross-linking to the negative charged groups of pectin chain. As a result, the oil droplets dispersed in the structure of the calcium cross-linked gel beads. The proposed model of emulsion-gelation process by which the oil-entrapped CaPG beads are formed is illustrated in Figure 1.

Figure 2 shows the appearance of oil-entrapped CaPG beads containing mineral oil (10% wt/wt). The beads containing mineral oil were spherical, transparent, and slightly yellowish, whereas those containing olive oil, soybean oil, corn oil, rice oil, peppermint oil, and sunflower oil were less transparent and light yellowish, and the beads containing sesame oil were dark brown in color. This result was owing to original color presented in an oil phase.

Particle Size Studies

The mean diameter of conventional CaPG beads was 1.27 ± 0.08 mm. The mean diameter of oil-entrapped CaPG beads containing different types and amounts of oil is shown in Table 1. The results show that the amount of oil in pectin solution affected the mean diameter of the beads. The size of the gel beads increased as the amount of oil used was increased. For example, when the amount of mineral oil was

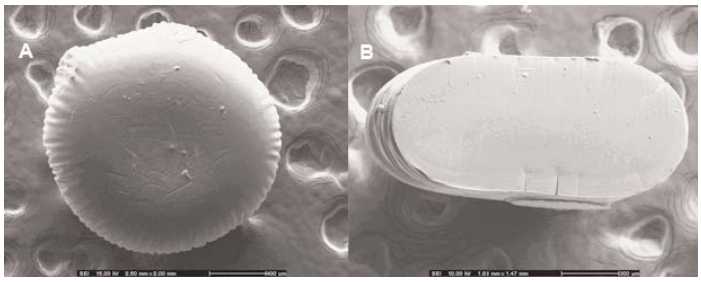

Figure 3. SEM of (A) external and (B) internal structure of a conventional calcium pectinate gel bead. The sizes of exposed area and scale bars are shown on the individual photographs.

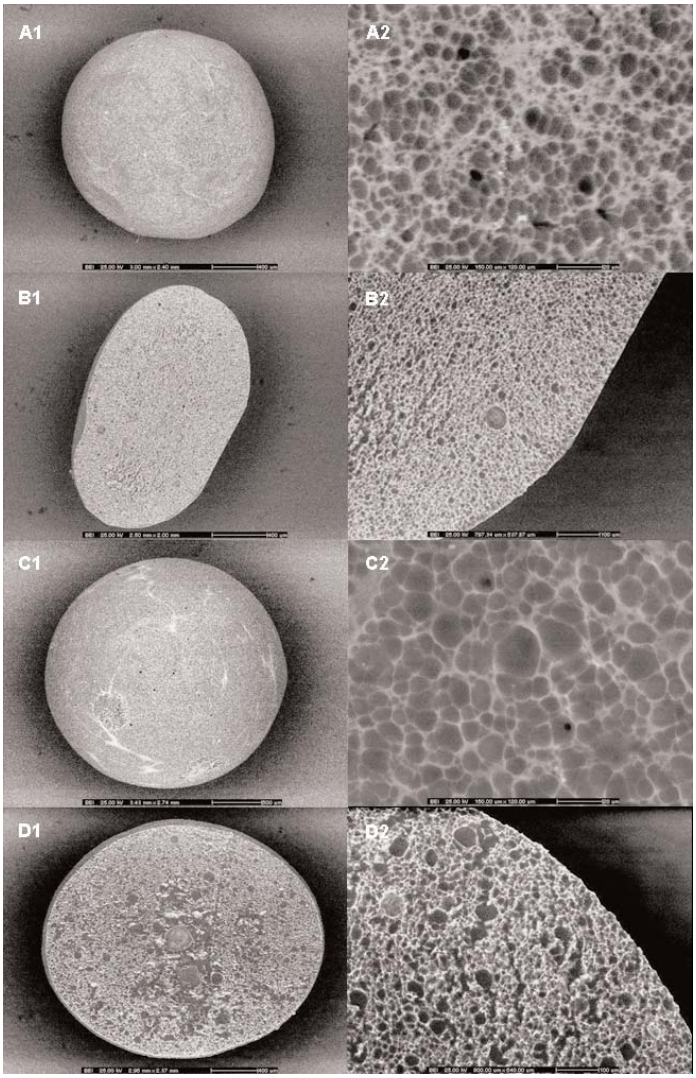

Figure 4. SEM of (A) external and (B) internal structure of an oil-entrapped calcium pectinate gel bead containing olive oil (5%); and (C) external and (D) internal structure of an oil-entrapped calcium pectinate gel bead containing olive oil (30%). The sizes of exposed area and scale bars are shown on the individual photographs.

increased from 5% wt/wt to 40% wt/wt, the size of the CaPG beads significantly increased from 1.65 ± 0.06 to 2.22 ± 0.11 mm. In addition, the size of CaPG beads made of different types of pectin was not significantly different ($P > .05$). The beads containing peppermint oil were smaller than those containing other oils when comparing at the same percentage of oil used. This is probably owing to the volatility of peppermint oil resulting in loss of mass during air drying.

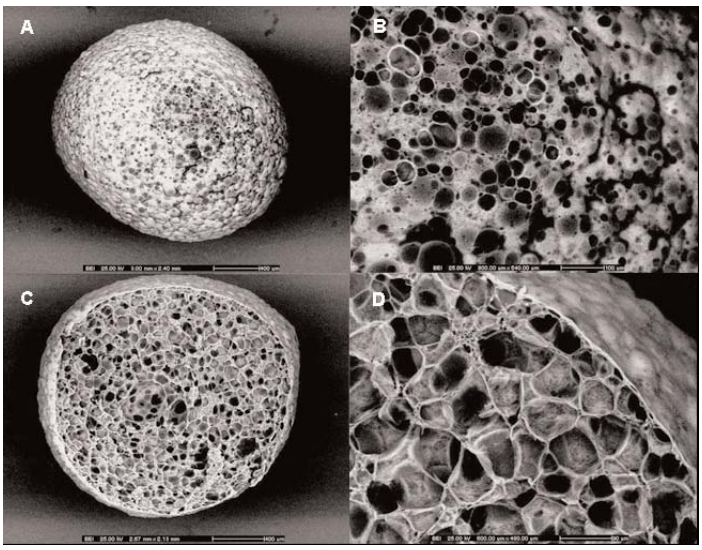

Figure 5. SEM of (A and B) external and (C and D) internal structure of an oil-entrapped calcium pectinate gel bead containing peppermint oil (30%). The sizes of exposed area and scale bars are shown on the individual photographs.

Morphology of Gel Beads

Samples were taken from different formulations and operating conditions for SEM observation. Typical image is shown in Figure 3 illustrating the external and internal structure of the dry conventional CaPG beads. Upon air drying, the conventional CaPG beads of all formulations became small, dense, and flattened with wrinkled circumference due to water diffusing gradually from the sphere under the drying process. The oil-entrapped CaPG beads were more spherical with no hollow at the middle of sphere surface (Figures 4 and 5). This spherical shape of oil-entrapped CaPG beads could be maintained with high concentration of oil. The oil-entrapped CaPG structure formed showed the sponge-like structure where the oil was entrapped. This sponge-like structure may correspond to the egg-box structure of calcium pectinate (as proposed in Figure 1), which was rigid and water insoluble.

The pores of the oil-entrapped CaPG beads represented the oil droplets, and their size was influenced by concentration of oil. Figure 4 shows the external and internal morphology of an oil-entrapped CaPG bead made of 5% and 30% olive oil. The morphology of the external and internal structure was identical. The surface of oil-entrapped CaPG beads showed small pores (5-40 μm) containing oil droplets dispersed all over the structure. The size of the pores found on the CaPG beads containing 5% olive oil was smaller than that of the beads containing 30% olive oil. This finding is probably due to the homogeneous dispersion of the small fraction of oil phase in pectin solution. The droplets of dispersed phase, therefore,

could separate to form a more stable emulsion (before extrusion into calcium chloride solution) than those of larger fraction of oil phase. Moreover, the large number of oil droplets in emulsion may stick to each other with a thin film between them to be flocculation or they may unite to a larger droplet to be coalescence.[20] As a consequence, larger oil-filled pores were obtained when a greater amount (30% wt/wt) of olive oil was used to prepare the oil-entrapped CaPG beads.

Furthermore, the structure of the oil-entrapped CaPG beads depends on the type of oil used. Although the macrostructure of all oil-entrapped CaPG beads appeared to be the same irrespective of oil type, the morphology determined by SEM showed different surface appearance. It was found that oil-entrapped CaPG beads made of peppermint oil were rougher than those of olive oil and had smaller pores dispersed all over the beads. Figure 5 shows the external and internal morphology of oil-entrapped CaPG beads made of peppermint oil (30%).

Buoyancy of Gel Beads

When conventional CaPG beads made of LM-104 were steeped in distilled water, normal saline solution, or SGF, they sank as shown in Table 1. In contrast, the oil-entrapped CaPG beads containing various oils floated immediately and remained floating for 24 hours if a sufficient amount of oil was used. The oil-entrapped CaPG beads containing 10% light mineral oil or 20% olive oil, soybean oil, corn oil, rice oil, sesame oil, or peppermint oil, or 30% sunflower oil, floated in the test solutions irrespective of the type of medium. The results appear to be related to their relative density (ie, 0.84 for light mineral oil, between 0.90 and 0.92 for peppermint oil, olive oil, soybean oil, corn oil, sesame oil, and rice oil, and more than 0.92 for sunflower oil). The results indicated that if the oil with lower relative density was used, a smaller amount of the oil was required to keep the beads afloat. In fact, each type of oil contains various fatty acids that link together and form linear or branch chains. These properties may affect the formation of emulsion with pectin and need to be further examined at a molecular level.

The floating behavior of the beads made of LM-101 (data not shown) was similar to that of LM-104. This indicates that degrees of esterification (ie, between 28% and 36%) of the pectin were not the main factor on floating property of the system.

Conclusion

A new floating system of oil-entrapped CaPG beads was designed and prepared by an emulsion-gelation method and its morphology and buoyancy were investigated in this study. The mean diameter of beads increased with the increased amount of oil phase. The pore size of oil-entrapped CaPG beads was affected by concentration of oil. The oil-entrapped CaPG beads showed excellent, immediate, and lasting buoyancy in the acidic environment of the gastric fluid as well as in distilled water or normal saline solution if they contained a sufficient amount of oil, depending on the relative density of the oil.

The enhanced buoyancy property of oil-entrapped CaPG beads makes them an excellent candidate for an intragastric floating drug delivery system. This property will be applicable to the gastro-retention of drug delivery systems by slowing down the gastric emptying of systems. The lasting intragastric buoyancy of a controlled release dosage form may also provide a suitable manner to deliver drugs that are locally active to the gastric mucosa in the stomach and, hence, achieve a sustained site-specific therapeutic action (eg, antibiotic administration for *Helicobacter pylori* eradication in the treatment of peptic ulcer disease).

In order to investigate the actual buoyancy of the system and its usefulness in sustaining drug release, such formulations will be selected for drug loading and release examination. We are continuing our experiments with these systems in an attempt to (1) keep the systems afloat in the gastric condition and (2) control the drug (eg, antimicrobial agent) release from the oil-entrapped CaPG beads.

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