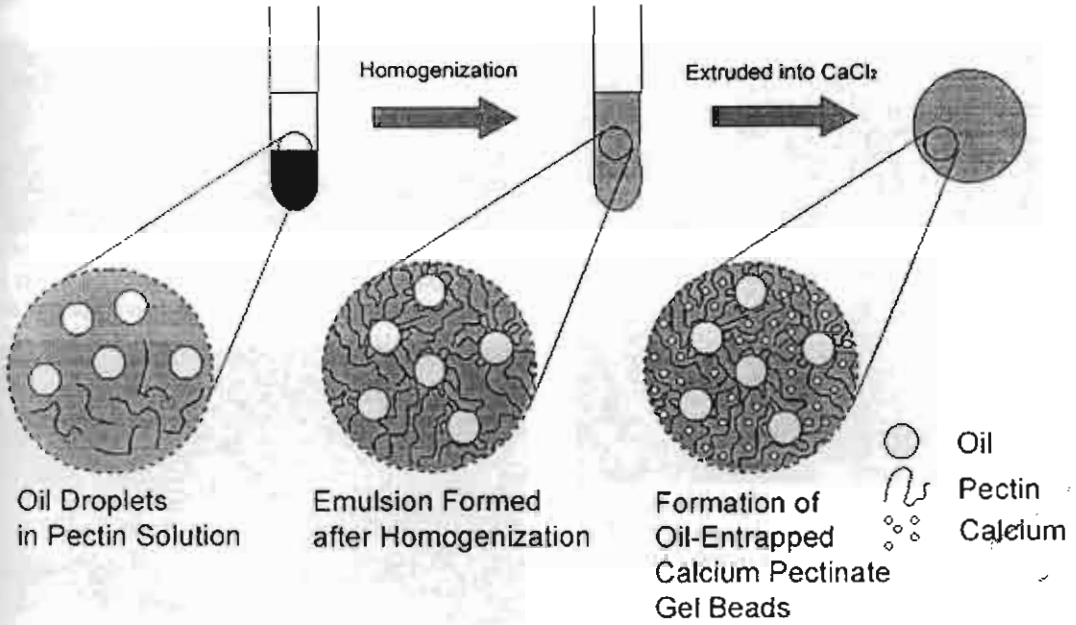
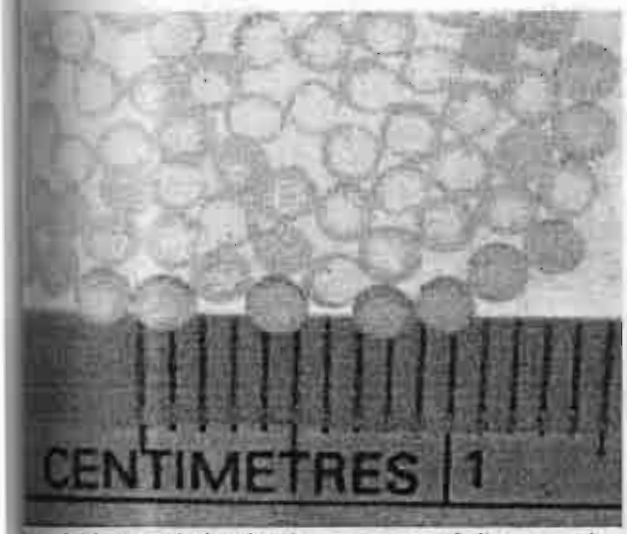
Table 1. Mean Diameter and Buoyancy of the Conventional and Oil-entrapped Calcium Pectinate Gel Beads Made of Pectin (LM-104) and Various Types of Oil in Different Test Solutions*

	Mean Diameter, mm ± SD (n = 50)	Buoyancy Behavior (n = 20)		
		Distilled Water	Normal Saline	SGF
[illegible] oil (conventional)	1.27 ± 0.08	S	S	S
[illegible]neral oil (relative density† = 0.84)				
[illegible]%	1.65 ± 0.06	S	S	S
[illegible]%	1.78 ± 0.04	F	F	F
[illegible]%	1.92 ± 0.04	F	F	F
[illegible]%	2.08 ± 0.06	F	F	F
[illegible]%	2.22 ± 0.11	F	F	F
[illegible] oil (relative density = 0.91)				
[illegible]%	1.46 ± 0.04	S	S	S
[illegible]%	1.60 ± 0.02	S	S	S
[illegible]%	1.73 ± 0.03	F	F	F
[illegible]%	1.84 ± 0.05	F	F	F
[illegible]%	2.04 ± 0.03	F	F	F
[illegible]flower oil (relative density = 0.94)				
[illegible]%	1.46 ± 0.03	S	S	S
[illegible]%	1.52 ± 0.04	S	S	S
[illegible]%	1.75 ± 0.02	S	S	S
[illegible]%	1.85 ± 0.03	F	F	F
[illegible]%	1.95 ± 0.06	F	F	F
[illegible]bean oil (relative density = 0.92)				
[illegible]%	1.65 ± 0.05	S	S	S
[illegible]%	1.82 ± 0.03	F	F	F
[illegible]%	1.91 ± 0.03	F	F	F
[illegible] oil (relative density = 0.92)				
[illegible]%	1.62 ± 0.05	S	S	S
[illegible]%	1.81 ± 0.07	F	F	F
[illegible]%	2.01 ± 0.04	F	F	F
[illegible] oil (relative density = 0.91)				
[illegible]%	1.62 ± 0.04	S	S	S
[illegible]%	1.84 ± 0.04	F	F	F
[illegible]%	1.93 ± 0.03	F	F	F
[illegible]me oil (relative density = 0.91)				
[illegible]%	1.60 ± 0.06	S	S	S
[illegible]%	1.73 ± 0.05	F	F	F
[illegible]%	1.85 ± 0.03	F	F	F
[illegible]tcoint oil (relative density = 0.90)				
[illegible]%	1.41 ± 0.07	S	S	S
[illegible]%	1.51 ± 0.04	F	F	F
[illegible]%	1.60 ± 0.03	F	F	F

*[illegible]dicates *USP* simulated gastric fluid without pepsin; S, sink; and F, float (immediately, and still afloat for at least 6 hours)
†[illegible] density was reported according to the manufacturer's specification.



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



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

hout homogenization, the oil separated from the pectin tion despite being mixed by stirrer. The homogenized ture gave the homogeneous texture of the combination se of the emulsifying property of pectin.[13] However, emulsifying property was limited when the oil concentra- was increased. As a consequence, oil began to leak from beads at 40% wt/wt even when a homogenized mixture achieved.

tin helped to emulsify the mixture of water and oil phas- during the homogenization process. Although there is no explanation about the origin of the emulsifying function ectin, 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

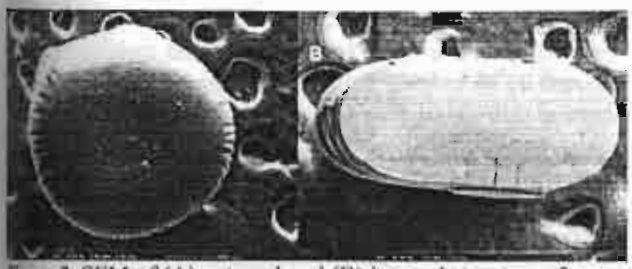


Figure 3. SEM of (A) external and (B) internal structure of a conventional calcium pectinate gel bead. The sizes of exposed area and scale bars are shown on the individual photographs.

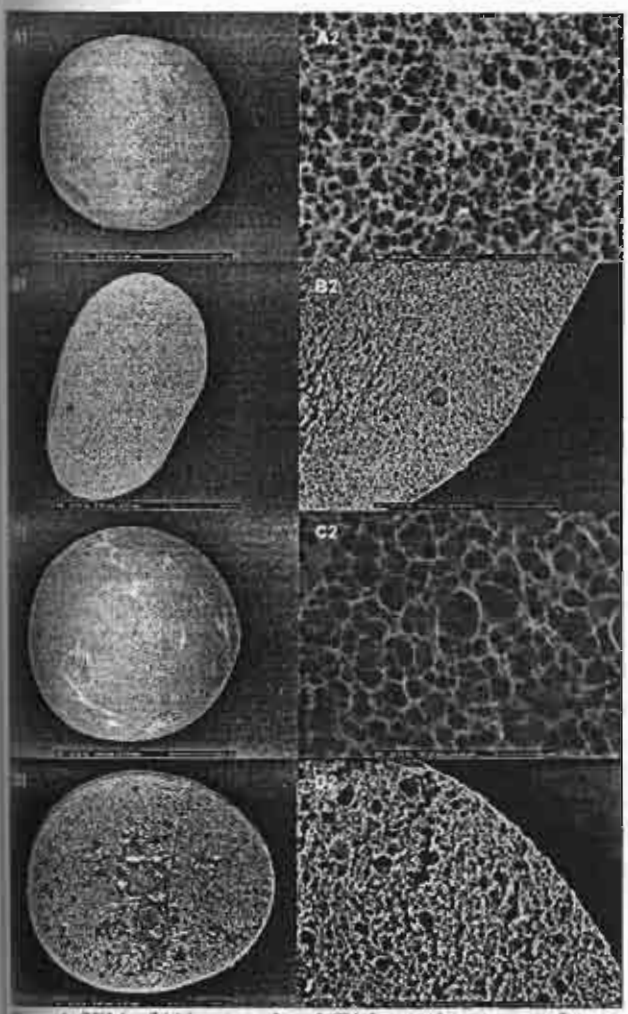


Figure 4. SEM of (A) external and (B) internal structure of an oil-entrapped calcium pectinate gel bead containing olive oil (5%); and (C) external and (D) internal structure of an oil-entrapped calcium pectinate gel bead containing olive oil (30%). The sizes of exposed area and scale bars are shown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

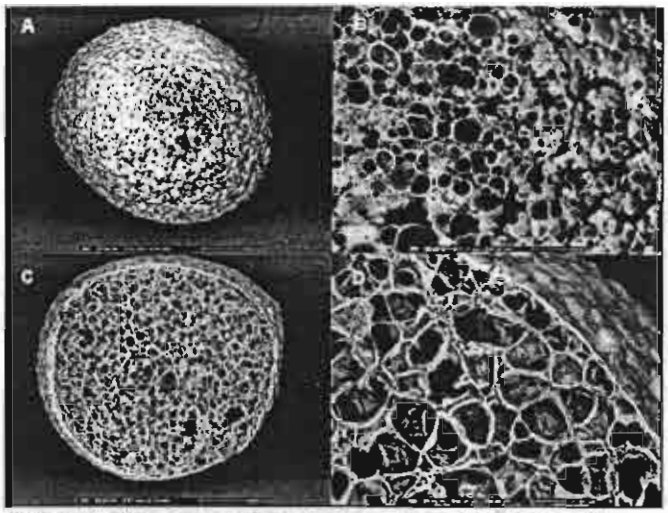


Figure 5. SEM of (A and B) external and (C and D) internal structure of an oil-entrapped calcium pectinate gel bead containing peppermint oil (30%). The sizes of exposed area and scale bars are shown on the individual photographs.

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,

separate to form a more stable emulsion (before extru- into calcium chloride solution) than those of larger frac- of oil phase. Moreover, the large number of oil droplets emulsion may stick to each other with a thin film between to be flocculation or they may unite to a larger droplet coalescence.[20] As a consequence, larger oil-filled pores obtained when a greater amount (30% wt/wt) of olive oil 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 oil-entrapped CaPG beads appeared to be the same irrespec- 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 immersed 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 bran, 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 (= 0.84 for light mineral oil, between 0.90 and 0.92 for pep- mint oil, olive oil, soybean oil, corn oil, sesame oil, and rice bran 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 float. 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.

ACKNOWLEDGEMENTS

This work was financially supported by the Thailand Research Funds (grant number MRG4680120). The authors gratefully acknowledge Mr Witoon Sae-Ngow from Silpakorn University Scientific and Technological Research Equipment Centre (SUSTREC) for technical assistance on SEM imaging. Thanks also go to Food and Cosmetic System Co, Ltd, (Bangkok,Thailand) for supplying pectin samples.

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Sriamornsak P, Thirawong N, Puttipipatkhachorn S. Emulsion gel beads of calcium pectinate capable of floating on the gastric fluid: Effect of some additives, hardening agent or coating on release behavior of metronidazole. *European Journal of Pharmaceutical Sciences* 2005, 24(4), 363-373.

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EUROPEAN JOURNAL OF PHARMACEUTICAL SCIENCES
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Emulsion gel beads of calcium pectinate capable of floating on the gastric fluid: effect of some additives, hardening agent or coating on release behavior of metronidazole

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Received 5 October 2004; received in revised form 2 December 2004; accepted 10 December 2004
Available online 23 January 2005

Abstract

Emulsion gel (EMG) beads of calcium pectinate capable of floating in the gastric condition were developed using an emulsion-gelation method and their release properties were investigated. Attempts to modify the drug release were made by applying some additives into the starting solution prior to bead formation, by hardening with glutaraldehyde, and by coating with polymer. The metronidazole-loaded EMG beads were found to float on simulated gastric fluid. Increasing the drug to pectin ratio in the beads slowed the drug release from the conventional and the EMG beads. However, the drug release from these beads was rapid, i.e., about 80% of drug loading released within 20–80 min. The additives (PEG10000, glyceryl monostearate and Eudragit® L) had a slight, insignificant, effect on the drug release. Using 2% glutaraldehyde as a hardening agent prolonged the drug release. Coating the beads with Eudragit® RL significantly sustained the drug release while the beads remained buoyant. The results suggest that EMG beads are suitable as a carrier for intragastric floating drug delivery and that their release behaviour could be modified by hardening with glutaraldehyde or by coating with Eudragit® RL.

Keywords: Calcium pectinate; Pectin; Emulsion gel; Beads; Floating; Additives; Sustained release

1. Introduction

Oral administration is always the preferred means of drug delivery to the systemic circulation. Many attempts have been made to develop sustained release preparations with extended clinical effects and reduced dosing frequency. A problem frequently encountered with conventional sustained release dosage forms is the inability to increase their residence time in the stomach and proximal portion of the small intestine. Retention of drug delivery systems in the stomach prolongs the overall gastrointestinal transit time, thereby resulting in improved oral bioavailability of the basic drugs that have poor solubility in higher pH, and of drugs susceptible to circadian variations (Moes, 1993). These systems are also appropriate for drugs which 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 (Cooreman et al., 1993).

Floating dosage forms can be used to retain the delivery system in the stomach to increase the gastric residence time. Various methods have been used to prepare the floating dosage forms (Hwang et al., 1998; Singh and Kim, 2000). 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 (Singh and Kim, 2000). Floating properties of dosage form can also be fabricated using oils, for example, tablets containing mineral oil-entrapped agar (Desai and Bolton, 1993).

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doi:10.1016/j.ejps.2004.12.004

The polysaccharide pectin is an inexpensive, non-toxic product extracted from citrus peels or apple pomaces, and has been used as a food additive, a thickening agent and a gelling agent (Rolin, 1993). In addition, pectin is capable of reducing interfacial tension between an oil phase and a water phase and can be effective in the preparation of emulsion (Sriamornsak et al., 2003). Pectin has a very complex structure which depends on both its source and the extraction process. Numerous studies have contributed to our understanding of the structure of pectin. Basically, it is a polymer of α-D-galacturonic acid with 1–4 linkages (Rolin, 1993). This chain is regularly interrupted by some rhamnogalacturonan segments which combine galacturonic acid residues and α-L-rhamnopyranose by a 1–2 linkage (Schols and Voragen, 1996). The galacturonic acid of the backbone is partially methyl-esterified. Low methoxy pectin with a degree of esterification less than 50%, can form rigid gels by the action of calcium ions or multivalent cations, which crosslink the galacturonic acid chains. Calcium pectinate hydrogels are stable in low pH solution, and are being investigated as a potential carrier material for different controlled release systems. In recent years, calcium pectinate gel (CaPG) beads 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 (Sriamornsak and Nunthanid, 1998, 1999) or for targeting drugs to the colon (Sriamornsak, 1999). We recently investigated the morphology and floating properties of oil-entrapped gel beads of calcium pectinate (Sriamornsak et al., 2004).

In this study, the drug-loaded emulsion gel (EMG) beads of calcium pectinate using selected oils were developed for floating drug delivery system, based on our previous report (Sriamornsak et al., 2004) and the drug release behavior of EMG beads capable of floating in the gastric fluid was studied. The effect of various release modification methods on drug release including; the use of additives in the starting solution prior to bead formation, hardening with glutaraldehyde, and coating with polymer, was also investigated.

2. Materials and methods

2.1. Materials

Low methoxy pectin with a degree of esterification of 25% (GENU pectin type LM-104 AS-FS) was the generous gift of CP Kelco (Lille Skensved, Denmark). Eudragit® L100 and Eudragit® RL100 (Röhm Pharma, Darmstadt, Germany), glyceryl monostearate (GMS), metronidazole (MZ) (IPC Drug Center, Bangkok, Thailand), calcium chloride, polyethylene glycol (PEG) 10000 (E. Merck, Darmstadt, Germany) and glutaraldehyde (Fluka Chemie, Buchs, Switzerland) were used as received. Light mineral oil, olive oil and peppermint oil and all other chemicals were standard pharmaceutical grade.

2.2. Preparation of conventional CaPG beads, EMG beads, and modified EMG beads

2.2.1. Conventional CaPG beads

Conventional CaPG beads were prepared by the ionotropic gelation method that was previously described (Sriamornsak and Nunthanid, 1998, 1999). Briefly, 5 g of pectin were dispersed in water with agitation to make a 100-g solution. Various amounts of MZ (80-mesh sieved) were dispersed in pectin solution to make different pectin to drug ratios (i.e., 1:1, 1:1.5 and 1:2, w/w). The dispersion was then extruded, using a needle of 0.80-mm inner diameter, into 0.34 M calcium chloride which was gentle stirred at room temperature. The distance from the needle to the surface of calcium chloride solution was fixed to 5 cm. The gel beads formed were allowed to stand in the solution for 20 min before being separated and washed with distilled water. The beads were dried at 37 °C for 12 h.

2.2.2. EMG beads of calcium pectinate

The EMG beads of calcium pectinate were prepared by emulsion-gelation method as previously reported (Sriamornsak et al., 2004). Five grams of pectin were dissolved in water with agitation. Different amounts (i.e., 10, 20, 30 g) of olive oil, light mineral oil or peppermint oil were added to the solution to make 100-g mixtures and homogenized using a homogenizer (Type XI020, Ystral GmbH. Dottingen, Germany), at 3000 rpm for 5 min. Various amounts of MZ were dispersed in an emulsion of oil and pectin mixture. The EMG beads were treated in the same manner as conventional CaPG beads. In some cases, the EMG beads were treated in 2% (v/v) glutaraldehyde for 2 h prior to washing and drying at 37 °C for 12 h.

2.2.3. Additive-added EMG beads

Different amounts of GMS, PEG10000, or Eudragit® L100 were dispersed in the homogenized emulsion mixture of pectin, oil and MZ (additive to pectin ratio was 0.25:1, 0.5:1 or 1:1, w/w) and mixed until the homogenous mixture was obtained. The mixture was used to prepare the additive-added EMG beads in the same manner as conventional CaPG beads

2.2.4. Polymer-coated EMG beads

The coating on drug-loaded EMG beads was performed by the air suspension method. The coating solution (8%, w/v) was prepared by dissolving Eudragit® RL100 in absolute alcohol to make a 100-ml solution. The EMG beads (10 g) were placed in a 250-ml round-bottomed flask. The coating solution was introduced to the cores using a spray gun at a rate of 0.2 g/min and a stream of drying air at 60 °C was applied to the surface of the cores. Coat application was continued until a 16% coating weight gain was achieved. The hot air was continuously blown in the chamber until all the solvent had evaporated. The coated beads were then collected and dried in a hot air oven at 50 °C for 12 h.

2.3. Study of particle size and morphology of gel beads

The mean diameter of 50 dried beads was determined by optical microscopy (BH-2, Olympus, Japan). The microscope eyepiece was fitted with a micrometer by which the size of the beads could be determined.

Morphological examination of the surface and internal structure of the dried beads was carried out using a scanning electron microscope (Model Maxim-2000, CamScan Analytical, Cambridge, England) equipped with secondary electron detector at an accelerating voltage of 15 keV. The samples were coated with gold to a thickness of about 30 nm in a vacuum evaporator. The internal structure of the beads was examined by cutting them in half with a steel blade.

2.4. Buoyancy of gel beads

The gel bead samples ($n = 20$) were placed in the Erlenmeyer flask filled with 50 ml of simulated gastric fluid USP without pepsin (SGF) test solution. The flask was shaken in a shaking incubator. The shaking speed was 100 rpm and the temperature was maintained at 37 °C. Their buoyancy was observed for 24 h. The preparation was considered to have buoyancy in the test solution only when all of the gel beads floated in it (Cooreman et al., 1993).

2.5. Determination of entrapment efficiency and drug release

Prior to the determination of MZ content, the beads must be dissolved by phosphate buffer (pH 7.4) containing 5 mM ethylenediamine tetraacetic acid. The content of MZ was later assayed by UV-spectrophotometer (Hitachi U-2000, Japan) in pH 7.4 phosphate buffer at 277 nm. The determinations were made in triplicate. The ratio of the actual drug content in the beads to the theoretical drug content was termed the entrapment efficiency (EE).

The in vitro release of MZ from the different formulations was examined using a USP dissolution apparatus 1 (Erweka, Germany) with 1000 ml of SGF (pH 1.2) and the basket rotation at 100 rpm. The temperature was controlled at 37 ± 0.1 °C. Samples were taken at appropriate time intervals and assayed spectrophotometrically at 277 nm. All dissolution runs were performed in triplicate.

3. Results and discussion

3.1. Preparation and morphology of gel beads

An aqueous solution of pectin was extruded into calcium chloride solutions and gel beads were formed instantaneously by ionotropic gelation (Sriamornsak and Nunthanid, 1998) in which intermolecular cross-links were formed between the divalent calcium ions and the negatively charged carboxyl groups of the low methoxyl pectin molecules. The conventional CaPG beads were easily manufactured without any sophisticated equipment (Sriamornsak and Nunthanid, 1998). The MZ-loaded CaPG beads can also be prepared by the same method but for this formulation MZ was dispersed in pectin solution prior to bead formation. Typical SEM images illustrating the external and internal structures of the MZ-loaded CaPG beads are shown in Fig. 1. The external surface of gel beads was rough and its shape remained spherical after the drying process. The MZ particles, homogenously dispersed in the calcium pectinate network, can also be seen in the image.

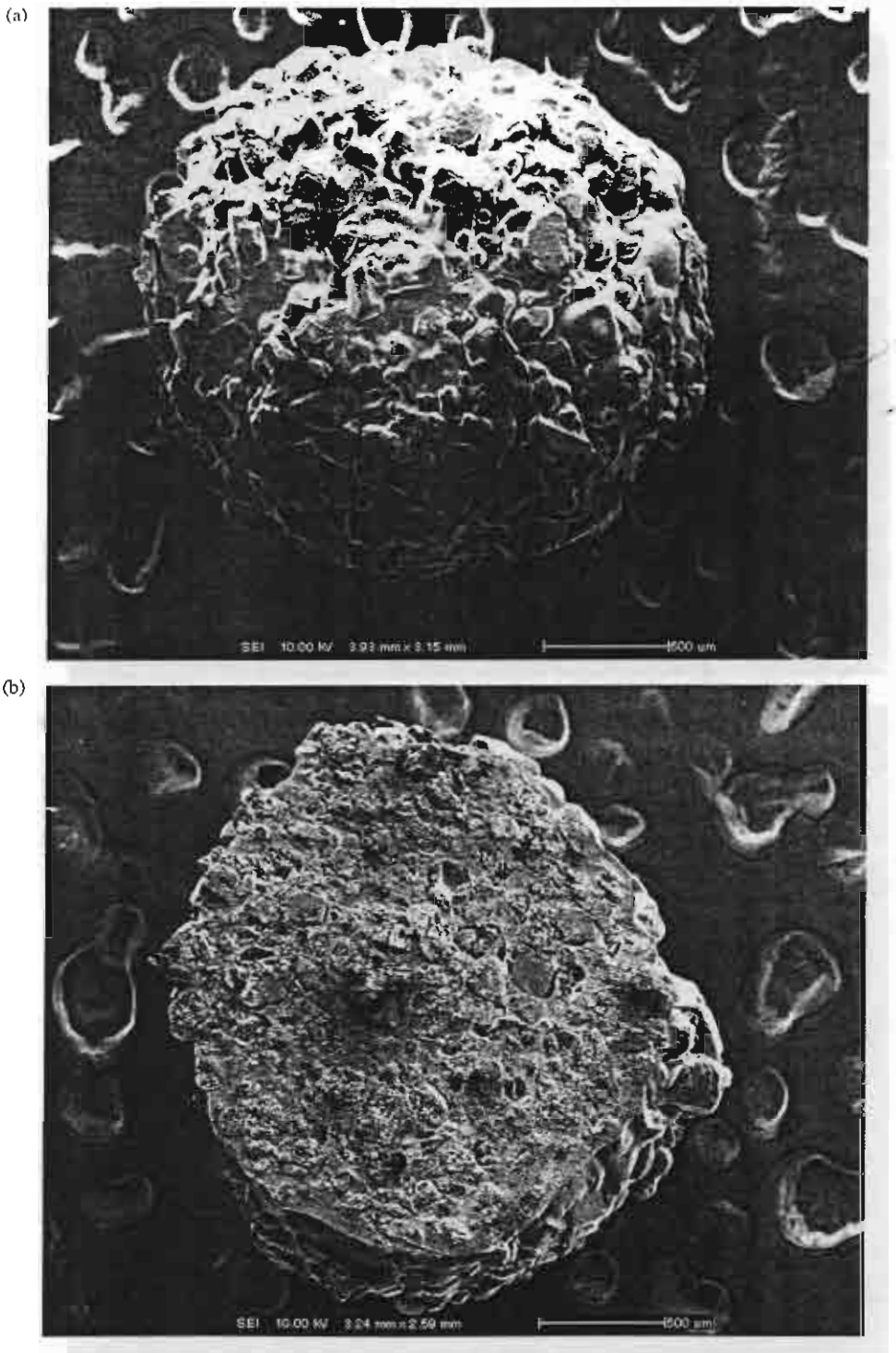
Pectin helped to emulsify the mixture of water and oil phases during the homogenization process. The emulsion stabilization property of pectin can be explained by its surface active ability to reduce the interfacial tension between an oil phase and a water phase (Leroux et al., 2003), or steric and mechanical stabilization mechanisms similar to other polysaccharides such as cellulose, guar gum and locust bean gum (Garti and Reichman, 1993). The MZ-loaded EMG beads were formed by the emulsion-gelation process (Sriamornsak et al., 2004) by which the MZ-loaded emulsion containing pectin was gelled by the action of calcium. Fig. 2 shows external and internal morphology of EMG beads containing 30% (w/w) olive oil. Distribution of the oil droplets in the structure of the calcium cross-linked gel beads was uniform but for the MZ particles, it was randomly scattered. The EMG beads were more spherical and exhibited a smoother surface than conventional CaPG beads. The morphology of all additive-added EMG beads containing 30% (w/w) olive oil was identical to that of EMG beads containing 30% (w/w) olive oil (data not shown). Fig. 3 shows external and internal morphology of MZ-loaded EMG beads containing 30% (w/w) peppermint oil. The beads were spherical in shape with a slightly rougher surface than those containing olive oil. The internal structure of an MZ-loaded EMG bead containing 30% (w/w) peppermint oil demonstrates the sponge-like nature (Fig. 3c) of the structure even though the beads were dried in the hot-air oven. It also shows that there was no oil droplet under the gold coating. This is due to the volatile property of peppermint oil, resulting in the pore formation during the drying process.

3.2. Mean diameter and EE of gel beads

The mean diameter and EE of the MZ-loaded CaPG beads, EMG beads and modified EMG beads at different pectin to MZ ratios is shown in Table 1. The mean diameter of the MZ-loaded gel beads ranges between 2.45 and 3.51 mm. The average size of the beads increased slightly as the amount of MZ and/or oils increased. The hardening agent caused a decrease in bead size as it promoted the formation of crosslinks between the pectin molecules (Sriamornsak and Nunthanid, 1999). The type of additives used insignificantly influenced the mean diameter of the modified EMG beads.

The EE of the beads was calculated from the fractional amount of drug remaining in the beads. As shown in Table 1, the EE of the conventional CaPG beads and EMG beads

anges from 64.30% to 84.13%, while that of the additive-dded EMG beads and coated EMG beads ranges from 9.66% to 66.17%. This is probably due to the high solu-ility of MZ, resulting in high drug loss (about 15–35%) into the solution during the preparation. The EE increased slightly with increased amount of drug loading. On the other hand, the EE decreased, by half, when the beads were soaked in the hardening agent for 2 h.



g. 1. Scanning electron micrographs of (a) external and (b) internal structures of conventional calcium pectinate gel beads. Magnifications and scale bars are own on the individual photographs.

3.3. Buoyancy of gel beads

Our previous study (Sriamornsak et al., 2004) showed that the conventional CaPG beads (with no drug) made of low methoxy pectin did not float in SGF. In contrast, if a sufficient amount of oil was added (i.e. 10% mineral oil, 20% olive oil or peppermint oil), the EMG beads floated immediately and remained floating for 24 h. The results appeared to be related to their relative density, i.e. 0.84 for light mineral oil, between 0.90 and 0.92 for peppermint oil and olive

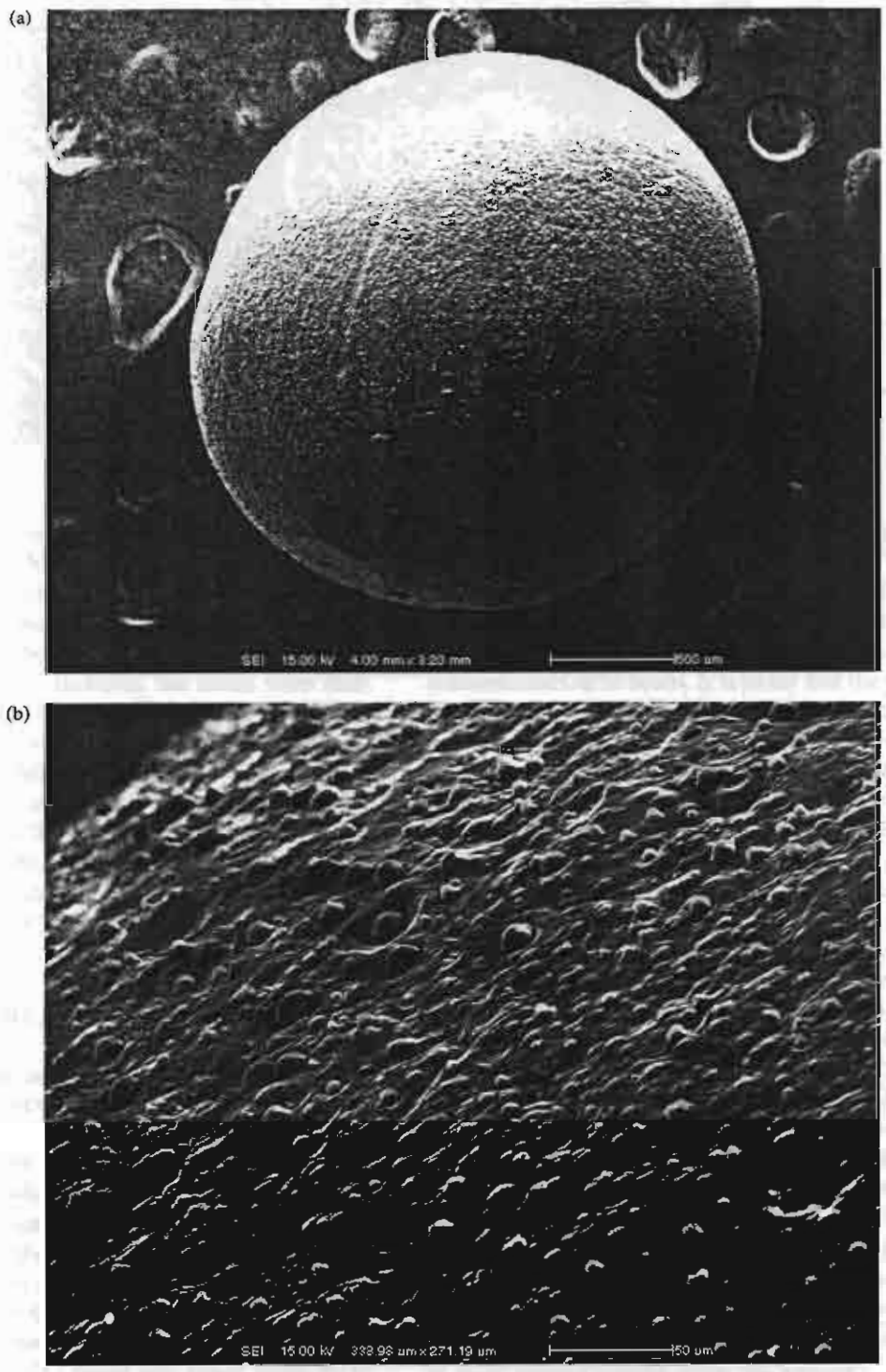


Fig. 2. Scanning electron micrographs of (a and b) external and (c) internal structures of emulsion gel beads of calcium pectinate containing olive oil (30%). Magnifications and scale bars are shown on the individual photographs.

(c)

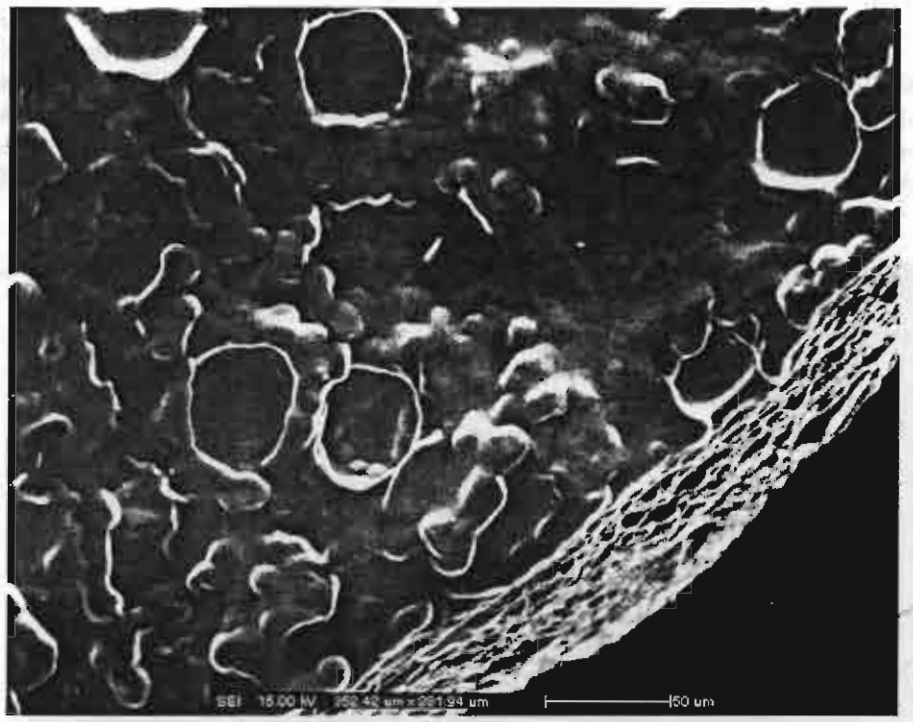


Fig. 2. (*Continued*).

Different results were obtained when MZ was loaded in conventional CaPG and EMG beads, that is, the MZ-loaded EMG beads containing mineral oil (10%), olive oil (20%), peppermint oil (20%) did not float immediately (Table 2). This is probably due to the increased density of the beads when the drug was added. However, the beads were then float after the drug was released from the beads. Additionally, the amount of oil required to keep the beads afloat was increased (Table 2). The MZ-loaded EMG beads containing mineral oil (20%) or olive oil (30%) or peppermint oil (30%) floated immediately in SGF. The buoyancy of EMG beads treated with glutaraldehyde, additive-added EMG beads, and polymer-coated EMG beads is also shown in Table 2. Good in vitro floating behavior in SGF was observed in all modified EMG beads.

In vitro release of MZ from gel beads

Release studies were carried out, only when the beads floated in SGF, in order to examine the suitability of the EMG beads as an intragastric floating drug delivery system. MZ, which is used for *H. pylori* eradication, was used as a model drug. The drug release profiles were presented by plotting the amount of MZ released against time.

Figs. 4a–c illustrates the release profiles of MZ-loaded conventional CaPG beads, and EMG beads containing 20% mineral oil and 30% olive oil, respectively. No lag time was observed in any of the formulations studied. The release of MZ from conventional CaPG beads was rapid; about 80% of MZ was released within 20–30 min, in any ratio of pectin to MZ. This is probably due to the fact that MZ dissolves quickly in water and diffuses through the calcium pectinate structure into the dissolution medium easily. The amount of MZ loaded did not significantly affect the release behavior. The EMG beads containing either 20% mineral oil or 30% olive oil produced slower drug release profiles, compared to conventional CaPG beads. It is likely that the oil, which was dispersed in the structure of EMG beads, obstructed the dissolution channel of MZ. Consequently, the drug release was prolonged; about 80% of MZ was released within 40–80 min. Since the drug release was still quick and was not harmonious for floating drug delivery, modification of the EMG bead formulations was need. Some treatments were done or some additives were added in the formulations in order to extend the drug release.

The hardening agent is commonly used to strengthen the bead structure as well as to modify the release behavior. In this study, a hardening agent was used in order to delay the drug release from EMG beads. Fig. 5 shows the effect of the hardening agent, glutaraldehyde, on MZ release from EMG beads containing various oils. The MZ release from EMG beads containing 30% olive oil, which were soaked in 2% glutaraldehyde for 2 h, was prolonged about two-fold. About 80% of drug release was achieved within 120 min, compared to about 50 min for non-hardened EMG beads. However, the release of MZ from EMG beads containing peppermint oil, which were soaked in glutaraldehyde, was not prolonged and showed similar results to that of non-soaked EMG beads (data not shown). The porous structure of these beads resulting from the volatility of the oil may have contributed to these insignificant differences in drug release.

Some pharmaceutical excipients, for example, enteric polymer (i.e. Eudragit® L), hydrophobic additive (i.e. GMS), and hydrophilic additive (i.e. PEG10000) were used as additives in the formulation, in order to modify the release behavior of the EMG beads. Fig. 6 shows the effect of additive : pectin ratio on MZ release from the additive-added EMG beads using Eudragit® L, GMS, and PEG10000. The drug release from EMG beads was slightly slower when Eudragit® L, was added into the beads containing 30% olive oil. Nevertheless, the release from EMG beads was not significantly different when the amount of Eudragit® L was increased (Fig. 6a). It was suggested that the Eudragit® L was only dispersed in

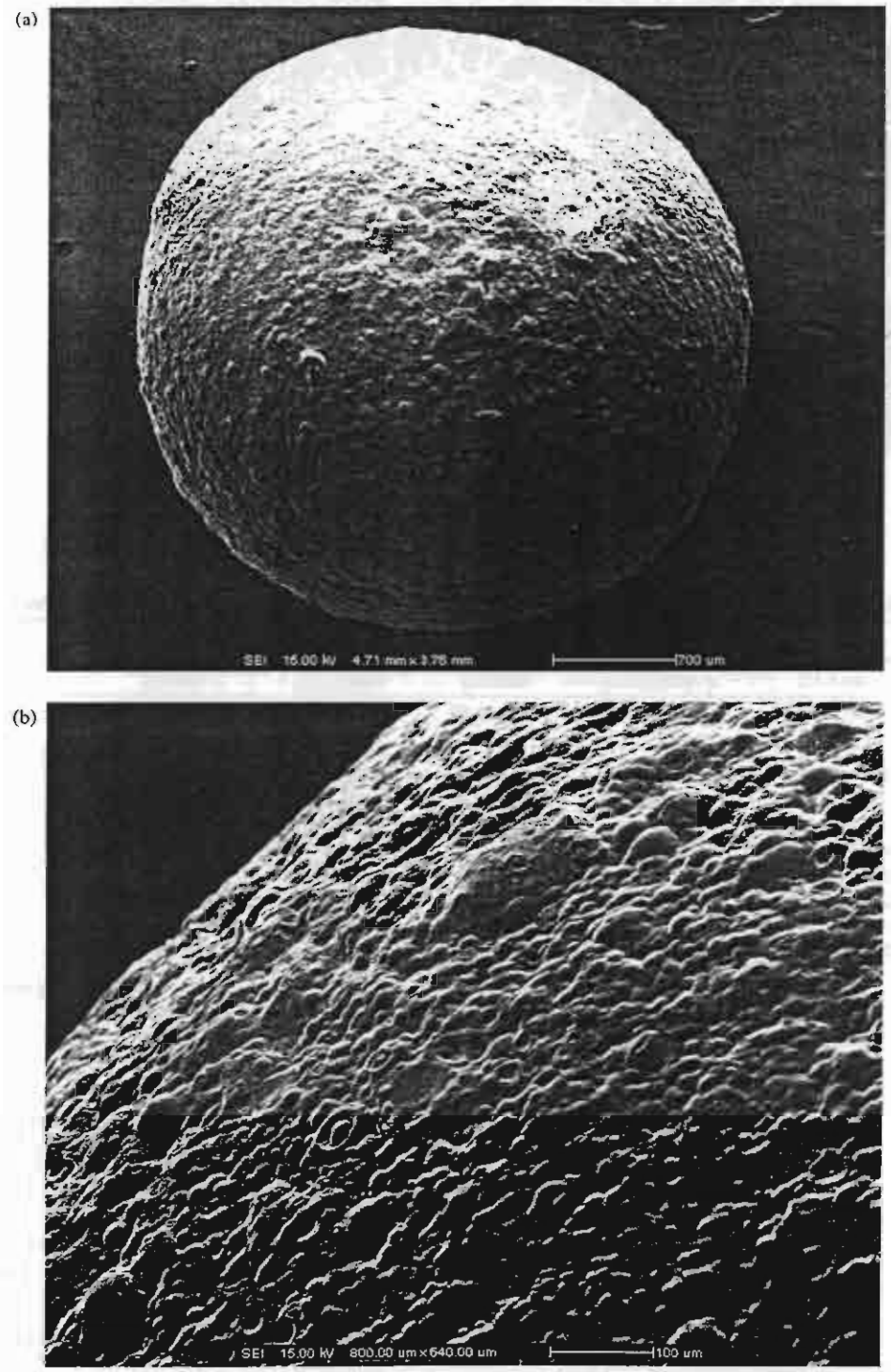


Fig. 3. Scanning electron micrographs of (a and b) external and (c) internal structures of emulsion gel beads of calcium pectinate containing peppermint oil (5%). Magnifications and scale bars are shown on the individual photographs.

(c)

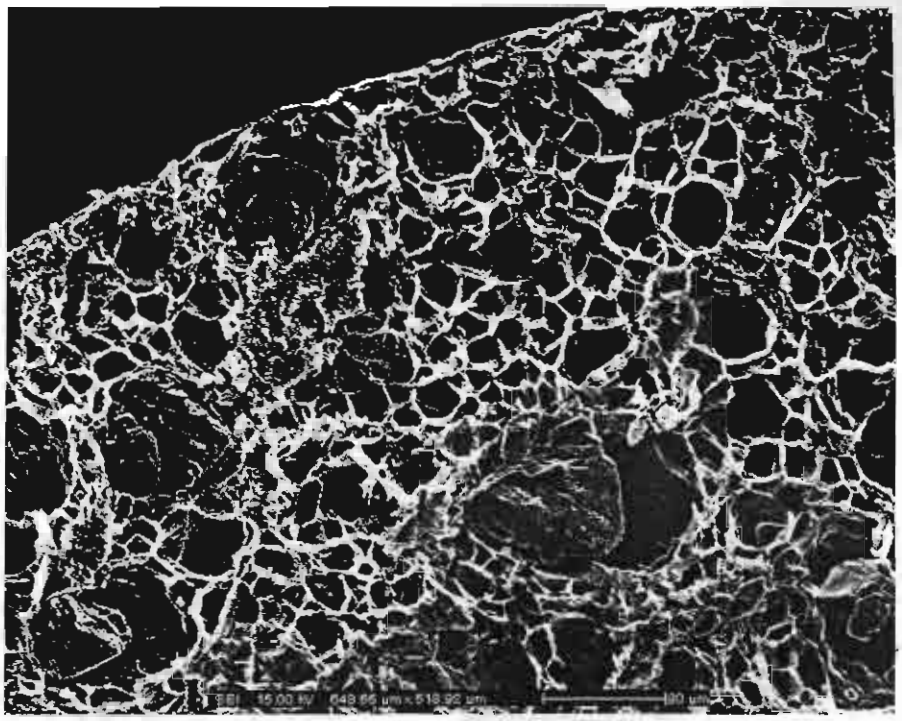


Fig. 3. (*Continued*).

Table 1
Mean diameter and entrapment efficiency of the MZ-loaded CaPG beads, EMG beads and modified EMG beads at different pectin to MZ ratios

Formulations	Mean diameter, mm ± S.D. ($n = 50$)			Entrapment efficiency, % ± S.D. ($n = 3$)		
	1:1 ratio	1:1.5 ratio	1:2 ratio	1:1 ratio	1:1.5 ratio	1:2 ratio
Conventional CaPG beads	2.45 ± 0.04	2.49 ± 0.11	2.54 ± 0.12	80.28 ± 1.12	78.68 ± 0.45	75.70 ± 1.34
EMG beads						
10% mineral oil	2.78 ± 0.04	2.82 ± 0.14	2.86 ± 0.09	70.16 ± 0.95	78.14 ± 1.42	75.58 ± 1.35
20% mineral oil	2.92 ± 0.11	2.94 ± 0.07	2.98 ± 0.14	69.09 ± 0.28	76.30 ± 0.97	78.05 ± 1.15
10% olive oil	2.60 ± 0.11	2.64 ± 0.08	2.66 ± 0.12	64.30 ± 0.15	69.88 ± 1.21	75.76 ± 0.95
20% olive oil	2.73 ± 0.03	2.78 ± 0.08	2.81 ± 0.11	69.78 ± 0.36	75.63 ± 1.38	80.64 ± 0.51
30% olive oil	2.84 ± 0.06	2.88 ± 0.15	2.96 ± 0.05	66.48 ± 0.21	70.74 ± 0.80	72.57 ± 0.15
10% peppermint oil	2.71 ± 0.07	2.77 ± 0.12	2.79 ± 0.08	72.90 ± 0.88	81.89 ± 0.62	78.46 ± 1.44
20% peppermint oil	2.97 ± 0.04	3.05 ± 0.14	3.12 ± 0.09	78.56 ± 0.64	80.13 ± 1.11	84.13 ± 0.94
30% peppermint oil	3.28 ± 0.03	3.40 ± 0.11	3.51 ± 0.14	72.84 ± 0.56	75.47 ± 1.22	77.57 ± 0.76
EMG beads hardening with glutaraldehyde						
20% mineral oil	2.72 ± 0.14	N/A	N/A	38.44 ± 0.36	N/A	N/A
30% olive oil	2.69 ± 0.09	N/A	N/A	28.80 ± 0.04	N/A	N/A
30% peppermint oil	2.98 ± 0.13	N/A	N/A	30.64 ± 0.02	N/A	N/A
Additive-added EMG beads containing 30% olive oil						
Eudragit® L100:pectin = 0.5:1	2.88 ± 0.09	N/A	N/A	59.66 ± 0.07	N/A	N/A
Eudragit® L100:pectin = 1:1	2.92 ± 0.14	N/A	N/A	62.03 ± 0.18	N/A	N/A
GMS:pectin = 0.25:1	2.90 ± 0.11	N/A	N/A	62.15 ± 0.75	N/A	N/A
GMS:pectin = 0.5:1	2.93 ± 0.13	N/A	N/A	63.66 ± 0.84	N/A	N/A
GMS:pectin = 1:1	2.94 ± 0.16	N/A	N/A	66.17 ± 1.41	N/A	N/A
PEG10000:pectin = 0.25:1	2.91 ± 0.07	N/A	N/A	61.82 ± 1.14	N/A	N/A
PEG10000:pectin = 0.5:1	2.97 ± 0.16	N/A	N/A	62.89 ± 0.72	N/A	N/A
PEG10000:pectin = 1:1	2.98 ± 0.13	N/A	N/A	65.18 ± 0.54	N/A	N/A
Polymer-coated EMG beads containing 30% olive oil						
Eudragit® RL100	3.20 ± 0.14	N/A	N/A	60.25 ± 0.40	N/A	N/A

Abbreviations: MZ = metronidazole, CaPG = calcium pectinate gel, EMG = emulsion gel, GMS = glyceryl monostearate, PEG = polyethylene glycol, N/A = not applicable.

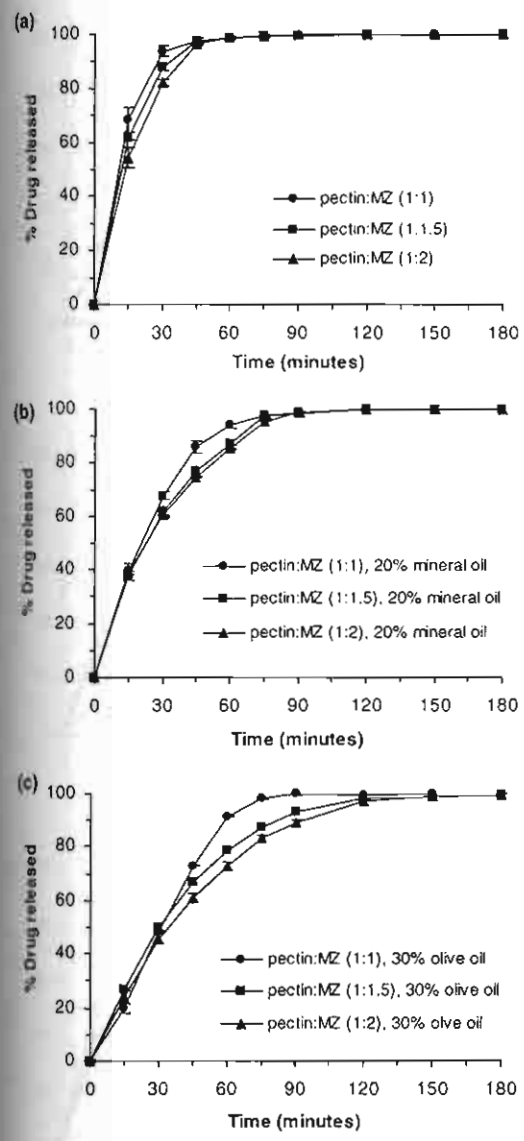


Fig. 4. Effect of pectin to drug ratio on metronidazole (MZ) release from (a) conventional calcium pectinate gel beads and emulsion gel beads containing (b) mineral oil and (c) 30% olive oil. The means and the standard deviation of triplicate data are plotted.

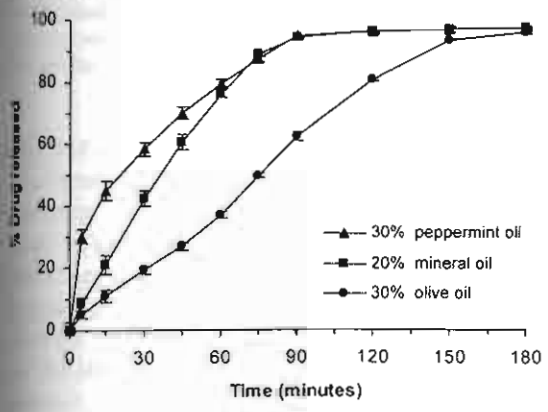


Fig. 5. Effect of hardening agent, 2% (v/v) glutaraldehyde, on metronidazole release from emulsion gel beads containing various oils. The means and the standard deviation of triplicate data are plotted.

Table 2
Buoyancy ($n = 20$) of the MZ-loaded CaPG beads, EMG beads and modified EMG beads in simulated gastric fluid USP without pepsin

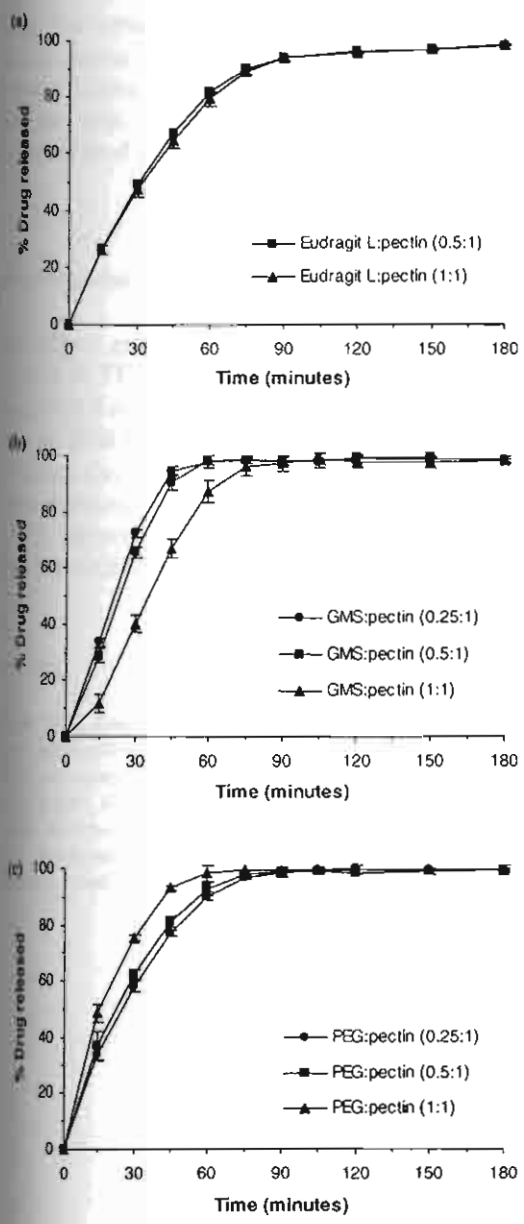
Formulations	Pectin:MZ ratio		
	1:1	1:1.5	1:2
Conventional CaPG beads	S	S	S
EMG beads			
10% mineral oil	S → F	S → F	S → F
20% mineral oil	F	F	F
10% olive oil	S	S	S
20% olive oil	S → F	S → F	S → F
30% olive oil	F	F	F
10% peppermint oil	S	S	S
20% peppermint oil	S → F	S → F	S → F
30% peppermint oil	F	F	F
EMG beads hardening with glutaraldehyde			
20% mineral oil	F	N/A	N/A
30% olive oil	F	N/A	N/A
30% peppermint oil	F	N/A	N/A
Additive-added EMG beads containing 30% live oil			
Eudragit® L100:pectin = 0.5:1	F	N/A	N/A
Eudragit® L100:pectin = 1:1	F	N/A	N/A
GMS:pectin = 0.25:1	F	N/A	N/A
GMS:pectin = 0.5:1	F	N/A	N/A
GMS:pectin = 1:1	F	N/A	N/A
PEG10000:pectin = 0.25:1	F	N/A	N/A
PEG10000:pectin = 0.5:1	F	N/A	N/A
PEG10000:pectin = 1:1	F	N/A	N/A
Polymer-coated EMG beads containing 30% olive oil			
Eudragit® RL100 (16% weight increase)	F	N/A	N/A

Abbreviations: S = sink, F = float (immediately, and still afloat for at least 24 h), S → F = sink immediately and then gradually float, N/A = not applicable, MZ = metronidazole, CaPG = calcium pectinate gel, EMG = emulsion gel, GMS = glyceryl monostearate, PEG = polyethylene glycol.

the EMG structure and did not interact with calcium ion or carboxylic group of pectin. Thus, it did not help to strengthen the network structure. In addition, the Eudragit® L did not encapsulate the drug particles or the beads so that the drug could release directly from the matrix bead structure.

GMS, a hydrophobic substance, was added to the starting solution prior to bead formation to delay the drug release. The drug release was slower when the GMS to pectin ratio was increased (Fig. 6b). Addition of GMS to the formulation at the ratio of 0.25:1 and 0.5:1 did not slow the drug release. Further addition of GMS at 1:1 ratio had a significant effect on the release of MZ in which about 80% of drug loading was released within 50 min. It is suggested that addition of GMS at higher amount gave the matrix with more hydrophobicity which consequently delayed the diffusion of drug from the beads.

Adding PEG10000 into the formulation, on the other hand, gave the opposite results. In the presence of PEG10000, the release of MZ was found to increase slightly. When PEG10000 to pectin ratio was increased, the drug release from the EMG beads was faster (Fig. 6c). It is likely that the PEG10000, a hydrophilic substance, enhances water uptake into the bead, and thus increases the dissolution of MZ.



Effect of additive to pectin ratio on metronidazole release from ...-added emulsion gel beads containing 30% olive oil; (a) Eudragit® L, ...ceryl monostearate (GMS), and (c) polyethylene glycol 10000 (PEG). ...means and the standard deviation of triplicate data are plotted.

...over, PEG was dissolved out and created porous matrix ...ich could enhance drug release. This result is in agreement ...Kumar et al. (2004) where the PEGs increase the re... of chlorpheniramine maleate and diazepam from glyc... monooleate matrices.

...It is apparent that both hydrophobic and hydrophilic ad... did not significantly help to prolong the drug release ...the EMG beads. The matrix structure (e.g. the drug parti... polymers, and additives homogenously dispersed in the ...ture) predominantly influenced the drug release. A dif... means to modify the drug release by coating the EMG ...was used. Fig. 7 illustrates the effect of coating with

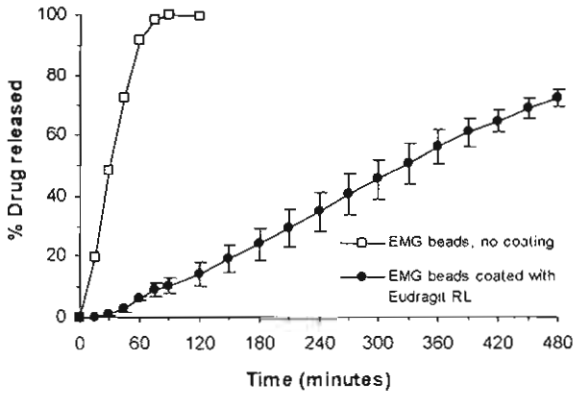


Fig. 7. Effect of coating with Eudragit® RL on metronidazole release from the emulsion gel (EMG) beads containing 30% olive oil. The means and the standard deviation of triplicate data are plotted.

Eudragit® RL on MZ release from the EMG beads containing 30% olive oil. Coating the EMG beads with Eudragit® RL slowed the release of MZ compared to uncoated EMG beads. After a lag time period, the release from coated beads is essentially constant; about 80% of drug loading were released at 8 h. It is generally known that the mode of drug release from beads or pellets coated with a water-insoluble membrane (i.e. reservoir type) is penetration of liquid into the beads, dissolution of the drug to form a saturated solution (as long as undissolved drug is present), partitioning of drug into the polymeric membrane, and diffusion of drug through the membrane.

4. Conclusion

The EMG beads of calcium pectinate were designed and prepared by emulsion-gelation method for use in floating drug delivery systems. MZ was used as a model drug for intragastric floating drug delivery system in order to *H. pylori* eradication in the treatment of peptic ulcer disease. The MZ released rapidly from conventional CaPG beads, but no significant change was seen with the different amounts of MZ loaded. The MZ-loaded EMG beads floated in an acidic environment of gastric fluid and the drug release from these beads was slightly slower than that from conventional CaPG beads. Modified EMG beads with Eudragit® L, GMS or PEG10000 as an additive, could not significantly prolong the drug release. However, the treatment with glutaraldehyde affected a slight reduction in the drug release. The drug release was dramatically prolonged by coating the EMG beads with Eudragit® RL. However, more information on the influence of different variables and coating conditions on the release need to be studied further. In addition, it is essential to consider the mechanisms implied in the release and the physicochemical properties of the active principles and polymers.

These systems can float in the gastric condition and can control the drug release from the EMG beads of calcium

pectinate. The enhanced buoyancy property of EMG beads of calcium pectinate makes them an excellent candidate for gastric floating drug delivery systems. Moreover, these systems may also provide a suitable means of delivery for drugs that are locally active to the gastric mucosa in the stomach.

Acknowledgments

The authors gratefully acknowledge Mr. Witoon Saeteow from SUSTREC (Silpakorn University Scientific and Technological Research Equipment Centre) for technical assistance on SEM imaging. Thanks also go to Food & Cosmetic System Co. Ltd. (Thailand) for supplying pectin samples manufactured by CP Kelco (Denmark). This work was financially supported by the Thailand Research Funds (grant number MRG4680120).

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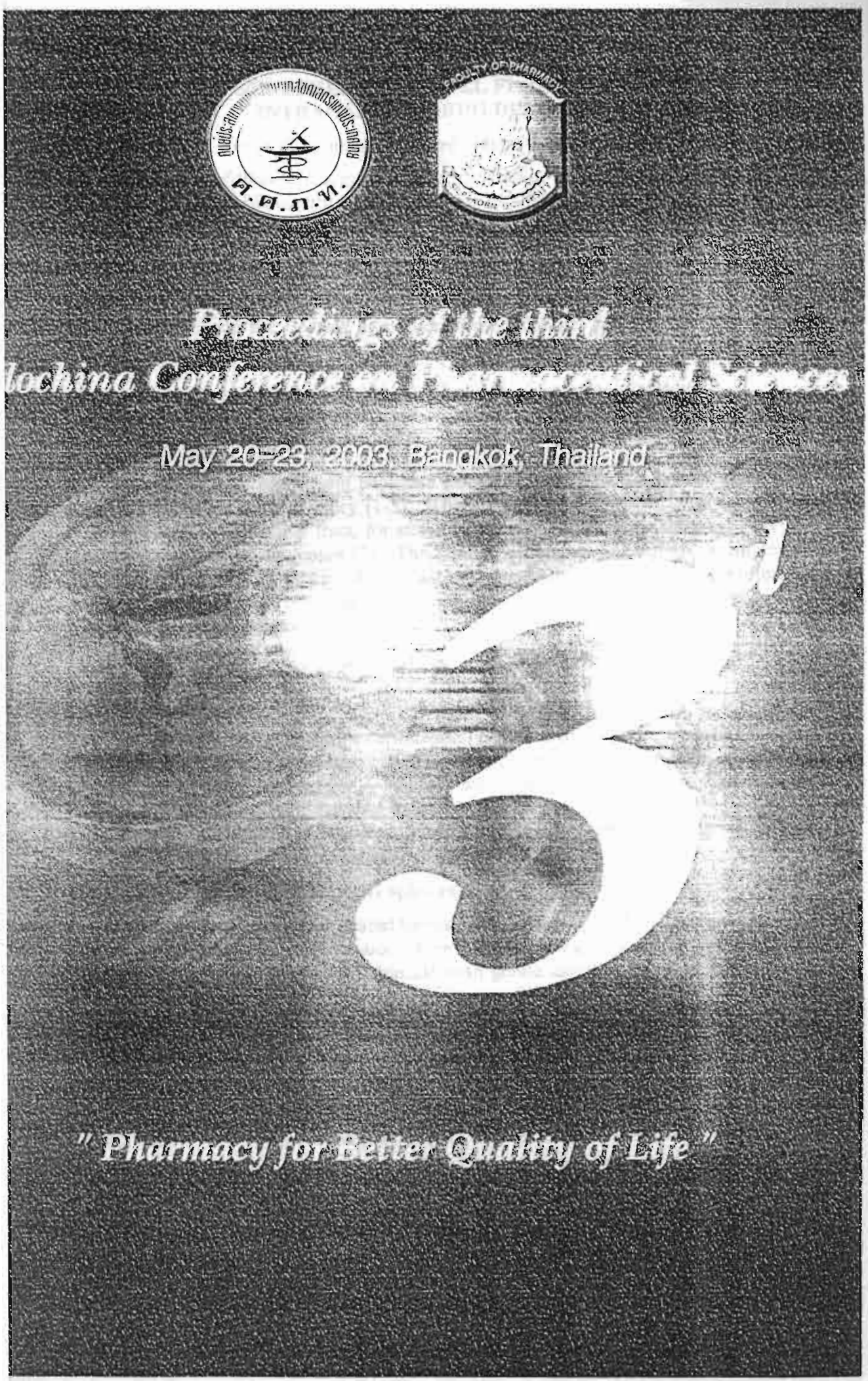
Proceedings of the third
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EMULSION GEL SPHERES: A NOVEL FLOATING SYSTEM FOR INTRAGASTRIC DRUG DELIVERY

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Abstract

A novel method to prepare emulsion gel spheres of calcium pectinate capable of floating in the gastric condition was designed. The effect of oil type and oil concentration on the floating properties of emulsion gel spheres has been tested. The buoyancy of gel spheres is attributed to the oil held in the gel matrix.

Keywords: calcium pectinate; pectin; oil; emulsion; gel spheres; floating

1. Introduction

Gel spheres of calcium pectinate have been developed in recent years as a unique vehicle for drug delivery [1-3]. The gel spheres have been used in various ways in the gastrointestinal tract, for example, for sustained release of drugs [1-2] or for targeting drugs to the colon [3]. The floating properties of calcium pectinate gel (CPG) spheres and their potential as a gastroretentive system has not yet been tested. In this study, selected oils were used to prepare emulsion gel spheres and their floating behavior was investigated.

2. Experimentals

Materials

Pectin with degree of esterification (DE) of 36% and degree of amidation (DA) of 14% (GENUpectin type LM-101 AS) and one with DE of 28% and DA of 20% (GENUpectin type LM-104 AS-FS) were the generous gift of Copenhagen Pectin (Denmark) and are referred to as LM-101 and LM-104 respectively. Light mineral oil, olive oil and sunflower oil were of standard pharmaceutical grade and all chemical reagents used were of analytical grade.

Preparation of conventional CPG spheres

CPG spheres were prepared by dissolving pectin (i.e., LM-101 and LM-104) in water with agitation. The solutions were extruded using a nozzle of 0.80-mm inner diameter into a 0.34 M calcium chloride with gentle agitation at room temperature. The CPG spheres formed were allowed to stand in the solution for 20 minutes, separated and washed with distilled water. The spheres were dried at 37°C for 12 hours.

Preparation of emulsion gel spheres

Both pectins were dissolved in water with agitation. Different amounts (i.e., 5, 10, 20, 30, 40 %w/w) of selected oils (i.e., light mineral oil, olive oil, sunflower oil) were added to the solution. Either the homogenized or non-homogenized mixture was extruded into calcium chloride at room temperature. The gel spheres formed were treated in the same manner as CPG spheres.

Study of particle size and morphology of gel spheres

The mean diameter of 50 dried spheres was determined by optical microscopy (BH-2, Olympus, Japan). The microscope eyepiece was fitted with a micrometer by which the size of the beads could be determined. A scanning electron microscope (Model MaXim, CamScan Analytical, England) was used to examine the structure of gel spheres.

Buoyancy of gel spheres

Specific gravity of the test solution (distilled water, simulated gastric fluid USP minus pepsin (SGF) and normal saline solution (i.e., 0.9% NaCl)) previously measured using standard pycnometer was 1.007, 1.013 and 1.014, respectively. The gel sphere samples (n=10) were steeped in 50 mL of each test solution and their buoyancy was observed visually. The preparation was considered to have buoyancy in the test solution only when all of the gel spheres floated in it [4].

3. Results and Discussion

Either aqueous solution of pectin or emulsion containing pectin and selected oils was dropped into calcium chloride solutions and gelled sphere was formed instantaneously by ionotropic gelation in which intermolecular cross-links were formed between the divalent calcium ions and the negatively charged carboxyl groups of the pectin molecules. The gel spheres were easily manufactured without any sophisticated equipment.

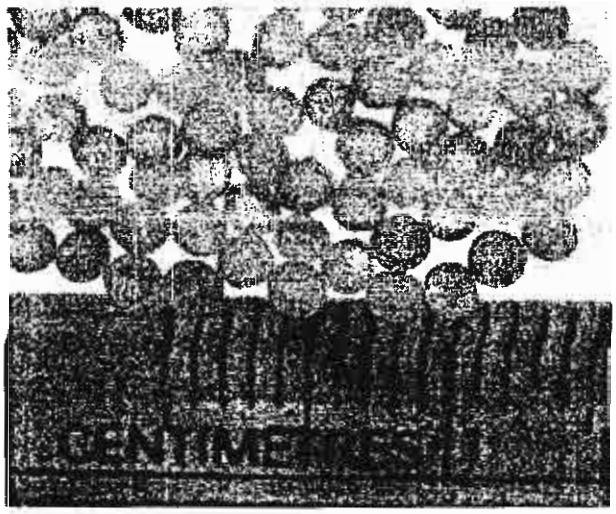

Figure 1. Photograph showing the appearance of emulsion gel spheres containing mineral oil (30%).

Figure 1 shows the appearance of emulsion gel spheres containing 10% of mineral oil. The spheres containing mineral oil were transparent and light yellowish whereas those containing olive oil and sunflower oil were less transparent and dark yellowish. Oil began to leak from the spheres with high concentration of oil (i.e. 40%). The mean diameter of emulsion gel spheres ranges between 1.46±0.04 and 2.22±0.11 mm while that of conventional gel spheres was 1.27±0.08 mm. Figure 2 demonstrated that the sphere diameter increased as the amount of oil used was increased. The mean diameter of spheres made of different types of pectin was not significantly different.

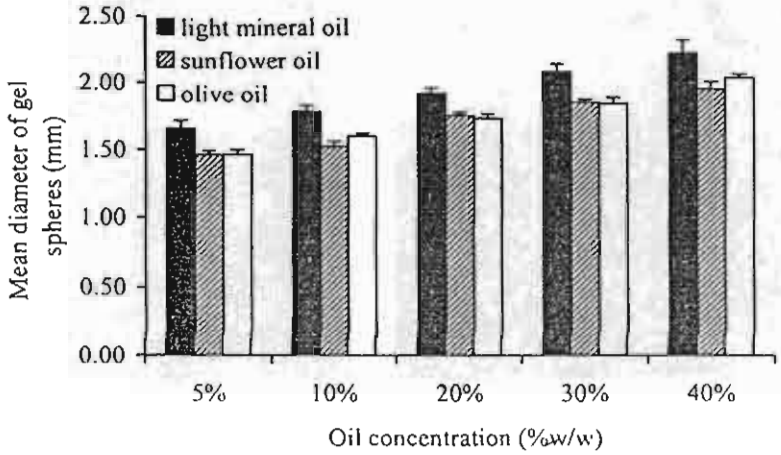

Figure 2. Mean diameter of emulsion gel spheres containing different oils and concentrations (n=50).

Samples were taken from different formulations and operating conditions for SEM observation. Some typical images are shown in Figure 3 to illustrate the external and internal structure of the dry conventional gel spheres. Upon air drying, the conventional gel spheres of all formulations became small, dense and had a hollow at the middle of surface. The emulsion gel spheres (Figure 4) were more spherical with no hollow at the middle of sphere surface. Figure 4 show the internal morphology of an emulsion gel sphere made of olive oil. The microstructure of all emulsion gel spheres appeared the same irrespective of oil type.

When conventional gel spheres were steeped in distilled water, normal saline solution or SGF, they sank as shown in Table 1. However, the emulsion gel spheres containing various oils were floated if the sufficient amount of oils were used. The emulsion gel spheres containing about 10% of mineral oil or 20% of olive oil or 30% of sunflower oil floated in the tested solutions. These results were due to the different relative densities of the oils used, i.e., 0.840, 0.913 and 0.921 for mineral oil, olive oil and sunflower oil, respectively. The results indicated that if the oil with lower relative density was used, the lower amount of the oil was required to keep the spheres float.

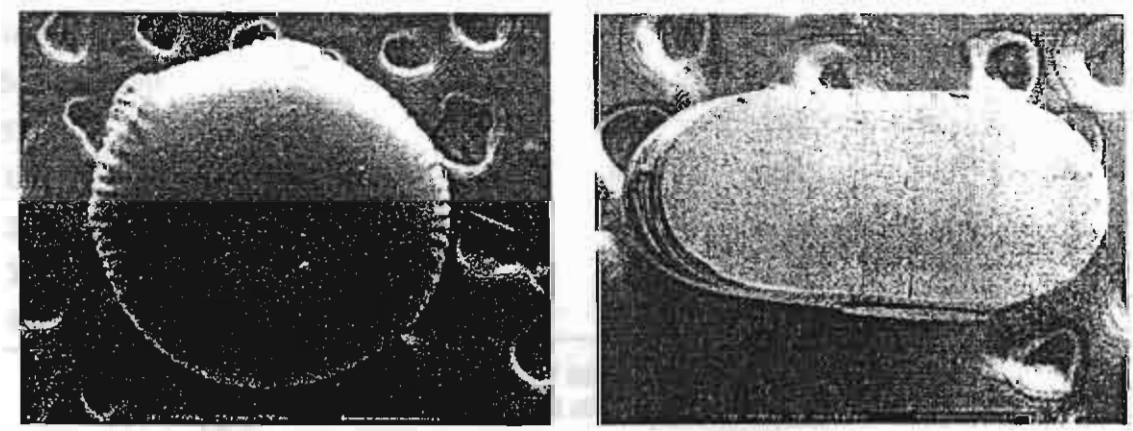

Figure 3. Scanning electron micrographs of (a) external and (b) internal structure of a conventional gel sphere.

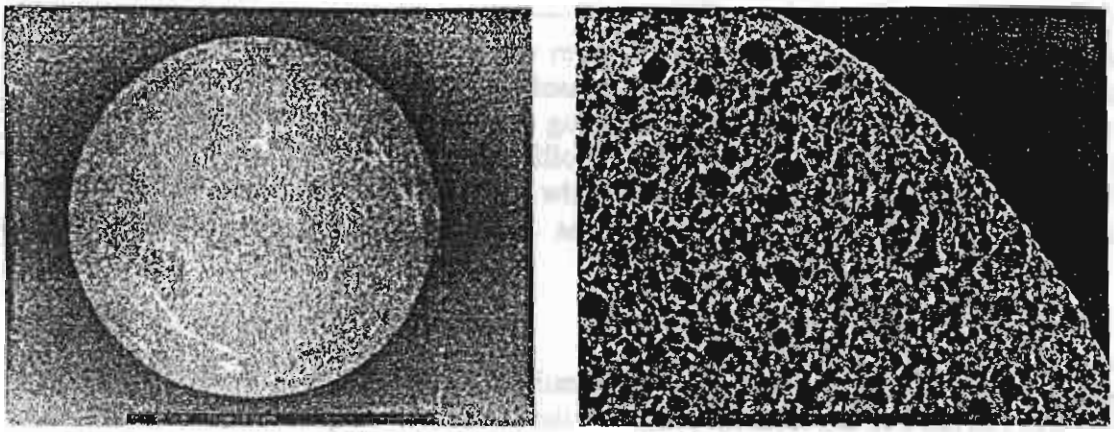

Figure 4. Scanning electron micrographs of (a) external and (b) internal structure of an emulsion gel sphere containing olive oil (30%).

Table 1. Buoyancy of the conventional and emulsion gel spheres in different tested solutions.

	Pectin LM-101			Pectin LM-104		
	Distilled water	Normal saline	SGF*	Distilled water	Normal saline	SGF*
No oil (conventional)	S	S	S	S	S	S
Mineral oil						
5%	S	S	S	S	S	S
10%	F	F	F	F	F	F
20%	F	F	F	F	F	F
30%	F	F	F	F	F	F
40%	F	F	F	F	F	F
Olive oil						
5%	S	S	S	S	S	S
10%	S	S	S	S	S	S
20%	F	F	F	F	F	F
30%	F	F	F	F	F	F
40%	F	F	F	F	F	F
Sunflower oil						
5%	S	S	S	S	S	S
10%	S	S	S	S	S	S
20%	S	S	S	S	S	S
30%	F	F	F	F	F	F
40%	F	F	F	F	F	F

* SGF = simulated gastric fluid USP minus pepsin
S = sink, F = float (immediately, and still float for 6 hours)

4. Conclusion

In this study, we designed a novel floating system of emulsion gel spheres and examined its buoyancy. The emulsion gel spheres floated in acidic environment of the gastric fluid as well as in distilled water or normal saline solution if they contained

sufficient amount of oil, depending on relative density of oil. These properties are applicable not only to the sustained release of drugs but also to the targeting of the gastric mucosa. The floating emulsion gel spheres appear to be a promising vehicle for delivering such preparations specifically to the region. And it will play an important role in therapy of diseases in which a gastric mucosa-specific drug delivery regimen should be considered, such as gastric ulcer with Helicobactor pylori infection.

5. References

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ประชุมวิชาการระดับนานาชาติ

Sriamornsak P, Thirawong N, Nunthanid J, Puttipipatkhachorn S, Kasantikul V and Keokitichai S. Novel emulsion gel spheres for floating drug delivery: Effect of selected factors on floating and drug release properties. ***The 1st European Federation in Pharmaceutical Science Conference on Optimising Drug Delivery and Formulation: New Challenges in Drug Delivery*****, Versailles, 29 September – 1 October 2003.**

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Novel emulsion gel spheres for floating drug delivery: Effect of selected factors on floating and drug release properties

Pornsak Sriamornsak[1], Nartaya Thirawong[1], Jurairat Nunthanid[1], Satit Puttipipatkhachorn[2], Vira Kasantikul[3], Sindhchai Keokitichai[3]

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Oral sustained drug delivery system is complicated by limited gastric residence times. Rapid gastrointestinal transit can prevent complete drug release in the absorption zone and reduce the efficacy of administered dose in the stomach and upper small intestine [1]. To overcome these limitations, a novel floating system of emulsion gel spheres with prolonged gastric residence times was designed and tested. The calcium pectinate gel spheres containing edible oil were prepared according to the method of Sriamornsak et al. [2-3] as follows. An oil phase and a water phase containing pectin (with 36% degree of esterification (DE) (LM-101) and 28% DE (LM-104), Copenhagen Pectin, Denmark) were either gently mixed or homogenized. The mixtures were then extruded into a 0.34 M calcium chloride with gentle agitation at room temperature. The gel spheres formed were allowed to stand in the solution for 20 minutes, separated and washed with distilled water. The spheres were dried at 37°C for 12 hours. The model drug, metronidazole, was used in this study. The effect of selected factors, such as type of oil, percentage of oil, type of pectin and pectin to drug ratio, on floating and drug release properties was investigated. In this study, the gel spheres were formed instantaneously by ionotropic gelation in which intermolecular cross-links were formed between the divalent calcium ions and the negatively charged carboxyl groups of the pectin molecules. The gel spheres were easily manufactured without any sophisticated equipment. The emulsion gel spheres containing various oils floated if the sufficient amount of oils were used. As percentage of oil increased, the floating time prolonged and the drug released slower. The gel spheres prepared from different types of pectin showed similar floating and drug release behavior. The increased drug to pectin ratio slightly influenced the drug release patterns. The results of these studies indicate that type and percentage of oil is more important than other factors studied. The enhanced buoyancy and prolonged release properties of emulsion gel spheres make them an excellent candidate for floating drug delivery system. These properties are applicable not only to the sustained release of drugs but also to the targeting of the gastric mucosa. The floating emulsion gel spheres appear to be a promising vehicle for delivering such preparations specifically to the region. And it will play an important role in therapy of diseases in which a gastric mucosa-specific drug delivery regimen should be considered, such as gastric ulcer with Helicobactor pylori infection.

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ประชุมวิชาการระดับนานาชาติ

Sriamornsak P, Thirawong N, and Puttipipatkhachorn S. A new intragastric floating system using calcium pectinate gel beads containing carbonates. *Proceedings of the Sixth NRCT-JSPS Joint Seminar on Natural Medicine in Pharmaceutical Sciences* 2003; 6: 224. [Bangkok, 2-4 December 2003]

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A NEW INTRAGASTRIC FLOATING SYSTEM USING CALCIUM PECTINATE GEL BEADS CONTAINING CARBONATES

Pornsak Sriamornsak[1], Nartaya Thirawong[1] and Satit Puttipipatkhachorn[2]

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A new intragastric floating drug delivery system using calcium pectinate gel beads containing carbonates, as gas-forming agents, was designed and tested in order to overcome the complication of limited gastric residence times of oral sustained drug delivery system. The calcium pectinate gel beads containing carbonates were prepared by dissolving or suspending carbonate salts (i.e., sodium bicarbonate, calcium carbonate, potassium carbonate or sodium carbonate) in pectin solution. The mixtures were then extruded into either neutral or acidified solution of calcium chloride with gentle agitation at room temperature. The gel spheres formed were then separated, washed with distilled water and dried. The effect of selected factors, such as type of carbonates, percentage of carbonates, type of pectin, type of gelation medium and drying condition, on physical and floating properties was investigated. The surface and cross-sectional morphology of the beads were examined with scanning electron microscopy. The beads containing potassium carbonate or sodium carbonate could not be prepared as the viscous gel formed before extrusion through the needle. Incorporation of sodium bicarbonate or calcium carbonate into pectin solution resulted in porous-structured beads. Acidity of gelation medium increased the pores in the structure. This is due to carbonate salts were reacted with acid to produce carbon dioxide. The evolving gas permeated through the calcium pectinate structure leaving gas bubbles or pores. As percentage of carbonates increased, the size and floating properties increased. The lyophilized beads gave more porous structure and increased buoyancy when compared to air-dried beads since the structure of lyophilized beads remained the same as wet beads. The enhanced buoyancy of calcium pectinate gel beads containing carbonates makes them an excellent candidate for intragastric floating drug delivery system. These properties are applicable not only to the sustained release of drugs but also to the targeting of the gastric mucosa.

ประชุมวิชาการระดับนานาชาติ

Sriamornsak P, Thirawong N and Puttipipatkhachorn S. Superporous gel spheres of calcium pectinate: a novel floating drug delivery. *The 2nd Pharmaceutical Sciences World Congress*, Kyoto, 29 May - 3 June 2004.

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P3A-IV-018 Superporous gel spheres of calcium pectinate: a novel floating drug delivery

Pornsak Sriamornsak[1], Nartaya Thirawong[1], Satit Puttipipatkhachorn[2]
[1]*Dept. of Pharmaceutical Technology, Fac. of Pharmacy, Silpakorn Univ., Thailand;* [2]*Dept. of Manufacturing Pharmacy, Fac. of Pharmacy, Mahidol Univ., Thailand*

Purpose: A new intragastric floating drug delivery system using superporous calcium pectinate gel spheres was designed and tested.
Methods: The superporous calcium pectinate gel (CPG) spheres containing carbonates, as gas-forming agents, were prepared by dissolving or suspending carbonates (i.e., $NaHCO_3$, $CaCO_3$, K_2CO_3 or Na_2CO_3) in pectin solution. The mixtures were then extruded into either neutral or acidified solution of $CaCl_2$ with gentle agitation. The CPG spheres formed were then separated, washed with distilled water and dried. The effect of selected factors, such as type of carbonates, percentage of carbonates, type of pectin, type of gelation medium and drying condition, on physical and floating properties was investigated. The surface and cross-sectional morphology of the dried CPG spheres were examined with SEM.
Results: The CPG spheres containing K_2CO_3 or Na_2CO_3 could not be prepared as the viscous gel formed before extrusion through the needle. Incorporation of $NaHCO_3$ or $CaCO_3$ into pectin solution resulted in porous-structured beads. Acidity of gelation medium increased the pores in the structure. This is due to carbonate salts reacted with acid to produce CO_2. The evolving gas permeated through the matrix structure leaving gas bubbles or pores. As percentage of carbonates increased, the size and floating properties increased. The lyophilized spheres gave superporous structure and increased buoyancy when compared to air-dried gel spheres since the structure of lyophilized spheres remained the same as wet beads.
Conclusion: The enhanced buoyancy of CPG spheres containing carbonates makes them an excellent candidate for intragastric floating drug delivery system. These properties are applicable not only to the sustained release of drugs but also to the targeting of the gastric mucosa.

P3A-IV-019 Novel chitosan particles and chitosan-coated emulsions inducing immune response via intranasal vaccine delivery

Yoshie Maitani[1], Takahiro Nagamoto[1], Yoshiyuki Hattori[1], Kozo Takayama[2]
[1]*Inst. of Medicinal Chemistry, Hoshi Univ., Japan;* [2]*Dept. of Pharmaceutics, Hoshi Univ., Japan*

Purpose: The mucosal vaccine delivery is very attractive for inducing a protective immune response and nasal mucosa is one of suitable immunized routes. The aim of this study was to prepare a novel vaccine carrier particulate system (nanoparticles and emulsions) with chitosan, and to evaluate the effect of this system on the immune response for intranasal delivery.
Methods: Chitosan nanoparticles (NP) and chitosan-coated emulsions (CC-Emul) were prepared by improvement of the method reported previously, and modified ethanol injection methods, respectively. The size, surface potential, and adsorbed amounts of ovalbumin (OVA) in NP and CC-Emul were measured. The rats were immunized with their particles adsorbed with OVA and cholera toxin (CT) by intranasal (i.n.) and intraperitoneal (i.p.) administration. IgG, IgA and anti-OVA IgG were measured.
Results: NP and CC-Emul could be prepared with particle diameter from about 0.4 μm to 3 μm. IgG induced by i.n. of NP was comparable with that by i.p., and IgA induced by i.n. of 0.4-μm and 1-μm sized NP was significantly higher than control (OVA and CT). IgG and IgA induced by i.n. of 2-μm sized CC-Emul were significantly higher than those with control.
Conclusions: The novel chitosan particles employed simple preparation methods showed high OVA adsorption. When administered intranasally, NP and CC-Emul induced systemic immune response in rats. These findings suggested that CC-Emul and the smaller sized (0.4 μm) NP are effective for targeting to nasal associated lymphoid tissues (NALT) in nasal vaccine delivery.

P3A-IV-020 The pH-sensitive polymeric micelle drug carriers designed for intracellular delivery

Younsoo Bae[1], Nobuhiro Nishiyama[2], Shigeto Fukushima[1], Kazunori Kataoka[1]
[1]*Graduate School of Engineering, The Univ. of Tokyo, Japan;* [2]*Graduate School of Medicine, The Univ. of Tokyo, Japan*

Purpose: During the last decade to devote considerable efforts, numerous kinds of drug carriers have been developed for cancer treatment. Even though great progress has been made in clearing the troublesome in vivo barriers such as rapid renal clearance, non-specific systemic spread and uptake of reticular endothelial system, the carriers still have difficulties to treat some intractable cancers that are resistant to anticancer agents due to the overexpression of drug-excreting P-glycoproteins (P-gp) on the tumor cell membrane. In order to clear this limit, we have designed a smart drug carrier that delivers the loaded drugs into cell interior without passing through P-gp.
Methods: Adriamycin, an anticancer drug, was conjugated to the amphiphilic block copolymers through acid-sensitive linkers, which self-assembled into core-shell micelle structure in aqueous solution with tens of nm size in diameter. The micelle was studied in vitro and in vivo by evaluating antitumor activity, toxicity, and biodistribution.
Results: Reversed phase liquid chromatograpy confirmed the micelle released the loaded drugs selectively in acidic condition below 6.0, corresponding to endosomes and lysosomes in the cell. The intracellular drug release and distribution of the micelle was observed by monitoring fluorescence change in intensity with confocal laser scanning microscopy, which demonstrated that the micelle was precisely functioning as designed interacting with live cells. In addition, characteristic delayed cytotoxicity was shown as coincubation time increased, which also reflects intracellular drug trafficking of the micelle. Subsequent animal test showed that the micelle was effectively suppressing tumor growth in tumor bearing mice with remarkably alleviated toxicity, which was considered to be due to tumor-specific drug delivery and release by drug release control.
Conclusions: With the ingenious design of chemical structures, an intelligent drug carrier, the pH-sensitive polymeric micelle, has prepared, which provided one of the most promising carrier models that not only change the bioavailability of loaded drugs but also broaden in vivo applications for the new type of cancer therapy in the future.

P3A-IV-021 Multi-targeting drug delivery system using thermo-responsive polymeric micelles combined with hyperthermia

Masamichi Nakayama[1], Masayuki Yokoyama[1], Teruo Okano[1], Kazuaki Sasaki[2], Ken-ichi Kawabata[2], Shin-ichiro Umemura[2]
[1]*Inst. Adv. Biomed. Eng. and Sci., Tokyo Women's Medical Univ., Japan;* [2]*Central Research Laboratory, Hitachi Ltd., Japan*

Purpose: By combining passive targeting drug carrier system with ON-OFF control of drug release by external stimuli in one carrier system, an ideal drug targeting is expected to be designed. In order to accomplish this intelligent drug targeting system, we have designed thermo-responsive polymeric micelles with poly(*N*-isopropylacrylamide) (PIPAAm) outer shells, and investigated control of anti-tumor activity by temperature change.
Methods: We synthesized thermally responsive polymeric micelles constructed with P(IPAAm-*co*-dimethylacrylamide)-*b*-poly(D,L-lactide) block copolymer, and the ADR loading was carried out by a dialysis method. In vitro cytotoxic activity of free ADR or the ADR-loaded micelles was assayed with human breast cancer MCF-7 at below or above the phase transition temperature (39.5℃) of polymeric micelles. Moreover, we investigated intracellular drug distribution by fluorescence microscopy in order to know these cytotoxic mechanisms modulated by temperature.
Results: In vitro cytotoxic activity of free ADR at 41℃ was compared with at 37℃. On the other hand, ADR-loaded polymeric micelles above the LCST (41℃) showed much higher cytotoxic activity than that below the LCST (37℃). In observation of intracellular drug distribution, free ADR which accumulated rapidly and selectively in nuclei without temperature effect, whereas ADR accumulation in the cells delivered by the polymeric micelles showed a significant temperature effect. The ADR delivered by the polymeric micelles distributed uniformly in whole cells above the phase transition temperature, while the ADR in the micelles showed slight accumulation in the cell below the LCST. This result shows that the thermo-responsive polymeric micelles delivered drug into the cell *via* triggered phase transition of the outer shell.
Conclusions: These results suggest that our thermo-responsive polymeric micelle system has a great potential in intracellular drug delivery control as well as for a multi-drug targeting system combining passive and active drug targeting.

ประชุมวิชาการระดับนานาชาติ

Sriamornsak P, Thirawong N and Puttipipatkhachorn S. Investigation of calcium pectinate gel beads as an intragastric floating drug delivery system: Using carbonates as a gas-forming agent. *The 31th Annual Meetings of the Controlled Release Society*, 12-16 June 2004.

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INVESTIGATION OF CALCIUM PECTINATE GEL BEADS AS AN INTRAGASTRIC FLOATING DRUG DELIVERY SYSTEM: USING CARBONATES AS A GAS-FORMING AGENT

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ABSTRACT SUMMARY

A new intragastric floating drug delivery system using calcium pectinate gel beads was designed. The effect of some formulation variables (e.g. carbonate salts, acidity of medium, pectin type, etc.) on buoyancy and morphology of the beads was studied. The beads floated in gastric conditions due to their porous structure.

Keywords: oral drug delivery, polysaccharides, gels

INTRODUCTION

Pectin is a naturally occurring water-soluble polysaccharide found in higher plant cell wall. Pectin can gel with calcium ions. Previously, calcium pectinate gel (CPG) beads were produced by ionotropic gelation for delivering drugs to small intestine[1-2] or colon[3]. In this study, we have designed a floating drug delivery system using CPG beads to target the drug to stomach in order to overcome the complication of limited gastric residence times of oral sustained drug delivery system. The floating drug delivery system employs carbonate salts as a gas-forming agent dispersed in a CPG matrix.

EXPERIMENTAL METHODS

Preparation of CPG beads

The CPG beads containing carbonates, as gas-forming agents, were prepared by dissolving or suspending carbonates (i.e., sodium bicarbonate, calcium carbonate, potassium carbonate or sodium carbonate) in pectin (GENUpectin, type LM-104 AS-FS, CP Kelco, Denmark) solution. The mixtures were then extruded into either neutral or acidified solution of calcium chloride with gentle agitation at room temperature. The gel beads formed were then separated, washed with distilled water and dried.

Scanning electron microscopy

A scanning electron microscope (CamScan Maxim 2000, England) was used to examine the structure of CPG beads. The effect of selected factors, such as type of carbonates, percentage of carbonates, type of pectin, type of gelation medium and drying condition, on morphology of the beads was investigated.

Buoyancy test

The buoyancy of the CPG beads was tested in simulated gastric fluid USP without pepsin (SGF), water, or 0.9% NaCl, at 37 °C using shaking incubator. The samples (n=10) were steeped in 50 mL of each test solution and their buoyancy was observed visually. The effect of some factors on buoyancy of the beads was also investigated.

RESULTS AND DISCUSSION

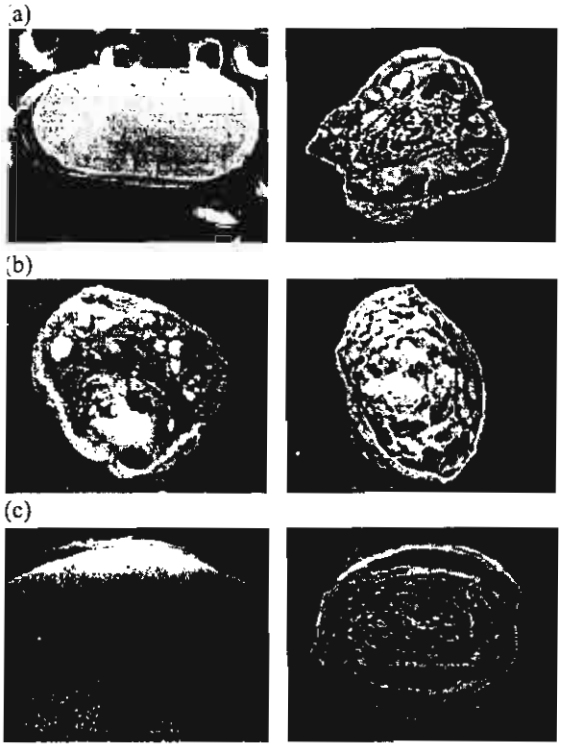
The CPG beads containing potassium carbonate or sodium carbonate could not be prepared as the viscous gel formed before extrusion through the needle. Incorporation of sodium bicarbonate or calcium carbonate into pectin solution resulted in porous-structured gel beads. Table 1 shows that both sodium bicarbonate and calcium carbonate significantly increased the size of the gel beads over the control (no gas-forming agent). As percentage of carbonates increased, the size of CPG beads increased. The size of lyophilized CPG beads was also larger than that of air-dried gel beads. This was because the lyophilization could maintain the structure of the gel beads to that of before drying while during air-drying the water evaporated from the structure and the beads became smaller.

Table 1. Mean diameter of dried CPG beads containing different carbonate salts (n=20).

Formulation	Mean diameter (mm ± SD)	
	Air-dried	Lyophilized
- / $CaCl_2$ (control)	1.17 ± 0.09	1.58 ± 0.16
5% $NaHCO_3$ / $CaCl_2$	1.26 ± 0.11	1.87 ± 0.10
5% $NaHCO_3$ / $CaCl_2$ + acetic â	-	-
10% $NaHCO_3$ / $CaCl_2$	1.29 ± 0.10	2.17 ± 0.18
10% $NaHCO_3$ / $CaCl_2$ + acetic â	-	-
5% $CaCO_3$ / $CaCl_2$	1.23 ± 0.07	1.60 ± 0.16
5% $CaCO_3$ / $CaCl_2$ + acetic â	1.37 ± 0.10	1.91 ± 0.16
10% $CaCO_3$ / $CaCl_2$	1.44 ± 0.06	1.73 ± 0.13
10% $CaCO_3$ / $CaCl_2$ + acetic â	1.51 ± 0.12	2.11 ± 0.16

Figure 1 shows the SEM photographs of the air-dried and lyophilized CPG beads containing no gas-forming agent, 5% sodium bicarbonate and 10% calcium carbonate. It was suggested that the presence of calcium ions contributed to homogenous CPG bead formation. Many large pores are present in the structure of CPG beads containing carbonate salts.

Figure 2 shows the SEM photographs of the air-dried and lyophilized CPG beads containing 10% calcium carbonate, gelled in neutral or acidified calcium chloride solution. Acidity of gelation medium increased the pores in the structure as the carbonate salts reacted with acid to produce CO_2. The evolving gas permeated through the calcium pectinate structure leaving gas bubbles or pores. Their size was also larger than that of gel beads prepared in neutral gelation medium (Table 1).



'igure 1. SEM photographs of the air-dried (left column) nd lyophilized (right column) CPG beads containing (a) .o gas-forming agent, (b) 5% sodium bicarbonate and (c) 0% calcium carbonate.

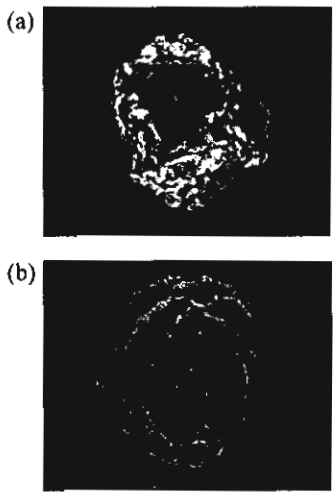


Figure 2. SEM photographs of the (a) air-dried and (b) yophilized CPG beads containing 10% calcium carbonate, gelled in acidified calcium chloride solution.

The buoyancy of prepared gel beads in SGF is shown in Table 2. Their buoyancy in water and 0.9% NaCl was similar to that in SGF. The carbonate-free beads sank completely in all media. The beads containing carbonate salts demonstrated excellent floating ability, especially the lyophilized beads and the beads gelled in acidified medium. The lyophilized beads had better floating ability than air-dried beads since the structure of the lyophilized beads was more porous (Figure 1). The floating ability of the CPG beads was not affected by the percentage of carbonates added. However, in some cases, the floating ability of the beads decreased as the percentage of carbonates increased.

Table 2. Buoyancy of the different CPG beads in simulated gastric fluid USP without pepsin (SGF). The numbers presented in the table are the percentage of beads floated at the time.

Formulation	Air-dried		Lyophilized	
	15 min	6 h	15 min	6 h
control	0	0	80	80
5% $NaHCO_3$ / $CaCl_2$	80	0	100	100
10% $NaHCO_3$ / $CaCl_2$	60	20	30	40
5% $CaCO_3$ / $CaCl_2$	0	0	100	100
5% $CaCO_3$ / $CaCl_2$ + acetic â	90	90	100	100
10% $CaCO_3$ / $CaCl_2$	0	0	30	40
10% $CaCO_3$ / $CaCl_2$ + acetic â	100	60	100	100

CONCLUSION

The enhanced buoyancy of gel beads containing carbonates makes them an excellent candidate for intragastric floating drug delivery system. These properties are applicable not only to the sustained release of drugs but also to the targeting to the gastric mucosa. The floating emulsion gel beads appear to be a promising vehicle for delivering such preparations specifically to the region. And it will play an important role in therapy of diseases in which a stomach-specific drug delivery regimen should be considered.

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ACKNOWLEDGMENTS

We would like to thank Mr. Witoon Sae-Ngow (SUSTREC) for SEM and Thailand Research Funds (grant number MRG4680120) for financial support.

ประชุมวิชาการระดับนานาชาติ

Sriamornsak P, Thirawong N and Puttipipatkhachorn S. Oil-entrapped calcium pectinate gel beads capable of floating on the gastric fluid – effect of some additives on release behaviour of metronidazole. ***Proceedings of the 20th Asian Congress of Pharmaceutical Sciences*** **2004; 20: IP-P-22. [Bangkok, 30 November - 3 December 2004]**

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ABSTRACTS

The 20th Congress of Federation of Asian Pharmaceutical Associations (FAPA)

November 30 - December 3, 2004
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The Pharmaceutical Association of Thailand under Royal Patronage

IP-P-22

OIL-ENTRAPPED CALCIUM PECTINATE GEL BEADS CAPABLE OF FLOATING ON THE GASTRIC FLUID – EFFECT OF SOME ADDITIVES ON RELEASE BEHAVIOUR OF METRONIDAZOLE

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Oil-entrapped calcium pectinate gel (CaPG) beads capable of floating in the gastric condition were developed using an emulsion-gelation method. The effect of various release modification methods on metronidazole release was also investigated. The metronidazole-loaded CaPG beads were found to float on simulated gastric fluid. Increasing the drug to pectin ratio in the beads decreased the release rate of the drug from either CaPG or oil-entrapped CaPG beads. However, the drug release from these beads was rapid, i.e., about 80% of drug loading released within 60 minutes. The attempts to modify the drug release were made by adding some additives into starting solution prior to bead formation, hardening with glutaraldehyde, or coating with polymer. The results demonstrated that addition of some additives (e.g., PEG 10000, glyceryl monostearate and Eudragit® L) insignificantly changed the release profiles while using 2% glutaraldehyde as a hardening agent prolonged the drug release about 2-fold. Coating the wet beads with Eudragit® RL significantly sustained the drug release and the beads still floated. The results suggested that oil-entrapped CaPG beads were promising as a carrier for intragastric floating drug delivery and their release behaviour could be modified by hardening with glutaraldehyde or coating with Eudragit® RL.

E-Proceeding of

20th FAPA Congress 2004, Thailand

Bangkok Convention Center (BCC), Bangkok

30 November - 3 December 2004

"Emerging Science and Profession in Pharmacy"

Songsak Srianujata, Ph.D.

Editor

Thanks to DSM Nutritional Products/Rovithai Ltd.

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ABSTRACT

Oil-entrapped calcium pectinate gel (OCP) beads capable of floating in the gastric condition were developed using an emulsion-gelation method. The effect of various release modification methods on metronidazole (MZ) release was also investigated. The MZ-loaded OCP beads were found to float on simulated gastric fluid. Increasing the drug to pectin ratio in the beads decreased the release rate of the drug from OCP beads. However, the drug release from these beads was rapid, i.e., about 80% of drug loading released within 60 min. The attempts to modify the drug release were made by adding some additives into starting solution prior to bead formation, hardening with glutaraldehyde, or coating with polymer. The results demonstrated that addition of some additives (e.g., polyethylene glycol, glyceryl monostearate and Eudragit® L) insignificantly changed the release profiles while using 2% glutaraldehyde as a hardening agent prolong the drug release about 2-fold. Coating the wet beads with Eudragit® RL significantly sustained the drug release and the beads still floated. The results suggested that OCP beads were promising as a carrier for intragastric floating drug delivery and their release behaviors could be modified by hardening with glutaraldehyde or coating with Eudragit® RL.

Keywords: oral drug delivery, pectin, gastro-retention, floating

INTRODUCTION

Oral administration is always the preferred means of drug delivery to the systemic circulation. Many attempts have been made to develop sustained release preparations with extended clinical effects and reduced dosing frequency. A problem frequently encountered with conventional sustained release dosage forms is the inability to increase their residence time in the stomach and proximal portion of the small intestine. Retention of drug delivery systems in the stomach prolongs the overall gastrointestinal transit time, thereby resulting in improved oral bioavailability of the basic drugs that have poor solubility in higher pH, and of drugs susceptible to circadian variations [1]. These systems are also appropriate for drugs which 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 [2]. Various approaches, such as bioadhesive delivery systems [3], density-controlled delivery systems [4], and floating dosage forms [5-6], have been tried as a means to retain the delivery system in the stomach with a view to increasing the gastric residence time.

Floating dosage forms can be made by a gelling process of hydrocolloid materials, or by incorporating a vacuum or gas-filled floatation chamber [7]. The most commonly used excipients are gel-forming or highly swellable cellulose type hydrocolloids, polysaccharides, and matrix forming polymers such as polycarbonate, polyacrylate and polystyrene [5]. A highly porous system as a carrier for intragastric floating drug delivery, e.g. hollow microspheres or microballoons [8], hydrophobic polypropylene foam powder with low density [9], coated calcium alginate beads containing air compartment [10] has been developed. Floating properties of dosage form can also be fabricated using oils, e.g., tablets composed of mineral oil-entrapped agar for controlled drug release [11].

The polysaccharide pectin is an inexpensive, non-toxic 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 is capable of reducing interfacial tension between an oil phase and a water phase and can be effective in the preparation of emulsion [13]. Pectin has a very complex structure which depends on both its source and the extraction process. Numerous studies have contributed to our understanding of 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 which 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 a degree of esterification less than 50%, can form rigid gels by the action of calcium ions or multivalent cations, which crosslink the galacturonic acid chains. Calcium pectinate hydrogels are stable in low pH solution, and are being investigated as a potential carrier material for different controlled release systems. In recent years, calcium pectinate gel (CP) beads have been developed as a unique vehicle for drug delivery. The CP 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]. We recently investigated the morphology and floating properties of oil-entrapped calcium pectinate gel (OCP) beads [18].

In this study, the drug-loaded OCP beads using selected oils were developed for floating drug delivery system, based on our previous report [18] and the drug release behavior of OCP beads capable of floating in the gastric fluid was studied. The effect of various release modification methods on drug release including; the use of additives in the starting solution prior to bead formation, hardening with glutaraldehyde, and coating with polymer, was also investigated.

MATERIALS AND METHODS

2.1 Materials

Low methoxy pectin with a degree of esterification of 28% (GENU pectin type LM-104 AS-FS) was the generous gift of CP Kelco (Denmark). Eudragit® L100 and Eudragit® RL100 (Röhm Pharma, Germany), glyceryl monostearate (GMS), metronidazole (MZ) (P.C. Drug Center, Thailand), calcium chloride, polyethylene glycol 10000 (PEG) (E. Merck, Germany) and glutaraldehyde (Fluka Chemie, Switzerland) were used as received. Light mineral oil, olive oil and peppermint oil and all other chemicals were standard pharmaceutical grade.

2.2 Preparation of conventional CP beads, OCP beads, and modified OCP beads

2.2.1 Conventional CP beads

Conventional CP beads were prepared by the ionotropic gelation method that was previously described [15-16]. Briefly, five grams of pectin were dispersed in water with agitation to make a 100-g solution. Various amounts of MZ (200-mesh sieved were dispersed in pectin solution to make different pectin to drug ratios (i.e., 1:1, 1:1.5 and 1:2 by weight). The dispersion was then extruded using a needle of 0.80-mm inner diameter into 0.34 M calcium chloride with gentle agitation at room temperature. The gel beads formed were allowed to stand in the solution for 20 min before being separated and washed with distilled water. The beads were dried at 37°C for 12 h.

2.2.2 OCP beads

The OCP beads were prepared by emulsion-gelation method [18]. Five grams of pectin were dissolved in water with agitation. Different amounts (i.e., 10, 20, 30 g) of olive oil, light mineral oil or peppermint oil were added to the solution and homogenized in high speed homogenizer to make 100-g emulsions. Various amounts of MZ were dispersed in an emulsion of oil and pectin mixture. The OCP beads were treated in the same manner as conventional CP beads. In some cases, the OCP beads were treated in 2%v/v glutaraldehyde for 2 h prior to washing and drying.

2.2.3 Additive-added OCP beads

Different amounts of GMS, PEG, or Eudragit® L100 were dispersed in the homogenized emulsion mixture of pectin, oil and MZ (additive to pectin ratio was 0.25:1, 0.5:1 or 1:1 by weight) and mixed until the homogenous mixture was obtained. The mixture was extruded into 0.34 M calcium chloride with gentle agitation at room temperature. The additive-added OCP beads formed were allowed to stand in the solution for 20 min and treated in the same manner as CP beads.

2.2.4 Polymer-coated OCP beads

The coating on drug-loaded OCP beads was performed by the air suspension method. The coating solution (8%w/v) was prepared by dissolving Eudragit® RL100 in absolute alcohol to make a 100-mL

solution. The coating solution was sprayed at a rate of 0.2 g/min until a 16% theoretical weight gain was achieved. The coated beads were then collected and dried in a hot air oven at 50°C for 12 h.

2.3 Study of morphology of gel beads

Morphological examination of the surface and internal structure of the dried beads was carried out using a scanning electron microscope (Model Maxim-2000, CamScan Analytical, England) at an accelerating voltage of 15 keV. The internal structure of the beads was examined by cutting them in half with a steel blade.

2.4 Buoyancy of gel beads

The gel bead samples (n=20) were steeped in 50 mL of simulated gastric fluid USP without pepsin (SGF) test solution and their buoyancy was observed for 24 h. The preparation was considered to have buoyancy in the test solution only when all of the gel beads floated in it [2].

2.5 Determination of drug release

The in-vitro release of MZ from the different formulations was examined using a USP dissolution apparatus 1 (Erweka, Germany) with 1000 ml of SGF (pH 1.2) and the basket rotation at 100 rpm. The temperature was controlled at 37±0.1°C. Samples were taken at appropriate time intervals and assayed spectrophotometrically at 277 nm. All dissolution runs were performed in triplicate.

3. RESULTS AND DISCUSSION

3.1 Preparation and morphology of gel beads

An aqueous solution of pectin was extruded into calcium chloride solutions and gel beads were formed instantaneously by ionotropic gelation [15] in which intermolecular cross-links were formed between the divalent calcium ions and the negatively charged carboxyl groups of the low methoxyl pectin molecules. The conventional CP beads were easily manufactured without any sophisticated equipment [15]. The MZ-loaded CP beads can also be prepared by the same method but for this formulation MZ was dispersed in pectin solution prior to bead formation. The external surface of gel beads was rough and its shape remained spherical after the drying process. The MZ particles, homogenously dispersed in the calcium pectinate network.

Pectin helped to emulsify the mixture of water and oil phases during the homogenization process. The emulsion stabilization property of pectin can be explained by its surface active ability to reduce the interfacial tension between an oil phase and a water phase [13], or steric and mechanical stabilization mechanisms similar to other polysaccharides such as cellulose, guar gum and locust bean gum [19]. When emulsion was formed, the MZ was added and properly mixed. The mixture was then extruded into calcium chloride solution and the gel formed by the action of calcium cross-linking to the negative charged groups of the pectin chain [18]. By this emulsion-gelation technique, the MZ-loaded OCP beads were formed. Figure 1 shows external and internal morphology of OCP beads containing 30%w/w olive oil. They show the oil droplets uniformly distributed. The OCP beads were spherical and exhibited a smoother surface than conventional CP beads (data not shown). Figure 2 shows external and internal morphology of MZ-loaded OCP beads containing 30%w/w peppermint oil. The beads were spherical in shape with a slightly rougher surface than those containing olive oil. The internal structure of an MZ-loaded OCP bead containing 30%w/w peppermint oil demonstrates the sponge-like nature of the structure even though the beads were dried in the hot-air oven. It also shows that there was no oil droplet under the gold coating. This is due to the volatile property of peppermint oil, resulting in the pore formation during the drying process.

Figure 1. SEM photographs of (a and b) external and (c) internal structures of OCP beads containing olive oil (30%).

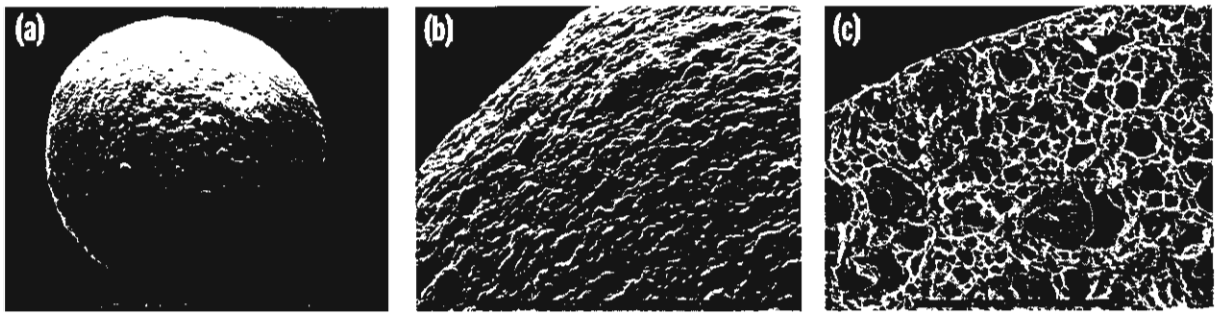

Figure 2. SEM photographs of (a and b) external and (c) internal structures of OCP beads containing peppermint oil (30%).

3.2 Buoyancy of gel beads

Our previous study [18] showed that the conventional CP beads (with no drug) made of low methoxy pectin did not float in SGF. In contrast, if a sufficient amount of oil was added (i.e. 10% mineral oil, 20% olive oil, and 20% peppermint oil), the OCP beads floated immediately and remained floating for 24 h. The results appeared to be linked to their relative density, i.e. 0.84 for light mineral oil, between 0.90 and 0.92 for peppermint oil and olive oil.

Different results were obtained when MZ was loaded in conventional CP beads, and OCP beads containing olive oil or mineral oil or peppermint oil. The MZ-loaded OCP beads containing mineral oil (10%), olive oil (20%), or peppermint oil (20%) did not float immediately. This is probably due to the density of the beads which increased when the drug was added. When drug was released from the MZ-loaded OCP beads, they gradually floated. As a result, the amount of oil required to keep the beads afloat was increased. The MZ-loaded OCP beads containing mineral oil (20%) or olive oil (30%) or peppermint oil (30%) floated immediately in SGF. The OCP beads treated with glutaraldehyde, additive-added OCP beads, and polymer-coated OCP beads floated in SGF.

3.3 In-vitro release of MZ from gel beads

Release studies were carried out, only when the beads floated in SGF, in order to examine the suitability of the OCP beads as an intragastric floating drug delivery system. MZ, used for *H. pylori* eradication, was used as a model drug. The drug release profiles were presented by plotting the amount of MZ released against time.

Figure 3 illustrates the release profiles of MZ-loaded conventional CP beads, and OCP beads containing 20% and 30% olive oil. No lag time was observed in any of the formulations studied. The release of MZ from conventional CP beads was rapid; about 80% of MZ was released within 20-30 min, in any ratio of pectin to MZ. This is probably due to the fact that MZ dissolves quickly in water and diffuses through the calcium pectinate structure into the dissolution medium easily. The amount of MZ loaded did not significantly affect the release behavior. The OCP beads containing either 20% mineral oil or 30% olive oil produced slower drug release profiles, compared to conventional CP beads. It was likely that the oil, which dispersed in the structure of OCP beads, obstructed the dissolution channel of MZ. Consequently, the drug release was prolonged; about 80% of MZ was released within 40-80 min. Since the drug release was still quick and was not harmonious for floating drug delivery, modification of the OCP bead formulations was needed. Some treatments were done or some additives were added in the formulations in order to extend the drug release.

Figure 4 shows the effect of the hardening agent, glutaraldehyde, on MZ release from OCP beads containing various oils. The MZ release from OCP beads containing 30% olive oil, soaking in 2% glutaraldehyde for 12 h, was prolonged about 2-fold. About 80% of drug release was achieved within 120 min, compared to about 50 min for non-hardened OCP beads. However, the release of MZ from OCP beads containing peppermint oil was not prolonged when soaking in glutaraldehyde and showed similar results to that of non-soaked OCP beads. The porous structure of these beads resulting from the volatility of the oil may have contributed to these insignificant differences in drug release.

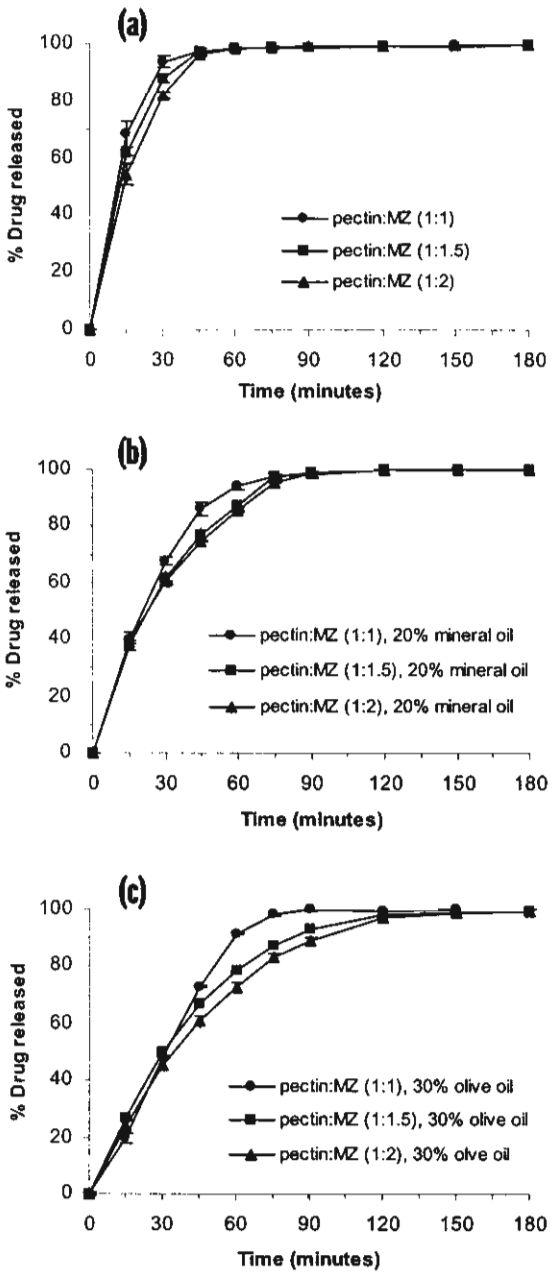

Figure 3. Effect of pectin to drug ratio on MZ release from (a) conventional CP beads, and OCP beads containing (b) 20% mineral oil and (c) 30% olive oil.

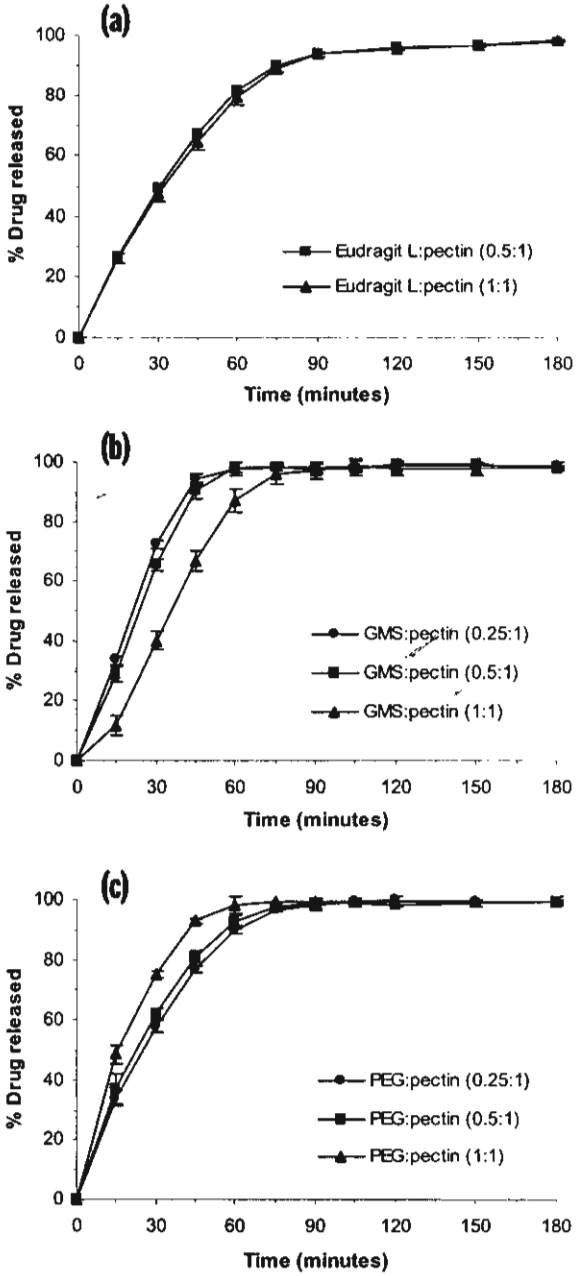

Figure 5. Effect of additive to pectin ratio on MZ release from additive-added OCP beads using (a) Eudragit L, (b) GMS, and (c) PEG.

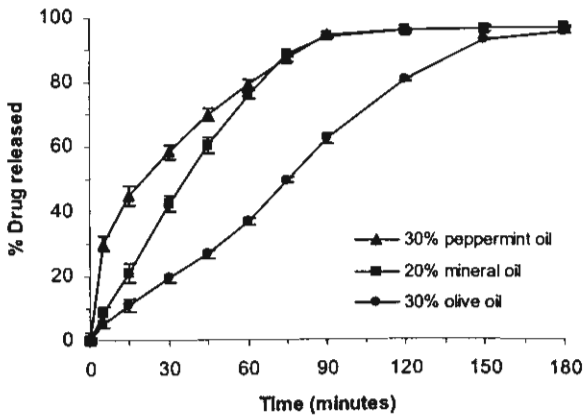

Figure 4. Effect of hardening agent, 2% glutaraldehyde for 2 h, on MZ release from OCP beads containing various oils

Figure 5 shows the effect of additive to pectin ratio on MZ release from the additive-added OCP beads using Eudragit® L, GMS, and PEG. The drug release from OCP beads was slightly slower when Eudragit® L was added into the beads containing 30% olive oil. Nevertheless, the release from OCP beads was not significantly different when the amount of Eudragit® L was increased (Figure 5a). This may be due to the Eudragit® L which only dispersed in the OCP structure and did not interact with calcium ion or carboxylic group of pectin. Thus, it did not help to strengthen the network structure. GMS, a hydrophobic substance, was added to the starting solution prior to bead formation to delay the drug release. The drug release was slower when the GMS to pectin ratio was increased (Figure 5b). Only the highest GMS to pectin ratio studied (1:1) had a significant effect on the release of MZ, although about 80% of drug loading was released within 50 min. Adding PEG into the formulation, on the other hand, gave the opposite results. When PEG to pectin ratio was increased, the drug release from the OCP beads was faster (Figure 5c). It is likely that the PEG, a hydrophilic substance, enhances water absorption into the bead, and thus the dissolution of MZ can occur easily. The drug release from all formulation of modified OCP beads using PEG was not dramatically extended.

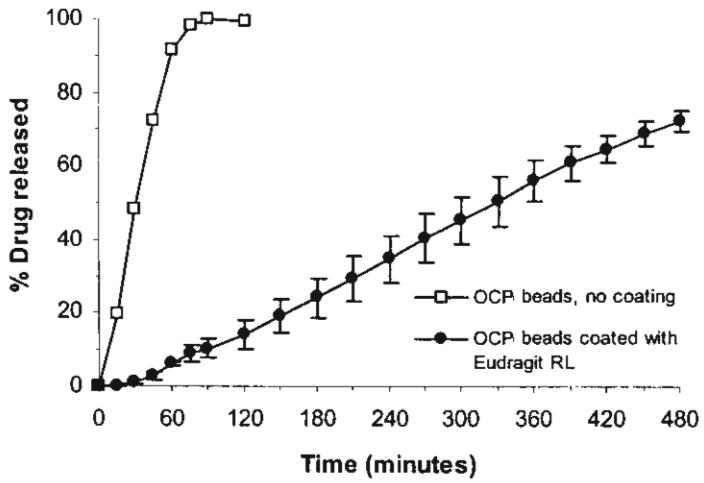

Figure 6. Effect of coating with Eudragit RL on MZ release from the OCP beads containing 30% olive oil.

Figure 6 illustrates the effect of coating with Eudragit® RL on MZ release from the OCP beads containing 30% olive oil. The amount of coating material was calculated as 0.16 g per 1 g of OCP beads (16% theoretical weight gain). Coating the OCP beads with Eudragit® RL slowed the release of MZ, compared to uncoated OCP beads. After a lag time period, the release from coated beads is essentially constant; about 80% of drug loading were released at 8 h. It is generally known that the mode of drug release from beads or pellets coated with a water-insoluble membrane is penetration of liquid into the beads, dissolution of the drug to form a saturated solution (as long as undissolved drug is present), partitioning of drug into the polymeric membrane and diffusion of drug through the membrane.

4. CONCLUSION

The OCP beads were designed and prepared by emulsion-gelation method for use in floating drug delivery systems. MZ was used as a model drug for intragastric floating drug delivery system in order to *Helicobacter pylori* eradication in the treatment of peptic ulcer disease. The MZ released rapidly from conventional CP beads, and no significant change was seen with the different amounts of MZ loaded. The MZ-loaded OCP beads floated in an acidic environment of gastric fluid and the drug release from these beads was slightly slower than that from conventional CP beads. Modified OCP beads with Eudragit® L, GMS or PEG as an additive, could not significantly prolong the drug release. However, the treatment with glutaraldehyde effected a slight reduction in the drug release. The drug release was dramatically prolonged by coating the OCP beads with Eudragit® RL.

These systems can float in the gastric condition and can control the drug release from the OCP beads. The enhanced buoyancy property of OCP beads makes them an excellent candidate for intragastric floating drug delivery systems. Moreover, this system may also provide a suitable means of delivery for drugs that are locally active to the gastric mucosa in the stomach.

ACKNOWLEDGMENTS

This work was financially supported by the Thailand Research Funds (grant number MRG4680120). The authors gratefully acknowledge Mr Witoon Sae-Ngow from SUSTREC (Silpakorn University Scientific and Technological Research Equipment Centre) for technical assistance on SEM imaging. Thanks also go to Food & Cosmetic System Co., Ltd. (Thailand) for supplying pectin samples.

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ประชุมวิชาการในประเทศ

Sriamornsak P, Thirawong N and Puttipipatkhachorn S. Evaluation of oil-entrapped calcium pectinate gel spheres as gastro-retentive drug delivery system. The RGJ Seminar Series XXIV: Pharmaceutics and Pharmaceutical Technology, Bangkok, 15-16 October 2003.

RGJ Seminar Series XXIV:
Pharmaceutics and Pharmaceutical Technology

จัดโดย

Thailand Pharmaceutics Education Network (TPEN)

ร่วมกับ โครงการปริญญาเอกกาญจนาภิเษก

วันที่ 15 –16 ตุลาคม พ.ศ. 2546

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P-2

Evaluation of Oil-entrapped Calcium Pectinate Gel Spheres as Gastro-retentive Drug Delivery System

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Objective

A novel gastro-retentive delivery system of oil-entrapped calcium pectinate gel spheres was designed and tested in order to overcome the complication of limited gastric residence times of oral sustained drug delivery system.

Methods

The calcium pectinate gel spheres containing edible oil were prepared by either gently mixed or homogenized an oil phase and a water phase containing pectin. The mixtures were then extruded into calcium chloride solution with gentle agitation at room temperature. The gel spheres formed were then separated, washed with distilled water and dried at 37°C for 12 hours. The model drug, metronidazole, was used in this study. The effect of selected factors, such as type of oil, percentage of oil, type of pectin and pectin to drug ratio, on floating and drug release properties was investigated.

Results

In this study, the gel spheres were formed instantaneously by ionotropic gelation in which intermolecular cross-links were formed between the divalent calcium ions and the negatively charged carboxyl groups of the pectin molecules. The gel spheres were easily manufactured without any sophisticated equipment. The oil-entrapped calcium pectinate gel spheres floated if the sufficient amount of oils were used. As percentage of oil increased, the floating time prolonged and the drug released slower. The gel spheres prepared from different types of pectin showed similar floating and drug release behavior. The increased drug to pectin ratio slightly influenced the drug release patterns.

Conclusion

The results of these studies indicate that type and percentage of oil is more important than other factors studied. The enhanced buoyancy and prolonged release properties of oil-entrapped calcium pectinate gel spheres make them an excellent candidate for floating drug delivery system. These properties are applicable not only to the sustained release of drugs but also to the targeting of the gastric mucosa.

Key words: calcium pectinate; oil-entrapped; floating; gastro-retentive; drug delivery

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Sriamornsak P, Thirawong N and Puttipipatkhachorn S. A novel floating emulsion gel sphere for stomach-specific drug delivery. Thai Journal of Pharmaceutical Sciences 2003; 27(supp): 21. (การประชุมเสนอผลงานวิจัยทางเภสัชศาสตร์ ครั้งที่ 20, วันที่ 1 ธันวาคม 2546, คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย)

ไทยเภสัชสาร

The Thai Journal of Pharmaceutical Sciences
Volume 27 Supplement Issue December 2003

กำหนดการและบทคัดย่อ

การประชุมเสนอผลงานวิจัยทางเภสัชศาสตร์ ครั้งที่ 20

1 ธันวาคม 2546

Scientific Program and Abstract Supplement
The 20th Annual Research Meeting in Pharmaceutical Sciences
December 1, 2003

ISSN 0125-4685

A NOVEL FLOATING EMULSION GEL SPHERE FOR STOMACH-SPECIFIC DRUG DELIVERY

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A novel method to prepare emulsion gel spheres of calcium pectinate capable of floating in the gastric condition was designed and tested. The gel spheres containing edible oil were prepared by either gently mixed or homogenized an oil phase and a water phase containing pectin. The mixtures were then extruded into calcium chloride solution with gentle agitation at room temperature. The gel spheres formed were then separated, washed with distilled water and dried at 37°C for 12 hours. The model drug, metronidazole, was used in this study. The effect of selected factors, such as type of oil and percentage of oil, on floating and drug release properties was investigated. The oil-entrapped calcium pectinate gel spheres floated if the sufficient amount of oils were used. As percentage of oil increased, the floating time prolonged and the drug released slower. The results of these studies indicate that type and percentage of oil is important to control the floating and drug release from calcium pectinate gel spheres.

ประชุมวิชาการในประเทศ

[นำเสนอผลงานวิจัยเรื่องนี้ เป็นส่วนหนึ่งของการบรรยาย - Invited Lecture]

Sriamornsak P. Use of citrus pectin in pharmaceutical production and drug delivery. *Proceedings of the 30th Congress on Science and Technology of Thailand* 2004; 30: 31.

บทคัดย่อ
ABSTRACTS

การประชุมวิชาการ
วิทยาศาสตร์และเทคโนโลยีแห่งประเทศไทย ครั้งที่ 30

19-21 ตุลาคม 2547

ณ ศูนย์แสดงสินค้าและการประชุมอิมแพ็ค เมืองทองธานี

30th Congress on Science and Technology of Thailand

19-21 October 2004

Impact Exhibition and Convention Center , Muang Thong Thani

สมาคมวิทยาศาสตร์แห่งประเทศไทยในพระบรมราชูปถัมภ์
THE SCIENCE SOCIETY OF THAILAND
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คณะวิทยาศาสตร์ มหาวิทยาลัยศรีนครินทรวิโรฒ
FACULTY OF SCIENCE
SRINAKARINWIROT UNIVERSITY

USE OF CITRUS PECTIN IN PHARMACEUTICAL PRODUCTION AND DRUG DELIVERY

พรศักดิ์ ศรีอมรศักดิ์

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บทคัดย่อ: เพคตินเป็นพอลิเมอร์จากธรรมชาติที่ผลิตได้จากกากผลไม้ เช่น เปลือกส้ม ซึ่งเป็นของเหลือทิ้งจากอุตสาหกรรมเกษตร มีการนำเพคตินมาใช้ในอุตสาหกรรมอาหารและเครื่องดื่มมายาวนาน ปัจจุบันมีการนำมาใช้ในอุตสาหกรรมทางเทคโนโลยีชีวภาพ ในเชิงเภสัชกรรมและการผลิตยาเพิ่มมากขึ้นเรื่อยๆ เพคตินมีคุณสมบัติเฉพาะที่ทำให้สามารถนำมาใช้เก็บกักหรือนำส่งยา โปรตีนหรือเปปไทด์ และเซลล์ บทความนี้ได้กล่าวถึงตัวอย่างงานวิจัยที่ใช้ประโยชน์เพคตินจากเปลือกส้มในการผลิตยาและเพื่อเป็นระบบนำส่งยา ที่มีการทำวิจัยในประเทศไทย เช่น การออกแบบยาเม็ดโดยใช้เพคตินเป็นสารก่อเจล เพื่อชะลอการปลดปล่อยตัวยาที่ให้โดยการรับประทาน การพัฒนาระบบยาเม็ดเคลือบฟิล์มชนิดคอมพอสิตระหว่างเพคตินกับพอลิเมอร์สังเคราะห์ที่ไม่ละลายน้ำเพื่อใช้ในการควบคุมการปลดปล่อยยาจากระบบนำส่งยาแบบนำวิถีไปยังอวัยวะเป้าหมายที่ลำไส้ใหญ่ การพัฒนาเทคโนโลยีการเคลือบฟิล์มยาเม็ดโดยอาศัยหลักการเกิดปฏิกิริยาทางเคมีที่ผิวประจันของเม็ดยากับเพคตินเกิดเป็นสารประกอบเชิงซ้อนเพื่อใช้ในการควบคุมการปลดปล่อยตัวยาออกจากเม็ดยา การออกแบบระบบเจลบีดเพื่อเก็บกักยาหรือยาโปรตีนสำหรับใช้ในการนำส่งยาที่ให้โดยการรับประทาน โดยอาศัยหลักการเกิดเจลระหว่างประจุ การพัฒนาเทคโนโลยีการควบคุมการปลดปล่อยยาจากเม็ดเจลบีดชนิดที่สามารถลอยตัวได้เพื่อให้ระบบคงอยู่ในกระเพาะอาหารนานขึ้นเพื่อใช้สำหรับยาที่ต้องการให้ออกฤทธิ์เฉพาะที่ในกระเพาะอาหาร การศึกษาโครงสร้างภายในของเม็ดเจลบีดโดยใช้เทคนิคการเกิดภาพของอิเล็กตรอนชนิดกระจายกลับ เป็นต้น เนื่องจากงานวิจัยและพัฒนาทางด้านเภสัชกรรมและระบบนำส่งยาที่ใช้เพคตินยังคงมีการดำเนินการอย่างต่อเนื่อง จึงคาดหมายได้ว่าจะมีนวัตกรรมและการประยุกต์ใช้ที่น่าสนใจในอนาคต

Abstract: Pectin, a naturally occurring polysaccharide, is almost exclusively derived from citrus peel, by-product from agro-industry. It has been used successfully for many years in the food and beverage industry. Pectin is finding increasing applications in the biotechnology and pharmaceutical industry. Pectin also has several unique properties that have enabled it to be used as a matrix for the entrapment and/or delivery of a variety of drugs, proteins and cells. This paper reviews the research works on the pharmaceutical (including drug delivery) application of citrus pectin those have been done by research groups in Thailand. The research works include the tablet design using pectin as a gel forming agent to prolong the drug release; pectin-Eudragit® composite film coating for colon-specific drug delivery; development of a new method of using pectin gel as a coating to avoid the use of organic liquids or elevated temperature to dry the coats. The comprehensive evaluation of the properties of calcium pectinate gel beads that may be used as a delivery system for drugs or therapeutic proteins has also been reported. The possibility of using calcium pectinate gel beads containing edible oils or carbonate salts as an intragastric floating drug delivery system has been evaluated. The extensive works on back-scattered electron imaging revealed the microscopic structure of the gel beads and the drug distribution through the matrix structure of gel beads. As research and development continues with delivery system using pectin, we expect to see many innovative and exciting applications in the future.

ประชุมวิชาการในประเทศ

Sriamornsak P, Thirawong N and Puttipipatkhachorn S. Oil-entrapped calcium pectinate gel beads as a gastroretentive drug delivery system. การประชุมนักวิจัยรุ่นใหม่พบเมธีวิจัยอาวุโส สกว., กาญจนบุรี, 14-16 มกราคม 2548.

ABSTRACTS

การประชุม

นักวิจัยรุ่นใหม่ ... พบ ... เมธีวิจัยอาวุโส สกว.

14 - 16 มกราคม 2548

โรงแรมเฟลิกซ์ ริเวอร์แคว กาญจนบุรี

สนับสนุนโดย

สำนักงานคณะกรรมการการอุดมศึกษา (สกอ.)

กระทรวงศึกษาธิการ

และ

สำนักงานกองทุนสนับสนุนการวิจัย (สกว.)

Oil-entrapped calcium pectinate gel beads as a gastroretentive drug delivery system

Pornsak Sriamornsak [a,*], Nartaya Thirawong [a] and Satit Puttipipatkhachorn [b]

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Abstract—Oral sustained drug delivery system is complicated by limited gastric residence times. Rapid gastrointestinal transit can prevent complete drug release in the absorption zone and reduce the efficacy of administered dose in the stomach and upper small intestine. To overcome these limitations, a novel gastroretentive delivery system of oil-entrapped calcium pectinate gel beads was designed and tested. The calcium pectinate gel beads containing edible oil were prepared by either gently mixed or homogenized an oil phase and a water phase containing pectin. The mixtures were 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 emulsion-gelation process to illustrate the formation of oil-entrapped calcium pectinate gel beads was proposed. Metronidazole was used as a model drug in this study. The effect of selected factors, such as type of oil, percentage of oil, type of pectin and pectin to drug ratio, on morphology, floating and drug release properties was investigated. The oil-entrapped calcium pectinate gel beads floated if the sufficient amount of oils was used. Scanning electron micrographs demonstrated very small pores, range between 5 and 40 μm, dispersed all over the beads. The type and percentage of oil play an important role to control the floating of oil-entrapped calcium pectinate gel beads. As percentage of oil increased, the floating time prolonged and the drug released slower. The gel beads prepared from different types of pectin showed similar floating and drug release behavior. The increased drug to pectin ratio slightly influenced the drug release patterns. The results of these studies indicate that type and percentage of oil is more important than other factors studied. The enhanced buoyancy of oil-entrapped gel beads makes them an excellent candidate for intragastric floating or gastroretentive drug delivery system. These properties are applicable not only to the sustained release of drugs but also to the targeting to the gastric mucosa. The floating gel beads appear to be a promising vehicle for delivering such preparations specifically to the region. And it will play an important role in therapy of diseases in which a stomach-specific drug delivery regimen should be considered.

Keywords—Gastroretention; Drug delivery system; Pectin; Calcium pectinate; Beads; Edible oil; Oil-entrapped beads

Output

1. Sriamornsak, P., Thirawong, N. and Puttipipatkhachorn, S. *Journal SPAA*, 2004. in press.

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