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Calcium pectinate gel beads for controlled release drug delivery: I. Preparation and in vitro release studies[1]

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Abstract

Calcium pectinate gel (CPG) beads of indomethacin, a poorly soluble drug, were prepared by dispersing indomethacin in a solution of pectin and then dropping the dispersion into calcium chloride solution. The droplets instantaneously formed gelled spheres by ionotropic gelation. The effect of several factors such as pectin type, the presence of a hardening agent and the drug loading were investigated on the percentage of drug entrapped, size distribution and drug release from the CPG beads. The release characteristics were studied using the rotating basket dissolution method. Strong spherical beads with narrow size distributions, high yields and good entrapment efficiencies could be prepared. All factors investigated have significantly affected the release of indomethacin from CPG beads. The mechanism of drug release from CPG beads followed the diffusion controlled model for an inert porous matrix. Therefore, calcium pectinate gel could be a useful carrier for controlled release drug delivery of poorly soluble drugs. © 1998 Elsevier Science B.V.

Keywords: Pectin; Calcium pectinate gel; Indomethacin; Controlled release; Drug delivery; Beads

1. Introduction

Pectins are important ionic polysaccharides found in plant cell walls. They consist mainly of linearly connected α-(1→4)-D-galacturonic acid residues which have carboxyl groups. The degree of esterification (DE) and degree of amidation (DA), which are both expressed as a percentage of carboxyl groups (esterified or amidated), are im-

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portant means to classify pectins. Low methoxy pectins (with DE < 50%) form rigid gels by the action of calcium, which cross-links the galacturonic acid chains (Rolin, 1993).

Combination of calcium salts and pectin has been used to prepare matrix tablets for colonic delivery of several model drugs (Rubinstein et al., 1993; Ashford et al., 1993, 1994). The rationale for this is that calcium pectinate will be degraded by colonic pectinolytic enzymes (Englyst et al., 1987), but will retard drug release in the upper gastrointestinal tract due to its insolubility and because it is not degraded by gastric or intestinal enzymes (Sandberg et al., 1983). Since pectin can react with calcium ions, we have investigated calcium pectinate as an insoluble hydrophilic coating for sustained release delivery by interfacial complexation (Sriamornsak et al., 1997a,b). Recently, pectin beads prepared by the ionotropic gelation method (Aydin and Akbuga, 1996; Sriamornsak et al., 1997b) have been investigated as a sustained release drug delivery system; however, the use of pectin beads has some drawbacks due to their rapid in vitro release.

In the present study, we developed the method for preparation of spherical calcium pectinate gel (CPG) beads containing a model drug, indomethacin. The aim of this study was to investigate the effect of some formulation variables (type of pectin, the presence of a hardening agent and the amount of drug loading) on CPG beads properties and on drug release characteristics.

2. Materials and methods

2.1. Materials

Amidated low methoxy (LM) pectin with DE of 36% and 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 P36 and P28 respectively. Indomethacin (China National Chemicals, China), calcium chloride, Trizma® base (Sigma, St. Louis, MO), and glutaraldehyde (Becthai, Thailand) were used as supplied and where applicable were AR grade. All other chemicals were of reagent grade.

2.2. Preparation of indomethacin-loaded CPG beads

CPG beads were prepared by dissolving amidated LM pectin in water with agitation. Indomethacin (100 mesh sieved) was dispersed in 5% (w/w) aqueous solution of both types of pectin, i.e. P36 and P28. The dispersions were dropped using a nozzle of 0.80 mm inner diameter into a 5% (w/v) solution of calcium chloride with gentle agitation at room temperature. The CPG beads formed were allowed to stand in the solution for 2 h, separated and washed with distilled water and consequently suspended in glutaraldehyde solution. The suspension was filtered, rewashed with water, then filtered and dried at 37°C for 12 h. A number of different variables were investigated and are summarised in Table 1.

2.3. Characterisation of the CPG beads

2.3.1. Particle size

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.

2.3.2. Drug content and entrapment efficiency

Prior to the determination of indomethacin content, the CPG beads must be dissolved by phosphate buffer (pH 7.4) containing 5 mM ethylenediaminetetraacetic acid. The content of indomethacin was later assayed by UV-spectrophotometer (Hitachi U-2000, Japan) in pH 7.4 phosphate buffer at 318 nm. The determinations were made in triplicate. The ratio of the actual indomethacin content in the CPG beads to the theoretical indomethacin content was termed the entrapment efficiency (EE).

2.3.3. Swelling properties

Thirty dried CPG beads were placed in a beaker to which 200 ml of distilled water or pH 7.4 Tris buffer was added, and then stirred with a

Table 1
Formulation, mean diameter and indomethacin loading capacity of CPG beads

Formulation	Type of pectin	Hardening agent	Drug loading (%)	Mean diameter (mm ± S.D.[a])	Entrapment efficiency[b] (% ± S.D.[c])
P36 blank	P36	—	—	1.353 ± 0.046	—
P28 blank	P28	—	—	1.360 ± 0.024	—
P36/5/No	P36	No	5	1.997 ± 0.047	85.63 ± 0.80
P36/5	P36	Yes	5	1.687 ± 0.042	94.12 ± 0.30
P28/5/No	P28	No	5	1.809 ± 0.032	88.40 ± 0.58
P28/5	P28	Yes	5	1.646 ± 0.042	98.14 ± 0.56
P28/2.5	P28	Yes	2.5	1.599 ± 0.037	96.75 ± 0.78
P28/10	P28	Yes	10	1.928 ± 0.029	94.25 ± 0.66
P28/15	P28	Yes	15	1.932 ± 0.040	90.96 ± 0.10
P28/20	P28	Yes	20	2.058 ± 0.068	90.73 ± 0.26

[a] S.D. was calculated from 50 measurements.
[b] Entrapment efficiency was calculated based on the theoretical drug loading.
[c] S.D. was calculated from three repeated measurements.

magnetic stirrer at a speed of 100 rev./min. After 24 h, the equilibrium swollen beads were observed and measured under an optical microscope. The magnitude of swelling was presented by the ratio of the mean diameter of swollen beads to the mean diameter of the dried beads before the test.

2.4. In vitro drug release

Drug release kinetics from the CPG beads were evaluated using the rotating basket dissolution method (USP dissolution apparatus 1; Pharmatest™, Germany). The baskets were rotated at 100 rev./min at 37°C. The dissolution medium used was pH 7.4 Tris buffer. The analytical wavelength was 318 nm and Beer's law was obeyed over the range of 0–100 mg/l. Drug release was measured from accurately weighed amounts of the CPG beads, equivalent to 75 mg of indomethacin, added to 750 ml of dissolution medium. All dissolution runs were performed in triplicate.

3. Results and discussion

3.1. Preparation and characterisation of indomethacin-loaded CPG beads

An aqueous solution of LM pectin containing indomethacin was dropped into calcium chloride solutions and gelled 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 LM pectin molecules. The CPG beads were easily manufactured without any sophisticated equipment.

As shown in Table 1, the mean diameter of indomethacin-loaded CPG beads ranges between 1.599 and 2.058 mm while CPG beads containing no drug had diameters of 1.360 and 1.353 mm for P28 and P36, respectively. The beads' diameter decreased as the hardening agent was used as the hardening agent promoted the formation of cross-links between the LM pectin molecules. Moreover, the higher the drug loading, the higher the average size of beads.

The percent EE of indomethacin in CPG beads was calculated from the fractional amount of drug remaining in the beads. Factors affecting the percent EE were the type of pectin, the presence of a hardening agent and the amount of drug loading. As shown in Table 1, the type of pectin affected the percent EE. The lower the DE of pectin, the higher the EE. Moreover, the EE increased when hardening agent was added. It is thought that the hardening agent cooperates to build a dense surface on the beads which prevents leakage of the drug into the counter ion solution during the preparation process. On the other

hand, the EE decreased with increased amount of drug loading. Due to the low aqueous solubility of indomethacin, the percent EE was rather high and showed good efficiency of drug entrapment.

The extent of swelling of the CPG beads upon rehydration during release was affected by the dissolution medium. A small number of charges present on the Tris buffer might allow the gel beads to swell more strongly than in water due to greater solvent penetration into the calcium pectinate network, followed by greater ion exchange between calcium and hydrogen ions. Hydrogen ions might displace calcium in the gelled structure and partially form soluble pectinic acid regions, which are more permeable (Sriamornsak et al., 1997b). The hydrated CPG beads were examined by optical microscopy after 24-h immersion in the different fluids and the ratio of the swollen diameter to the dried diameter was calculated. The equilibrium swelling ratios are given in Fig. 1 which shows that there is a small influence of hardening agent on the swelling of the beads in water and Tris buffer. The type of pectin strongly affected the swelling of CPG beads. The lower the DE of the pectin the lower the swelling ratio and the looser the structure of gel beads. It is surprising that in the presence of drug the swelling ratio increased in Tris buffer while there was almost no difference in water (Fig. 1a). Furthermore, when the drug loading was increased, the swelling ratio decreased due to the decreased amount of pectin polymer in the beads (Fig. 1b). In all cases the gels remained intact, although in the Tris buffer they appeared slightly softer.

3.2. In vitro release and mechanism of drug release from CPG beads

Release studies were carried out to examine the suitability of using the calcium pectinate matrix gel beads for prolonged drug release. The release pattern was represented by plotting the cumulative percent drug released against time. Different formulation variables such as type of pectin, the presence of hardening agent and the amount of drug loading were investigated and their effects on the release of indomethacin are shown in Fig. 2.

Fig. 2a shows the effect of the type of pectin and the presence of a hardening agent on drug release from CPG beads containing 5% w/v drug. The release of indomethacin from the CPG beads was significantly slower than the dissolution of indomethacin powder. The result was due to the application of a rate controlling polymer matrix. The release of indomethacin from CPG beads with P28 was slower than those with P36. It could be clearly seen that the drug release from the hardened formulations was slower than those from the non-hardened formulations. It is thought that this is due to the promotion of cross-links between pectin chains by the hardening agent.

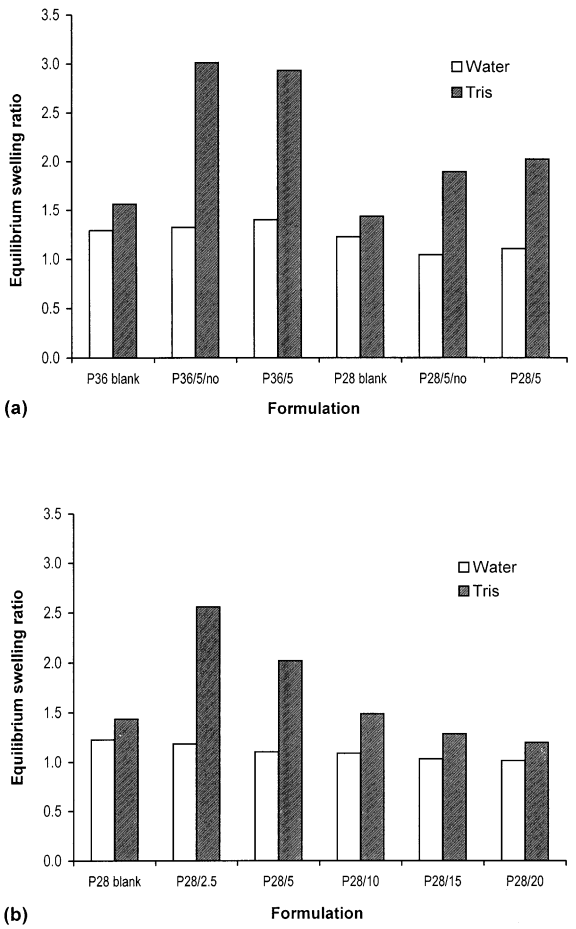

Fig. 1. The equilibrium swelling ratios of CPG beads in water and pH 7.4 Tris buffer; (a) effect of the type of pectin and presence of hardening agent and (b) effect of drug loading.

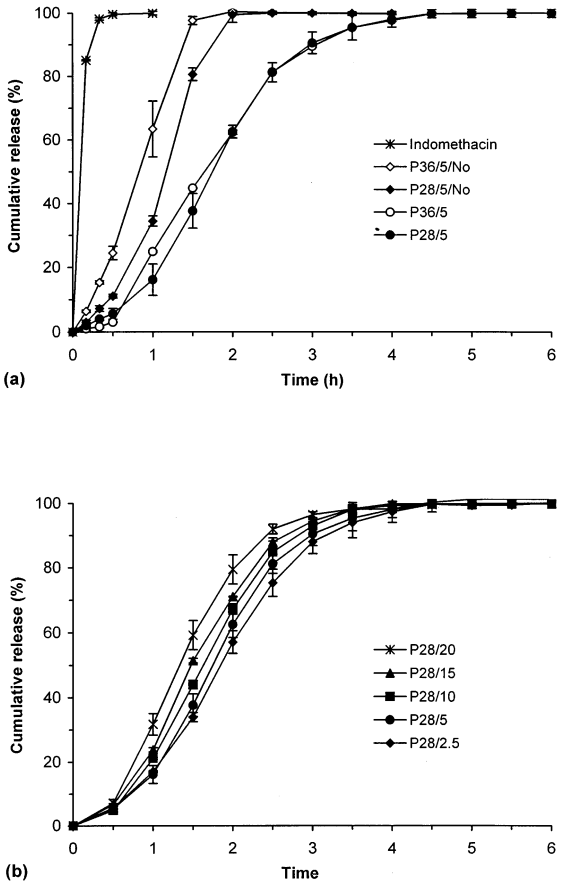

Fig. 2. Release profiles of indomethacin from CPG beads; (a) effect of the type of pectin and presence of hardening agent and (b) effect of drug loading. Each point represents the mean ($\pm$ S.E.) for at least three determinations.

The release data shown in Fig. 2b, showed that the CPG beads containing 20% w/v drug gave the highest drug release. Fifty percent of drug was released in 80 min while those with 2.5% w/v drug released 50% of drug over 112 min. The increase in drug release from beads containing high drug concentrations may be due to the decrease in the polymer/drug ratio.

The drug release from an inert matrix is described by Higuchi's equation (Higuchi, 1963) as follows:

$$Q = [D\epsilon/\tau(2A - \epsilon C_S)C_S t]^{\frac{1}{2}} \quad (1)$$

in which Q is the amount of drug released per unit area of exposed surface of the matrix, D is the diffusion coefficient of the drug in the matrix, τ is the tortuosity, ϵ is the total porosity of the matrix after the drug has been extracted, A is the amount of drug in the matrix, C_S is the solubility of the drug in the polymeric matrix, and t is the time. An approximation of Higuchi's equation can be obtained by plotting the percent of drug released versus the square root of time as expressed by Eq. (2).

$$Q = Kt^{\frac{1}{2}} \quad (2)$$

Eq. (2) yields a straight line when Q is plotted against square root of time.

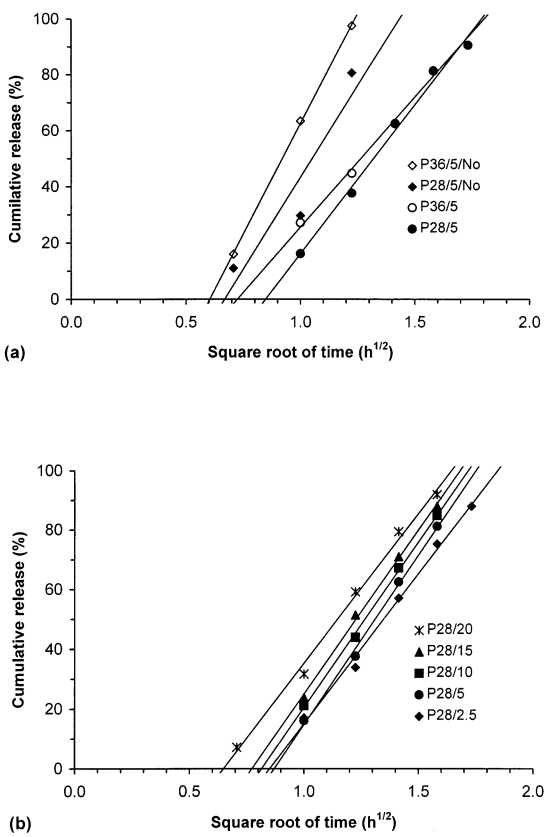

Fig. 3. The cumulative release of indomethacin from CPG beads as a function of the square root of time; (a) effect of the type of pectin and presence of hardening agent and (b) effect of drug loading.

Fig. 3a,b shows the linearity of plots for the cumulative percent drug released versus square root of time. All r^2 values were greater than 0.97. These data indicated that the drug release from CPG beads containing indomethacin followed the diffusion controlled model for an inert porous matrix as described by Higuchi's square root of time equation.

In conclusion, the indomethacin-loaded CPG beads were successfully prepared using an ionotropic gelation technique and appear to provide a controlled release delivery system. Strong spherical beads with narrow size distributions could be prepared with high yields and good entrapment efficiencies. All investigated factors have significantly affected the percentage of drug entrapment, the size of beads and the release of indomethacin from CPG beads. The mechanism of drug release from CPG beads followed the diffusion controlled model for an inert porous matrix. Therefore, calcium pectinate gel could be a useful carrier for the controlled release of poorly soluble drugs.

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