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# Use of pectin as a carrier for intragastric floating drug delivery: Carbonate salt contained beads ☆

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**Abstract**

Pectin has been investigated as a carrier for an intragastric floating drug delivery by a means of calcium pectinate gel (CaPG) beads. The CaPG beads containing carbonate salt, as a gas-forming agent, were prepared by dispersing carbonate salt in pectin solution and then extruding into either neutral or acidified solution of calcium chloride. The effect of selected factors, such as type of carbonates, percentage of carbonates, degree of methylesterification (DE) of pectin, type of gelation medium, drug loading and drying method, on morphology, floating and release properties was investigated. Incorporation of sodium bicarbonate into pectin solution resulted in porous structured beads. Acidity of gelation medium increased the pores in the structure of beads containing calcium carbonate. This is due to carbon dioxide generated from reaction of carbonate salts with acid. Drug release from CaPG beads is clearly dependent upon the formulation and processing variables studied. It is obvious that the highly porous of the freeze-dried beads showed a good floating ability with fast drug release. The drug release could be prolonged by using pectin with lower DE, 10% calcium carbonate, acidified gelation medium, and high drug loading. However, their floating ability seemed to be decreased. It is suggested that the optimization of formulation and processing variables is further needed to obtain a good floating ability and a prolonged drug release.


*Keywords:* Pectin; Calcium pectinate; Carbonate salts; Gel beads; Floating; Intragastric drug delivery; Gastroretention

## 1. Introduction

Pectin is an inexpensive, non-toxic polysaccharide extracted from citrus peels or apple pomaces, and has been used as a food additive, a thickening agent and a gelling agent (May, 1990; Rolin, 1993). Pectin is widely used in traditional therapy of irritated mucous membranes in the respiratory tract, chronic bronchitis, dry cough and other irritations of the buccal region (Smart, Kellaway, & Worthington, 1984). It also has bioadhesive properties towards other gastrointestinal tissues (Schmidgall & Hensel, 2002; Sriamornsak, Thirawong, Nunthanid, & Puttipipatkhachorn, 2006), which can be used as a drug delivery device on a specific site for targeted release and optimal drug delivery due to intimacy and duration of contact. This may be the starting point for new considerations in development of gastrointestinal-specific drug delivery systems.

Pectin has a very complex structure which depends on both its source and the extraction process. Numerous studies contributed to elucidate the structure of pectin. Basically, it is a polymer of α-D-galacturonic acid with 1–4 linkages (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 & Voragen, 1996). The galacturonic acid of the backbone is partially methyl-esterified. Low

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methoxy pectin with 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 carrier material for different controlled release systems. In recent years, gel beads of calcium pectinate have been developed as a unique vehicle for drug delivery. The calcium pectinate gel (CaPG) beads have been used in various ways in the gastrointestinal tract, for example, for sustained release of drugs (Sriamornsak & Nunthanid, 1998, 1999), for targeting drugs to the colon (Sriamornsak, 1999).

Oral administration of drugs by means of controlled release delivery systems should ideally enable to obtain the required plasma levels and to keep them steady for a prolonged period of time. Unfortunately, this ideal therapeutic target cannot systemically be achieved (Rouge, Buri, & Doelker, 1996); this in spite of the progresses accomplished today in formulation and control of drug release kinetics from such type of dosage forms. The main limitations come from the variability of gastrointestinal transit time and from the non-uniformity of drug absorption throughout the gastrointestinal tract. For example, multiple-unit dosage forms (e.g., beads, pellets) are emptied from the stomach to the intestine within 30–60 min (Parr et al., 1990). These physiological limitations could be overcome by prolonging the gastric residence time of the dosage forms. A number of different means has been investigated to slow down the gastric emptying of a dosage form, e.g., more particularly, the use of floating dosage forms having a bulk density lower than that of gastric fluid (Moes, 1993; Singh & Kim, 2000).

Floating oral dosage forms are expected to remain buoyant in a lasting way upon the gastric contents and to consequently enhance the bioavailability of all drugs which are well-absorbed from the upper gastrointestinal tract. The lasting gastroretentive 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 site-specific therapeutic action, for example, antibiotic administration for *Helicobacter pylori* eradication in the treatment of peptic ulcer disease (Cooreman, Krausgrill, & Hengels, 1993). Moreover, the drugs that are less soluble in or are degraded by the alkaline pH of small intestine may benefit from prolonged gastric retention (Moes, 1993). Floating dosage forms can be made by a gelling process of hydrocolloid materials or by incorporating a floatation chamber, vacuum or gas filled (Chien, 1992). The most commonly used excipients are gel-forming or highly swellable cellulose type hydrocolloids, polysaccharides, and matrix forming polymers (Singh & Kim, 2000). A highly porous matrix based on hydrocolloid materials, as a carrier for gastroretentive floating drug delivery, e.g. calcium alginate beads containing air compartment or gas forming agents (Choi, Park, Hwang, & Park, 2002; Iannuccelli, Coppi, Bernabel, & Cameroni, 1998), oil-entrapped calcium pectinate gel beads (Sriamornsak, Thirawong, & Puttipipatkhachorn, 2004, 2005) has been developed.

One of disadvantages of floating dosage forms is that they require a sufficient high level of fluids in the stomach for the systems to float and to release drug locally in the stomach (Singh & Kim, 2000). This limitation can be overcome by coating the dosage form with bioadhesive polymers, thereby enabling them to adhere to the mucous membrane of the stomach wall (Chitnis, Malshe, & Lalla, 1991). Thus, an approach based on floating and bioadhesion was designed using pectin, which also has a bioadhesive property. Also, in this study, pectin has been used as a drug carrier in the form of the CaPG beads. The CaPG beads are multiple-unit systems which may be more advantageous than single-unit systems by avoiding "all-or-none" emptying from the stomach during migrating myoelectric complex (MMC) motility of the stomach. In this study, a floating system employing carbonate salts as gas-forming agents dispersed in a CaPG matrix was prepared, in order to target the drug, metronidazole (an antibiotic used for eradication of *H. pylori*), to stomach. The effect of selected formulation and processing factors, including type and amount of carbonates, degree of methylesterification of pectin, type of gelation medium and drying method on formation and physical characteristics of beads were investigated. Floating and *in vitro* drug release properties of the obtained beads were also studied.

## 2. Materials and methods

### 2.1. Materials

Low methoxy pectin with degree of methylesterification (DE) of 36% (GENUpectin type LM-101 AS) and one with DE of 28% (GENUpectin type LM-104 AS-FS) were the generous gift of CP Kelco (Denmark) and are referred to as LM-101 and LM-104, respectively. Sodium bicarbonate ($NaHCO_3$), sodium carbonate ($Na_2CO_3$), calcium carbonate ($CaCO_3$) and potassium carbonate ($K_2CO_3$) were purchased from Merck (Germany). Metronidazole and all other chemicals were standard pharmaceutical grade or analytical grade.

### 2.2. Preparation of blank CaPG beads

#### 2.2.1. Conventional CaPG beads

The conventional CaPG beads were prepared by ionotropic gelation method that was previously described (Sriamornsak & Nunthanid, 1998, 1999). Briefly, pectin (i.e., LM-101 and LM-104) was dissolved in water with agitation. The solutions (5% w/w) were extruded using a nozzle 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, separated and washed with distilled water. The beads were air-dried at 37 °C for 12 h or freeze-dried.

*2.2.2. CaPG beads containing carbonate salt*

The CaPG beads containing carbonate salt were prepared by dissolving or suspending carbonate salt (i.e., $NaHCO_3$, $CaCO_3$, $K_2CO_3$ or $Na_2CO_3$) in pectin solution. The mixture was extruded using a nozzle of 0.80-mm inner diameter into either neutral or acidified (containing 0.1 M hydrochloric acid (pH 1.2) or 10% v/v acetic acid (pH 3–4)) solution of 0.34 M calcium chloride with gentle agitation at room temperature. The CaPG beads formed were then separated, washed and air-dried at 37 °C for 12 h or freeze-dried.

*2.3. Preparation of drug-loaded CaPG beads*

The drug-loaded CaPG beads were prepared by suspending metronidazole (i.e. 2.5% and 5.0% w/w) in the pectin solution or mixture of pectin and carbonate salt, in order to make pectin to drug ratio of 1:0.5 and 1:1 w/w, respectively. The mixture was extruded into either neutral or acidified solution of calcium chloride and the beads formed were then treated in the same manner as blank CaPG beads.

*2.4. Study of particle size and morphology of CaPG beads*

The mean diameter of 50 dried beads was determined by optical microscopy (Model BH-2, Olympus, Japan). The microscope eyepiece was fitted with a micrometer by which the size of the beads could be determined. Analysis of variance (ANOVA) and Levene's test for homogeneity of variance were performed using SPSS version 10.0 for Windows (SPSS, USA). *Post hoc* testing ($p < 0.05$) of the multiple comparisons was performed by either the Scheffé or Games–Howell test depending on whether Levene's test was insignificant or significant, respectively.

Morphological examination of the surface structure of the dried beads were carried out using either a digital camera (Model S602Zoom, Fujifilm, Japan) equipped with Super-EBC Fuji Nonlens (6×) optical zoom or a scanning electron microscope (Model Maxim-2000, CamScan Analytical, England) equipped with back-scattered electron detector at an accelerating voltage of 25 keV. For examination of the internal structure of the beads, they were cut in half with a steel blade and then examined by a scanning electron microscope.

*2.5. Study of floating properties of gel beads*

Specific gravity of the test solution (water, 0.9% w/v sodium chloride) and simulated gastric fluid without pepsin (SGF, pH 1.2) previously measured using standard pycnometer was 1.007, 1.014 and 1.013, respectively. Floating properties of the gel beads was studied at $37 \pm 0.5$ °C by soaking 20 beads in 50 mL of each test solution. Each vessel was shaken at 100 rpm using Environmental Shaker – Incubator (Model ES-20, Biosan, Latvia). The percentage of floating samples was measured by visual observation. All the data were the average of at least three determinations.

*2.6. In vitro drug release studies*

Release studies were performed in triplicate using the USP basket method at 100 rpm and 37 °C in 1000 mL of test medium (i.e., SGF). Approximately 50 beads were used for each experiment. Samples were taken at appropriate

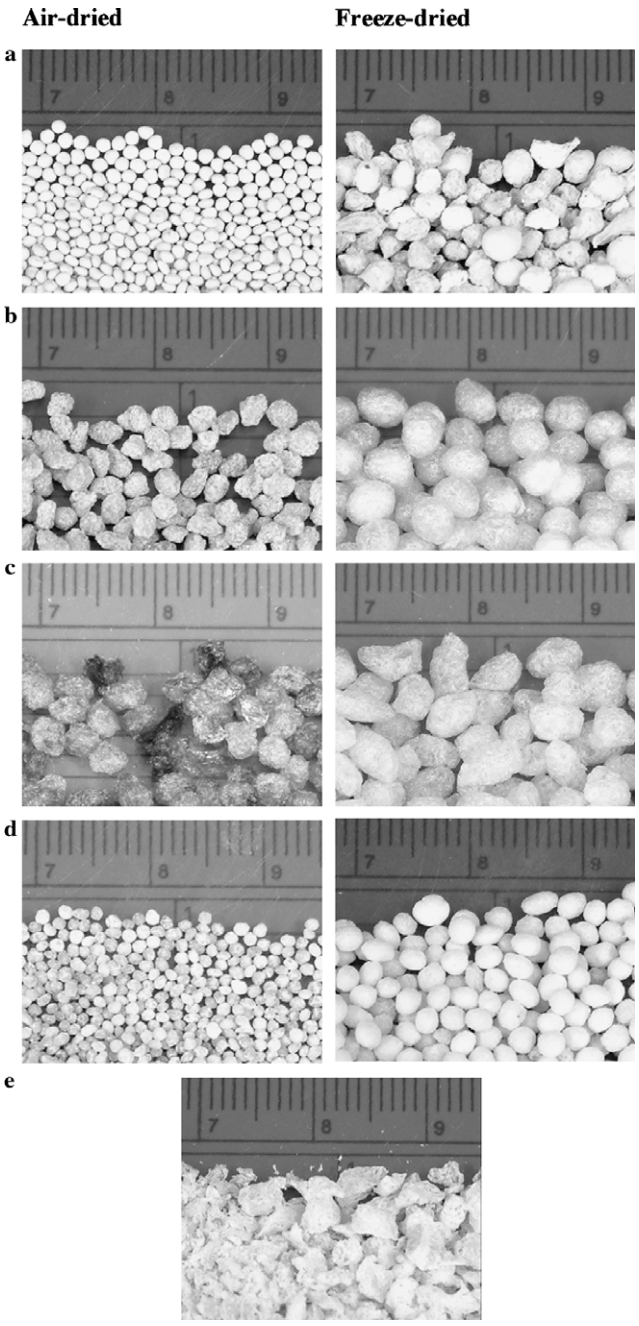


Fig. 1. Photo images of the air-dried (left column) and freeze-dried (right column) CaPG beads (using LM-104) containing 5% calcium carbonate gelled in (a) $CaCl_2$, (b) $CaCl_2$ + acetic acid, (c) $CaCl_2$ + HCl, those containing (d) 5% and (e) 10% sodium bicarbonate gelled in $CaCl_2$.

time intervals and assayed spectrophotometrically at 277 nm.

## 3. Results and discussion

### 3.1. Preparation of gel beads and their morphology

Various carbonate salts, i.e., $NaHCO_3$, $CaCO_3$, $K_2CO_3$ or $Na_2CO_3$, were incorporated into the pectin solution and the mixture was then extruded into a solution of cation (i.e., calcium chloride solution). The CaPG beads containing $NaHCO_3$ or $CaCO_3$ were produced 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 (Sriamornsak & Nunthanid, 1998). The gel beads were easily manufactured without any sophisticated equipment. The CaPG beads containing $K_2CO_3$ and $Na_2CO_3$, however, could not be produced as a viscous gel formed before extrusion through the needle.

Incorporation of $NaHCO_3$ (5% w/w) into pectin solution prior to bead formation resulted in porous-structured gel beads while the beads containing $CaCO_3$ showed the dense, non-porous structure if the beads were air-dried (Figs. 1 and 2). Using 10% w/w of $NaHCO_3$, the beads could not be formed (Fig. 1e) because the released gas burst the bead before the wall was sufficiently hardened. During the formation of the CaPG beads containing $CaCO_3$ using acidified gelation medium, carbonate salts are reacted with acid (acetic acid or hydrochloric acid) to produce carbon dioxide. The evolving gas permeates through the calcium pectinate structure leaving gas bubbles or pores (Figs. 1b and c), resulting in the highly porous and fragile beads. Most of the beads were predominantly spherical in appearance, although some were found to be elongated or irregular. The irregular

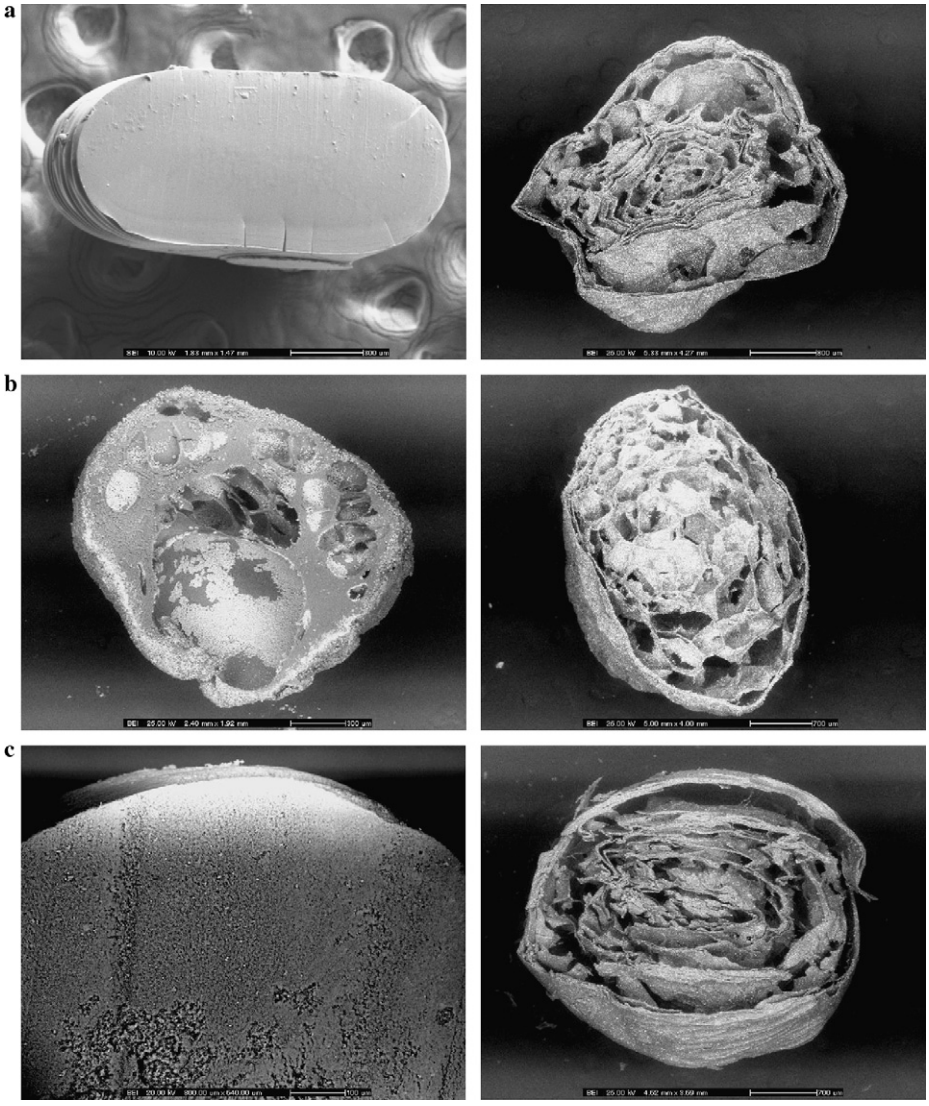


Fig. 2. SEM pictures of the air-dried (left column) and freeze-dried (right column) CaPG beads (using LM-104:MZ of 1:0.5 w/w) containing (a) no gas-forming agent, (b) 5% sodium bicarbonate, and (c) 10% calcium carbonate, gelled in $CaCl_2$.

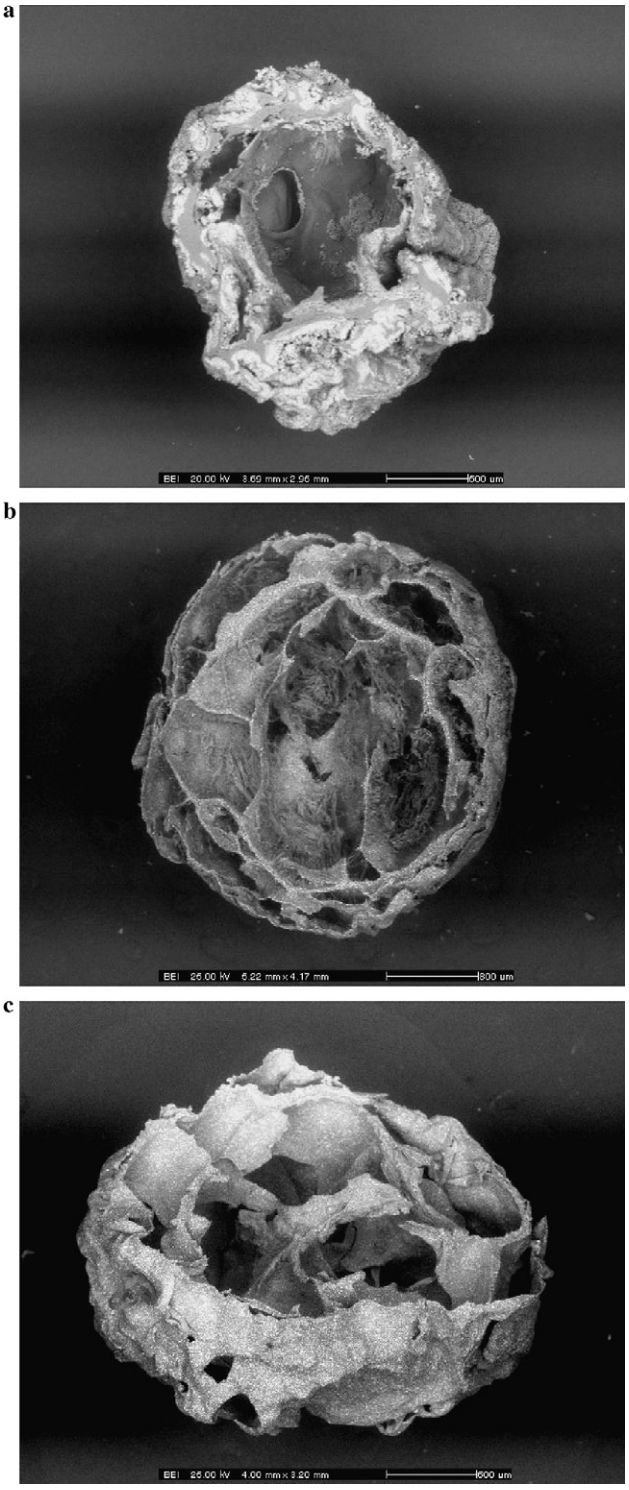


Fig. 3. SEM pictures of the internal structure of the CaPG beads (using LM-104) containing 10% calcium carbonate; (a) air-dried, gelled in $CaCl_2$ + acetic acid, (b) freeze-dried, gelled in $CaCl_2$ + acetic acid and (c) freeze-dried, gelled in $CaCl_2$ + HCl.

shape of the beads was observed after air-drying. This is due to the consequence of the shrinkage occurring during the drying process of the resultant beads. In many drying processes, migration of soluble solid or moisture to the peripheral layers of the solid as the solvent is removed,

Table 1
Mean diameter (mm ± SD) of dried CaPG beads containing different carbonate salts ($n = 50$)

| Formulation (carbonate salts/gelation medium) | LM-104 (DE28%) | | | | LM-101 (DE36%) | | | |
|---|---|---|---|---|---|---|---|---|
| | Air-dried | Freeze-dried | | | Air-dried | Freeze-dried | | |
| | No MZ[a] | No MZ | 1:0.5[b] | 1:1[b] | No MZ | No MZ | 1:0.5 | 1:1 |
| –/$CaCl_2$ (control) | 1.17 ± 0.09 | 1.58 ± 0.16 | 1.85 ± 0.11 | 1.99 ± 0.16 | 1.21 ± 0.08 | 1.58 ± 0.14 | 1.87 ± 0.18 | 2.01 ± 0.19 |
| 5% $NaHCO_3$/$CaCl_2$ | 1.26 ± 0.11 | 1.87 ± 0.17 | 1.94 ± 0.19 | 1.97 ± 0.11 | 1.49 ± 0.13 | 2.04 ± 0.16 | 1.59 ± 0.13 | 2.04 ± 0.10 |
| 5% $NaHCO_3$/$CaCl_2$ + acetic acid | Irregular | Irregular | N/A | N/A | Irregular | Irregular | N/A | N/A |
| 5% $NaHCO_3$/$CaCl_2$ + HCl | Irregular | Irregular | N/A | N/A | Irregular | Irregular | N/A | N/A |
| 10% $NaHCO_3$/$CaCl_2$ | Irregular | Irregular | N/A | N/A | Irregular | Irregular | N/A | N/A |
| 10% $NaHCO_3$/$CaCl_2$ + acetic acid | Irregular | Irregular | N/A | N/A | Irregular | Irregular | N/A | N/A |
| 10% $NaHCO_3$/$CaCl_2$ + HCl | Irregular | Irregular | N/A | N/A | Irregular | Irregular | N/A | N/A |
| 5% $CaCO_3$/$CaCl_2$ | 1.23 ± 0.07 | 1.60 ± 0.16 | 2.01 ± 0.17 | 2.03 ± 0.17 | 1.32 ± 0.11 | 1.70 ± 0.15 | 1.75 ± 0.11 | 1.80 ± 0.15 |
| 5% $CaCO_3$/$CaCl_2$ + acetic acid | 1.47 ± 0.10 | 1.91 ± 0.16 | 2.04 ± 0.17 | 2.05 ± 0.09 | 1.69 ± 0.18 | 2.09 ± 0.14 | 2.19 ± 0.18 | 2.23 ± 0.11 |
| 5% $CaCO_3$/$CaCl_2$ + HCl | 1.48 ± 0.07 | 2.04 ± 0.17 | 2.15 ± 0.18 | 2.18 ± 0.12 | 1.68 ± 0.07 | 2.07 ± 0.16 | 2.16 ± 0.07 | 2.25 ± 0.16 |
| 10% $CaCO_3$/$CaCl_2$ | 1.44 ± 0.06 | 1.73 ± 0.13 | 1.97 ± 0.19 | 2.01 ± 0.13 | 1.47 ± 0.11 | 1.56 ± 0.15 | 1.72 ± 0.13 | 1.83 ± 0.14 |
| 10% $CaCO_3$/$CaCl_2$ + acetic acid | 1.51 ± 0.12 | 2.11 ± 0.16 | 2.02 ± 0.07 | 2.13 ± 0.15 | 1.59 ± 0.14 | 2.27 ± 0.11 | 2.41 ± 0.16 | 2.47 ± 0.11 |
| 10% $CaCO_3$/$CaCl_2$ + HCl | 1.49 ± 0.06 | 2.02 ± 0.12 | 2.03 ± 0.12 | 2.12 ± 0.12 | 1.60 ± 0.17 | 2.30 ± 0.18 | 2.35 ± 0.15 | 2.42 ± 0.17 |

[a]MZ = metronidazole; [b]pectin:drug (MZ) ratio by weight.

is a common phenomenon. The use of freeze-drying should theoretically minimize this since water is removed by sublimation from the ice crystals and does not move as a liquid containing dissolved drug to the surface of the solid. The freeze-dried beads, therefore, can maintain the structure during the drying process, resulting in the more spherical beads.

Samples were taken from different formulations and operating conditions for SEM observation. The scanning electron micrographs of air-dried and freeze-dried CaPG beads containing no drug (with or without gas-forming agent) are shown in Figs. 2 and 3. The air-dried conventional beads (with no gas-forming agent) showed the smooth surface and dense structure (Fig. 2a), resulting from water evaporation. The freeze-dried conventional beads demonstrated the cabbage-like, multilayered structure, which was the result of the formation of calcium pectinate layer by layer at the interface. Incorporation of $NaHCO_3$ produced the porous-structured beads, regardless of air-drying or freeze-drying, as $NaHCO_3$ can dissociate in neutral medium and readily produce the $CO_2$ gas. The beads containing $CaCO_3$ gelled in different media showed different structures. The air-dried beads gelled in neutral medium illustrated the dense structure due to the insolubility of $CaCO_3$ in neutral medium while freeze-dried beads showed multilayered structure similar to those of conventional beads. In contrast, the $CaCO_3$-incorporated beads those gelled in acidified medium showed the highly porous structure regardless of drying methods. In acidic conditions, the $CaCO_3$ dissociates to release divalent calcium cations that contributed to more homogeneous CaPG bead formation and $CO_2$ gas that could be entrapped in the matrix gel beads. The porous nature of the $CaCO_3$-contained beads those gelled in acidified medium as shown by SEM (Fig. 3) may contribute to the floatation of the beads. The use of hydrochloric acid as acidifying agent reduced the pH of the medium to lower pH than that of acetic acid and might help to accelerate the dissociation rate of $CaCO_3$.

### 3.2. Particle size of gel beads

The mean diameter of dried CaPG beads containing different carbonate salts is shown in Table 1. Both $NaHCO_3$ and $CaCO_3$ significantly increased the size of gel beads over the control (no gas-forming agent). Increased amount of $NaHCO_3$ produced irregular shaped and ruptured beads. As percentage of $CaCO_3$ increased from 5% to 10%, the size of the air-dried CaPG beads increased whereas that of the freeze-dried beads did not changed significantly. This is because the freeze-drying process maintains the structure of the gel beads to that of before drying while the beads shrank down during air-drying due to water evaporation from the structure. Therefore, the size of freeze-dried CaPG beads was larger than that of air-dried gel beads. The gas entrapped in the bead structure, resulting from the bead formation in acidic medium, prior to freeze-drying resulted in the larger beads. The air-dried beads were, however, smaller due to the collapse of the porous structure (Fig. 3a). Degree of methylesterification had little effect on the size of CaPG beads prepared. The size of CaPG beads increased slightly when the drug loading was increased.

### 3.3. Floating property of CaPG beads

Floating tests were performed in SGF as well as in water and 0.9% w/v sodium chloride. The results showed that the conventional beads sank immediately on immersion in all test media. The beads containing carbonate salts showed different floating properties depending on the type and amount of the carbonate salt added in the formulation as well as the drying method (Fig. 4 and Table 2). Fig. 4a demonstrated the floating kinetics of some formulations in SGF. The floating behavior of the beads containing carbonate salts was related to their porous structure. The

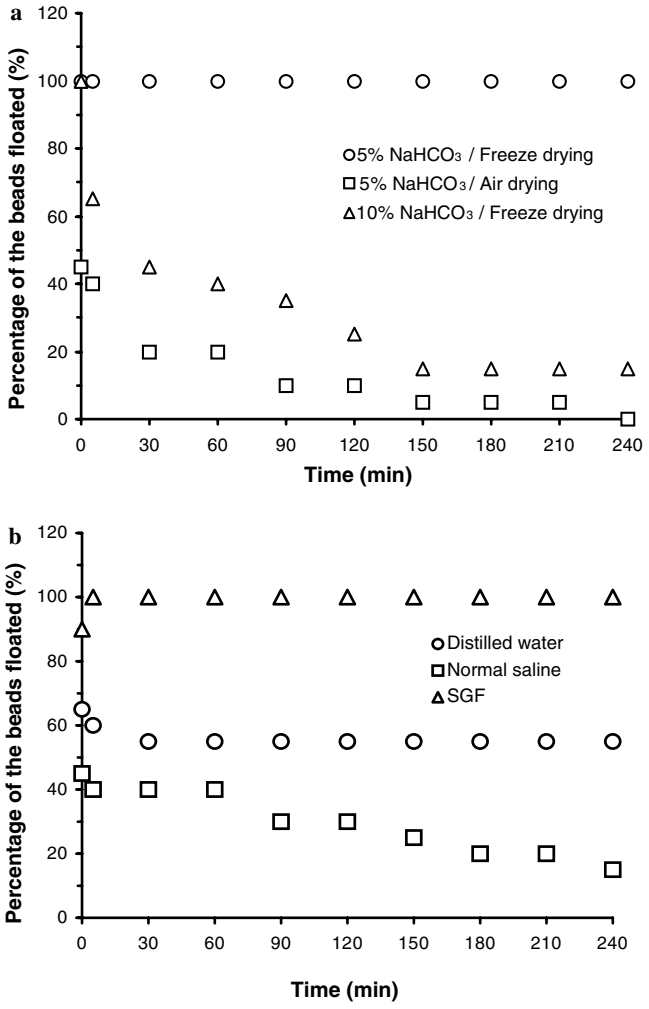


Fig. 4. Floating kinetics of (a) air-dried and freeze-dried CaPG beads (LM-104:MZ = 1:0.5 w/w) containing 5% or 10% $NaHCO_3$ tested in SGF and (b) freeze-dried CaPG beads (LM-104:MZ = 1:0.5 w/w) containing 10% $CaCO_3$.

Table 2
Floating property of drug-loaded CaPG beads containing different carbonate salts, using LM-104 (DE28%), $n = 20$

| Formulation | % Beads floated | | | | | |
|---|---|---|---|---|---|---|
| | Distilled water | | Normal saline | | SGF | |
| | 5 min | 6 h | 5 min | 6 h | 5 min | 6 h |
| Pectin to drug ratio = 1:0.5 w/w | | | | | | |
| 5% $NaHCO_3/CaCl_2$/air-dried | 0 | 0 | 0 | 0 | 10 | 0 |
| 5% $NaHCO_3/CaCl_2$/freeze-dried | 100 | 100 | 100 | 100 | 100 | 100 |
| 10% $NaHCO_3/CaCl_2$/freeze-dried | 10 | 10 | 15 | 0 | 60 | 10 |
| 5% $CaCO_3/CaCl_2$/air-dried | 0 | 0 | 0 | 0 | 0 | 0 |
| 5% $CaCO_3/CaCl_2$/freeze-dried | 35 | 35 | 35 | 30 | 100 | 100 |
| 10% $CaCO_3/CaCl_2$/freeze-dried | 60 | 55 | 40 | 15 | 100 | 100 |
| 5% $CaCO_3/CaCl_2$ + acetic acid/air-dried | 100 | 100 | 100 | 100 | 100 | 100 |
| 5% $CaCO_3/CaCl_2$ + acetic acid/freeze-dried | 100 | 100 | 100 | 100 | 100 | 100 |
| 10% $CaCO_3/CaCl_2$ + acetic acid/freeze-dried | 100 | 100 | 100 | 100 | 100 | 100 |
| 5% $CaCO_3/CaCl_2$ + HCl/air-dried | 100 | 100 | 100 | 100 | 100 | 100 |
| 5% $CaCO_3/CaCl_2$ + HCl/freeze-dried | 100 | 100 | 100 | 100 | 100 | 100 |
| 10% $CaCO_3/CaCl_2$ + HCl/freeze-dried | 100 | 100 | 100 | 100 | 100 | 100 |
| Pectin to drug ratio = 1:1 w/w | | | | | | |
| 5% $NaHCO_3/CaCl_2$/freeze-dried | 100 | 100 | 100 | 100 | 100 | 100 |
| 10% $NaHCO_3/CaCl_2$/freeze-dried | 10 | 10 | 15 | 0 | 65 | 15 |
| 5% $CaCO_3/CaCl_2$/freeze-dried | 100 | 100 | 90 | 95 | 100 | 100 |
| 10% $CaCO_3/CaCl_2$/freeze-dried | 85 | 85 | 85 | 85 | 100 | 100 |
| 5% $CaCO_3/CaCl_2$ + acetic acid/freeze-dried | 100 | 100 | 100 | 100 | 100 | 100 |
| 10% $CaCO_3/CaCl_2$ + acetic acid/freeze-dried | 100 | 100 | 100 | 100 | 100 | 100 |
| 5% $CaCO_3/CaCl_2$ + HCl/freeze-dried | 100 | 100 | 100 | 100 | 100 | 100 |
| 10% $CaCO_3/CaCl_2$ + HCl/freeze-dried | 100 | 100 | 100 | 100 | 100 | 100 |

more porous structure of the freeze-dried beads demonstrated the higher floating ability than that of air-dried beads, e.g., the freeze-dried beads containing 5% $NaHCO_3$ were able to float immediately on immersion in gastric fluid and remained buoyant after the buoyancy test period (6 h) while the percentage of the air-dried beads floated decreased over time. The rupture of bead structure when using 10% $NaHCO_3$, as shown by photo image (Fig. 1e), may cause the decreasing buoyancy of the beads (Fig. 4a). In gastric fluid, the beads containing 5% and 10% $CaCO_3$ exhibited a good floating ability; 100% beads floated after the lag time of 5 min (e.g., Fig. 4b). This can be explained by dissociation potency of $CaCO_3$ in acidic medium, i.e., the $CaCO_3$ dissociates to release $CO_2$ gas that could be entrapped in the matrix gel beads. However, the lower floating ability of these beads was observed in neutral media because of no $CO_2$ gas formation. The beads containing $CaCO_3$ those gelled in acidified gelation medium, on the other hand, exhibited a good floating ability (100% floating) in all test media, irrespective of pH of the medium. Their buoyancy was not influenced by the amounts of drug added as nearly all of the porous beads remained buoyant after the buoyancy test period (Table 2).

### 3.4. In vitro drug release studies

#### 3.4.1. Effect of formulation variables on drug release

In vitro drug release studies were carried out to examine the suitability of the calcium pectinate to use as matrix gel beads for intragastric floating drug delivery. Release profiles were represented by plotting the cumulative amount of drug release in SGF against the time. Fig. 5 demonstrates the effect of formulation variables, i.e. pectin type, pectin to drug ratio (or drug loading), and type of carbonate salts, on drug release from air-dried CaPG beads. The release of metronidazole from CaPG beads was significantly slower than the dissolution of metronidazole powder (data not shown) because of the application of a rate controlling polymer matrix. The release of metronidazole from CaPG beads is clearly dependent upon all the formulation variables tested. Drug release from CaPG beads made of LM-104 was significantly slower than those made of LM-101 (Fig. 5a), as has been shown previously (Sriamornsak & Nunthanid, 1998). It is thought that the promotion of cross-links between pectin chains is due to the more available free carboxyl group of LM-104 (pectin with lower DE) for cross-linking with calcium, often known as an 'egg-box' structure (Grant, Morris, Rees, Smith, & Thom, 1973).

The effect of drug loading (pectin to drug ratio) on the release behaviour is shown in Fig. 5b. The greater the drug loading (i.e., pectin to drug ratio of 1:1), the slower the drug release. A similar trend in the drug release profiles of theophylline-loaded calcium alginate gel beads and gel capsules has been reported (Tomida, Nakamura, Yoshitomi, & Kiryu, 1993). This finding indicates that the metronidazole release rate can be controlled by the drug loading in the beads. Fig. 5c shows the drug release from CaPG beads with different types and amounts of carbonate salts. Metronidazole was exhausted from the beads containing $NaHCO_3$ more quickly than from those containing $CaCO_3$ due to their more porous structure. The beads containing $NaHCO_3$

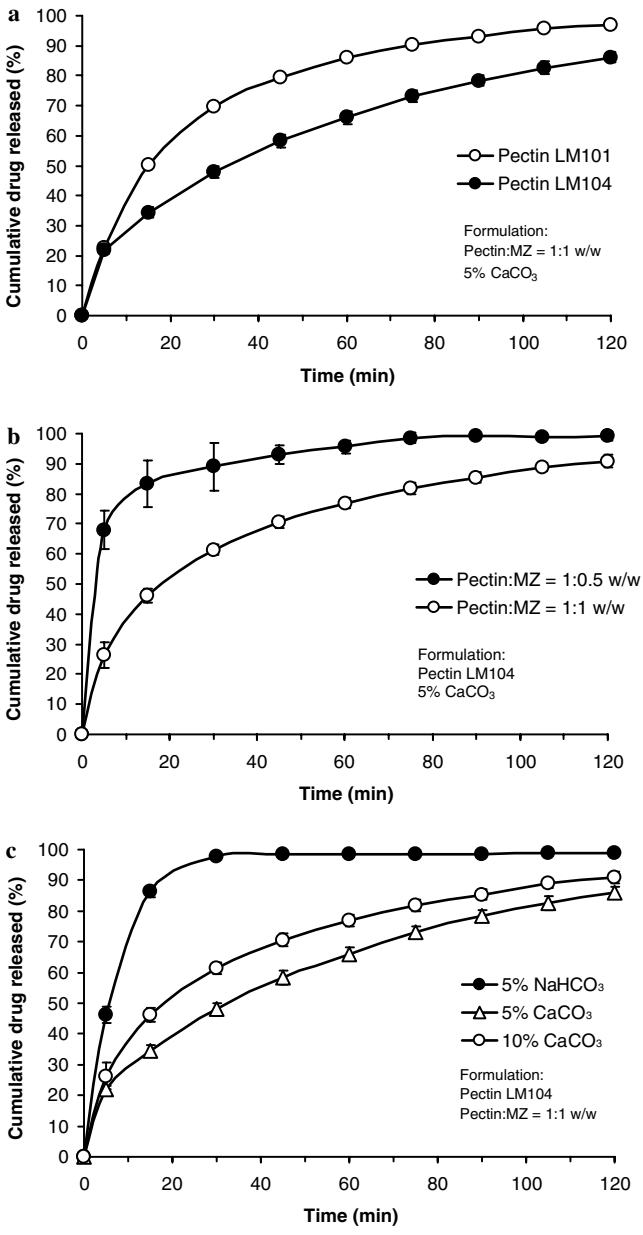


Fig. 5. Effect of formulation variables on metronidazole release from air-dried CaPG beads (gelled in $CaCl_2$) containing carbonate salt; (a) effect of pectin type, (b) effect of drug loading (pectin to drug ratio), and (c) effect of type and concentration of carbonate salt. The means and standard deviation of triplicate data are shown.

disintegrate more readily in the test media due to the increased water uptake. The ion-exchange phenomenon between sodium and calcium ions may also loosen the matrix structure of the beads. Sriamornsak (2002) found that a significant difference in the amount of calcium remaining in calcium pectinate films after exposure to 0.1 M NaCl at different time intervals; about 10–30% calcium was left in the films after 4 h exposure to 0.1 M NaCl. Although $CaCO_3$ is present as an insoluble dispersion in medium with neutral pH, it becomes water-soluble and produces $CO_2$ in acidic media resulting in porous structure. Thus, the faster drug release from the beads containing 10% $CaCO_3$ may be resulted from more porous structure than those with 5% $CaCO_3$ (Choi et al., 2002). The ionized calcium ions then promoted internal gelation by cross-linking with carboxyl group of pectin chains. This may play a role on the stability of the beads after release studies. The results indicate that $CaCO_3$ is superior to $NaHCO_3$ as a gas forming agent in CaPG bead preparations.

### 3.4.2. Effect of processing variables on drug release

Fig. 6a shows the effect of medium used for bead formation on drug release from air-dried CaPG beads containing 5% $CaCO_3$. The CaPG beads containing 5% $CaCO_3$ those gelled in acidified medium released the drug faster than those gelled in neutral medium. It is most likely that acidified gelation medium helps to produce $CO_2$ gas, resulting in the porous structure. The beads those gelled in mixture of $CaCl_2$ and hydrochloric acid released the drug more quickly, comparing to those gelled in $CaCl_2$ and acetic acid. It is possible that the calcium ions in the matrix structure were replaced faster by protons from the stronger acid solution of hydrochloric acid. A dramatic decrease in calcium cross-linking caused a more permeable gel structure (Tomida et al., 1993). These results are in agreement with previous work

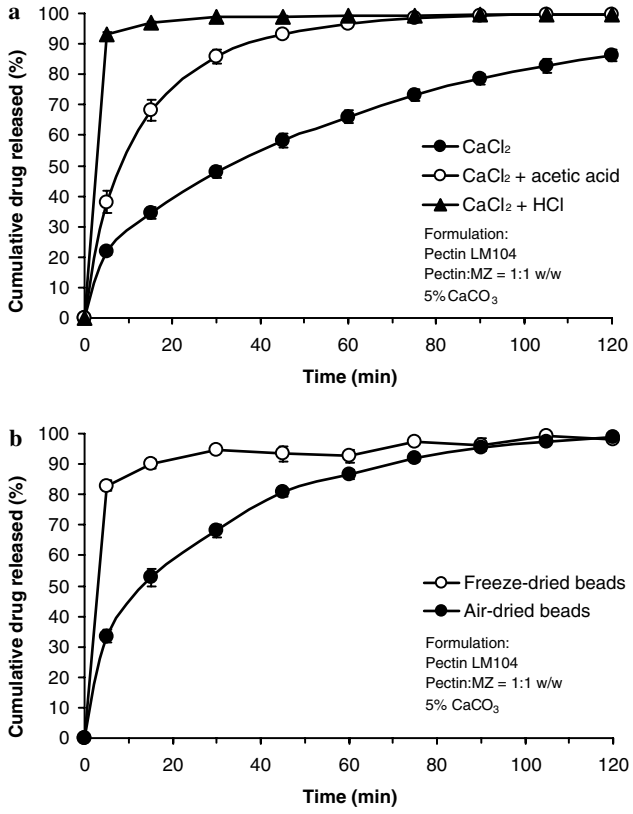


Fig. 6. Effect of processing variables on metronidazole release from CaPG beads containing carbonate salt; (a) effect of gelation medium (air-dried beads), and (b) effect of drying method (gelled in $CaCl_2$). The means and standard deviation of triplicate data are shown.

(Sriamornsak, 2002) which, in acidic media (0.1 M hydrochloric acid and SGF), negligible amount of calcium remained in the calcium films after 5 min exposure to these media. A total displacement of calcium could be due to proton-calcium ion exchange phenomenon. Lim and Kennedy (1997) also observed that 100% calcium were lost from calcium alginate films (prepared by immersing cast alginate film in calcium solution) exposed to SGF or 0.07 M hydrochloric acid.

The effect of drying method on drug release from CaPG beads (gelled in $CaCl_2$) is shown in Fig. 6b. As expected, the freeze-dried beads showed very high initial burst release fractions with rapid release kinetic profiles, while the air-dried beads demonstrated a lower burst and slower drug release. The faster drug release from the freeze-dried beads is probably explained by their higher porosity (Figs. 2 and 3). The freeze-dried beads tended to float on the release medium while the air-dried beads gathered at the bottom of the vessels and released drug slowly.

## 4. Conclusion

An intragastric floating drug delivery system using CaPG beads containing carbonate salt was designed and tested. The CaPG beads containing $NaHCO_3$ or $CaCO_3$ could be prepared by ionotropic gelation and showed porous and dense structure, respectively. Acidifying of gelation medium increased the pores in the structure of CaPG beads containing $CaCO_3$. This is because $CaCO_3$ reacted with acid to produce $CO_2$, and the evolving gas permeated through the matrix structure leaving gas bubbles or pores. Freeze drying of the beads resulted in porous structure and increased floating ability of the beads in test media. The release of metronidazole from CaPG beads containing carbonate salts depended on the formulation and processing variables tested. It is obvious that the highly porous beads showed a good floating ability and a fast drug release.

The results indicated that the beads using LM-104, $CaCO_3$, acidified gelation medium, and high drug loading could prolong the drug release. However, floating ability of the beads using these conditions, in some cases, seemed to be decreased. It is suggested that the optimization of formulation and processing variables is needed to obtain a good floating ability and a prolonged drug release. In order to achieve this, such formulations will be further modified and tested. We are continuing the experiments with these systems in an attempt to (1) keep the systems afloat in the gastric condition and (2) prolong the drug release from the CaPG beads.

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