MECHANICAL CHARACTERISATION OF CALCIUM PECTINATE HYDROGEL FOR CONTROLLED DRUG DELIVERY

Calcium pectinate beads, a particulate hydrogel system, is an attractive drug carrier for oral delivery. In this study, a poorly water-soluble model drug indomethacin was incorporated into calcium pectinate beads made of different pectin concentrations, which were produced by an extrusion method. The effect of pectin concentration on bead size, circularity, swelling behaviour, and mechanical properties, as well as in vitro drug release profile was investigated. The mechanical properties of calcium pectinate beads were determined by a micromanipulation technique. The drug release profile was measured using a standard British Pharmacopoeia method. It was found that the beads made of higher pectin concentration in general had a less permeable matrix structure and greater mechanical rigidity, although they swelled more after hydration. However, such an effect was not significant when the pectin concentration was increased to above 8%. Micromanipulation measurements showed that there was significant relaxation of the force being imposed on single hydrated beads when they were held, but this phenomenon did not occur on dry beads, which means that the force relaxation was dominated by liquid loss from the beads. The rate of the force relaxation was determined, and has been related to the release rate of the model drug entrapped in the calcium pectinate beads.

Hydrogel has emerged as a promising drug carrier for oral delivery. Generally, hydrogel is a polymeric jelly-like material that swells in water, but is not soluble, and consists of polymers that can be combined with water to create a solid with certain permeability [1]. Pectin is a natural water-soluble hydrogel, a polysaccharide that can be found in plant cells. It consists of linear chains of (1→4)-D-galacturonic acid residues that contain carboxyl groups. Low methoxyl pectin (degree of esterification < 50%) is capable of forming insoluble hydrogel such as calcium pectinate when the acid chains are cross-linked with a divalent cation, calcium, which can be utilised for drug delivery purpose [2].

Calcium pectinate (CaP) hydrogel is non-toxic, it can be degraded by colonic bacteria and is able to remain intact in the upper gastrointestinal tract [3]. It has been investigated as a carrier for controlled drug release and the protection of drugs against acidic gastric environment [4–7], which includes the production method, size, the permeability of calcium pectinate in particles and the drug release rates. However, little is known about the mechanical properties of CaP hydrogel although this information is essential for the production, handling and application of the drug delivery system. Moreover, the permeability of such hydrogel may strongly depend on their mechanical properties. This has been demonstrated for hydrated dextran microspheres [8], and it has been found that the pseudo Young’s modulus of hydrated dextran microspheres loaded with the model drugs myoglobin, ovalbumin, BSA and IgG was directly correlated to the pore size of the particles and the release rate of the model drugs. This work aims to characterise the mechanical properties of calcium pectinate (CaP) hydrogel beads by a micromanipulation technique [9,10] and to establish a relationship between their mechanical properties and permeability, which is inferred from the release rate of a model drug entrapped in them.

MATERIALS AND METHODS

Preparation of CaP beads loaded with indomethacin

CaP beads were produced by a simple extrusion method [11]. Pectin solutions 5, 7, 8 and 10% w/v were prepared with 5% indomethacin, a poorly water-soluble drug, which is intended to be delivered to the human small intestine. Extrusion was carried out by a Nisco Encapsulation unit (Var J1, SPA-00195, Nisco Engineering Inc, Switzerland), with a coaxial air stream to pull the pectin droplets from a needle with an internal diameter of 0.4 mm. The pectin solution was pumped into the encapsulator and the droplets formed landed into a calcium chloride (1.5 w/v %) cross-linking solution. The coaxial airflow rate was fixed at 1.5 l/min. The formed CaP beads were allowed to immerse in the cross-linking solution for 30 min before being washed three times with distilled water and dried in an oven at 37°C for 24 h. All chemicals were purchased from the Sigma–Aldrich Company Ltd. UK.
**Size, circularity and swelling of beads**

The size and circularity of the calcium pectinate beads were measured using an image analysis system (Quantimet Q570 Image Analyser, Leica Ltd, UK). The swelling of the beads was indicated by the swelling ratio, which is defined as the ratio between the volume of hydrated beads and that of dried ones.

**In vitro drug release rate**

The in-vitro drug release profile was determined using a standard British Pharmacopoeia (BP) basket apparatus at 37°C with a rotating speed of 100 rpm. Beads of 100 mg were immersed into 600 ml of in-vitro solution in a jacketed round-bottom dissolution vessel by means of a basket stirrer. 4 ml of the solution were withdrawn from the vessel at regular times and the same amount of fresh medium was introduced in order to maintain a constant volume for the entire run. The amount of drug released was measured by a UV spectrophotometer at 268 nm (Cecil 1020, Cecil Instruments, UK). To mimic the human gastrointestinal (GI) tract, the beads were first immersed in a simulated gastric fluid (0.1N HCl) for 2 h, and then transferred to a simulated intestinal fluid (Hank’s solution, pH 7.4) [11].

**Mechanical properties of beads**

The mechanical properties of the CaP beads were determined by a micromanipulation technique [10]. The principle of this technique was to compress single beads between two flat surfaces. The force being imposed on single beads and their deformation were measured simultaneously. Details of this technique are described elsewhere [10].

The size of each bead was measured from its microscope image. The force probe was made of a glass rod of 2.5 mm in diameter, which was connected to the output tube of a force transducer (500 g, Kulite, USA). Single beads (both wet and dry) were compressed and held, and compressed to different deformations, and the force being imposed on them were measured simultaneously. The compression speed used was 60 μm/s. Twenty beads from each sample were measured in order to obtain statistically meaningful results. The experiment was conducted at room temperature (21°C).

**RESULTS AND DISCUSSION**

**Size and swelling of the CaP beads**

Fifty CaP beads made of pectin concentrations of 5%, 7%, 8% and 10%, respectively, were exposed to the simulated gastric fluid and Hank’s solution. Table 1 shows the mean size and circularity of the beads before and after the rehydration. It may be seen that the pectin concentration did not have any significant effect on the mean diameter of the dried beads. After the dried beads were immersed in the simulated gastric fluid for 2 h, they swelled due to rehydration. The hydrated beads were then transferred to Hank’s solution, and were immersed for 2 h. It was observed that the beads swelled further in Hank’s solution. This might be due to limited pectin ionisation in the simulated gastric fluid (pH 1.2). This phenomenon reduced the repulsion and resulted in a closer network structure of the hydrogel, i.e. less swellable beads [12]. As the pH was increased to 7.4 in Hank’s solution, the ionisation of pectin was favoured and led to a more swellable network structure.

It was shown that the higher the pectin concentration from which the beads were made, the more significantly the beads swelled. Pectin contains ester, hydroxyl and carboxyl groups that can easily form hydrogen bonds with water molecules upon hydration. A higher proportion of pectin in the beads may cause stronger hydrogen bonding, and it is likely for the beads to take up more water and swell to a greater extent due to their ability to form excessive hydrogen bonds with water and, therefore, the beads become larger.

**In-vitro drug release rate**

The release profile was represented by plotting the cumulative increase of the drug concentration in dissolution liquids (in terms of percentage release) with time. As shown in Figure 1, there was very little drug

| Table 1. The size and circularity of calcium pectinate beads made of different pectin concentrations |
|---|---|---|---|---|
|   | Pectin concentration |
|   | 5% | 7% | 8% | 10% |
| **Mean diameter ± standard error, mm** |   |   |   |   |
| Dry beads | 1.02 ± 0.01 | 0.98 ± 0.01 | 0.97 ± 0.01 | 1.00 ± 0.01 |
| Beads in HCl for 2h | 1.27 ± 0.01 | 1.43 ± 0.01 | 1.38 ± 0.01 | 1.54 ± 0.01 |
| Beads then in Hank’s solution for 2h | 1.57 ± 0.01 | 1.65 ± 0.01 | 1.75 ± 0.01 | 1.85 ± 0.01 |
| **Circularity** |   |   |   |   |
| Dry beads | 1.19 ± 0.01 | 1.17 ± 0.01 | 1.14 ± 0.01 | 1.18 ± 0.01 |
| Beads in HCl for 2h | 1.23 ± 0.02 | 1.22 ± 0.02 | 1.22 ± 0.06 | 1.21 ± 0.02 |
| Beads then in Hank’s solution for 2h | 1.34 ± 0.02 | 1.23 ± 0.02 | 1.18 ± 0.02 | 1.25 ± 0.02 |
(<5%) released into the simulated gastric fluid at the end of the exposure for beads made of different concentrations. When they were exposed to Hank’s solution, it could be clearly noticed that the higher the pectin concentration, the slower the drug release rate. For comparison, the dissolution rate of pure indomethacin (IMC) powders in Hank’s solution is also shown in the figure, which is much faster the release rate of the drug entrapped in the CaP beads. This is believed to be due to the presence of a calcium pectinate polymer matrix that altered the drug release profile. Overall, most of the drug (>85%) was released in Hank’s solution after the beads were exposed to it for 5 h.

To quantitatively determine the rate of drug release to Hank’s solution, an exponential equation with one adjustable parameter was used to fit the corresponding profiles in Figure 1

\[ \frac{M_t}{M_{\infty}} = 1 - \exp(-ct) \]  

where \( \frac{M_t}{M_{\infty}} \) was the amount of drug released, c the drug release rate constant and t time. It was found that the equation fitted all the curves very well, and their regression coefficients were all greater than 0.97. The value of c versus the pectin concentration is presented in Figure 2. Clearly, the value of c decreases with pectin concentration, which might result from the fact that a higher pectin concentration led to a less permeable structure of calcium pectinate beads, hence lower drug release rate.

**Mechanical Properties of the Calcium Pectinate Beads**

Figure 3 shows a typical force versus time curve for the compression and holding of a CaP bead immersed in the simulated gastric fluid for 2 h and just immersed in Hank’s solution. Curve AB corresponds to the bead being compressed up to a pre-set displacement. Holding commenced at point B. It can be seen that the force decreased significantly when the bead was held (curve BC). The force relaxation curve BC was fitted by an equation with two adjustable parameters (\( F_\infty/ F_0 \) and k),

\[ \frac{F}{F_0} = \frac{F_\infty}{F_0} + (1 - \frac{F_\infty}{F_0}) \cdot \exp(-kt) \]  

where \( F \) was the force imposed on the CaP bead, \( F_0 \) the force at the beginning of holding (time \( t = 0 \)), \( F_\infty \) the residual force (i.e. corresponding to infinite time), k the force decay constant, and t the holding time. \( F_\infty/ F_0 \) represents the relative residual strength of the bead.

In order to compare the mechanical rigidity of CaP beads made of different pectin concentrations and how they changed after rehydration in the simulated gastric and intestinal fluids, from the force – displacement data.

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Figure 1. Release profiles of indomethacin (IMC) entrapped in calcium pectinate beads made of different pectin concentrations. The error bars represent the standard error of the mean of three repeated measurements.

Figure 2. Variation of the drug release rate constant with pectin concentration. The error bars represent the standard error of the mean of three repeated measurements.

Figure 3. The force versus sampling time for compression at 60 \( \mu \)m/s and holding of a calcium pectinate bead of 1.5 mm in diameter hydrated in the simulated gastric solution for 2 h and just immersed in Hank’s solution (--- equation 2).
measured by micromanipulation (curve AB), the corresponding pseudo stress versus deformation for the calcium pectinate beads made of different concentrations of pectin were determined [8], and shown in Figure 4. No rupture, but only deformation of the beads under compression was recorded. This is because the hydrogel matrix was monolithic and permeable to water. It can be seen from Figure 4, as the pectin concentration was increased, the beads were stronger (greater pseudo stress for a given deformation), particularly when the pectin concentration varied from 5% to 7%. The increase in pseudo stress was not significant when the pectin concentration was increased from 7 to 10%. It was also noticed that the beads after being immersed in Hank’s solution for 2 h became significantly weaker than those just being immersed in the solution (0 h).

The force relaxation (curve BC) in Figure 3 may be due to the visco-elastic nature of the bead or liquid loss from the bead when it was compressed. In order to distinguish the dominating factor, single dried beads were also compressed and held, and the force being imposed on them was determined. A typical force versus time for compressing and holding single dried calcium pectinate beads is shown in Figure 5. The force increased when the bead was compressed, as expected. However, the force did not relax significantly when the bead was held. This demonstrates that the force relaxation of hydrated CaP beads resulted from the loss of liquid since the bead material itself is not viscous in nature.

The force relaxation curves (represented by BC in Figure 3) for CaP beads after being exposed to the gastric and intestinal fluids were fitted by equation 2, and the values of the two parameters (F∞/F0 and k), which were found to be independent of the deformation of the particles (data not shown) are given in Table 2. The values of all the regression coefficients (R) were greater than 0.95, which indicated good fitting. The relative residual strength (F/F0) of the beads after being exposed to the gastric fluid for 2 h (or 0 h in Hank’s solution) increased with pectin concentration, while the value of k decreased. The change in these parameters

![Figure 4. Pseudo stress–deformation profiles for compressing single CaP beads made of different pectin concentrations. H0 indicates beads having been exposed to the simulated gastric fluid for 2 h, and just immersed in Hank’s solution, H2 indicates beads in the simulated gastric fluid for 2 h and then in Hank’s solution for 2 h. The error bars represent the standard error of the mean of 20 micromanipulation measurements.](image)

![Figure 5. Typical force versus time for the compression and holding a dried CaP bead of 600 μm in diameter.](image)

<table>
<thead>
<tr>
<th>Pectin</th>
<th>Relative Residual Strength</th>
<th>k (s⁻¹)</th>
<th>R*</th>
<th>Relative Residual Strength</th>
<th>k (s⁻¹)</th>
<th>R*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5%</td>
<td>0.41 ± 0.01</td>
<td>0.28 ± 0.02</td>
<td>0.95</td>
<td>0.37 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>0.96</td>
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<tr>
<td>7%</td>
<td>0.57 ± 0.02</td>
<td>0.18 ± 0.01</td>
<td>0.98</td>
<td>0.40 ± 0.01</td>
<td>0.25 ± 0.01</td>
<td>0.98</td>
</tr>
<tr>
<td>8%</td>
<td>0.64 ± 0.01</td>
<td>0.19 ± 0.01</td>
<td>0.98</td>
<td>0.30 ± 0.01</td>
<td>0.30 ± 0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>10%</td>
<td>0.64 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.99</td>
<td>0.47 ± 0.01</td>
<td>0.28 ± 0.01</td>
<td>0.98</td>
</tr>
</tbody>
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*Regression coefficient.
was rather insignificant as the pectin concentration was increased from 7 to 10%. After the beads were further immersed in Hank’s solution for 2 h, the beads had lower residual strengths. However, the value of $k$ increased, which indicated that the loss of liquid from the beads was faster.

The change in the value of $k$ should reflect the relative magnitude of the permeability of the CaP beads, which might be linked to the magnitude of the drug release rate constant ($c$ in equation 1). Their relationship is plotted in Figure 6. Clearly, $c$ increases with $k$. This is strong evidence to show that there is indeed a relationship between the mechanical properties of the CaP beads and their permeability. This also means that the micromanipulation technique could be applied to investigate the permeability of hydrogels in addition to their mechanical properties.

By compiling all the results attained from various characterisations, it can be noticed that the properties of the calcium pectinate beads are very much governed by their magnificent swelling behaviour. As the amount of pectin was increased, the structure of the beads became denser with more cross-linking between pectin and the calcium cation, resulting in their higher mechanical rigidity. After the drug was incorporated, its release rate was reduced. However, the increase in pectin concentration caused more swelling of the beads during their hydration due to ionisation of the pectin and the increased availability to form external hydrogen bonding. This accelerated the release of the drug and caused a decrease in the mechanical rigidity of the beads as pectin under this circumstance tended to bond with external water molecules to partially "degrade" their structure, instead of keeping them rigidly intact [13]. These two factors offset each other and resulted in a fairly similar drug release rate and mechanical rigidity of the beads for pectin concentrations of 7 to 10%.

Small intestinal fluid (Hank’s solution) contains a chelating agent such as the phosphate ion [11]. It can weaken the calcium cross-linking, degrade the hydrogel, and thus weaken the bead structure. This is the reason why the beads further immersed in Hank’s solution for 2 h showed a significantly lower mechanical rigidity (a greater force decay constant $k$ and a smaller residual force) than those in the simulated gastric fluid, as the beads swelled more, lost liquid faster under compression and became weaker.

**CONCLUSION**

It was been demonstrated that when the poorly water-soluble drug indomethacin was entrapped in CaP beads, there was very little release (<5%) of the drug to the simulated gastric fluid and the rate of its release in Hank’s solution decreased with increase in the concentration of pectin from which the CaP beads were made. Most of the drug (>85%) was released to Hank’s solution after 5 h.

It was also found that an increase in the pectin concentration caused more swelling of the CaP beads, but resulted in a less permeable and mechanically stronger matrix. The relationship between the drug release rate constant ($c$) and the force decay constant ($k$) was established. It is believed that the findings can be further exploited to formulate drug delivery systems with desired properties.

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**REFERENCES**

IZVOD

KARAKTERIZACIJA MEHANIČKIH OSOBINA KALCIJUM PEKTINATNOG HIDROGELA KAO NOSAČA LEKOVA SA KONTROLISANIM OTPUŠTANJEM AKТИVNE MATERIJE

(Naučni rad)

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Čestice kalcijum pektinata predstavljaju hidrogel koji je veoma pogodan kao nosač aktivne supstance (leka) odnosno pripremu leka sa kontrolisanim dejstvom za oralnu upotrebu kod ljudi. U ovom radu je koničen malo u vodi restvorilj indometacin koji je inkorporiran u zmca kalcijum pektinata načinjenih od pektina različite koncentracije i proizveden procesom ekstruzije. Procena je uticalj koncentracije pektina na veličinu zmca, njihovu sferičnost, osobine bubrenja, mehaničke osobine kao i na brzinu otpuštanja aktivne supstance (leka) in vitro. Mehaničke osobine zmca kalcijum pektinata su određivane primenom metode mikromanipulacije. Profil brzine otpuštanja leka je meren prema standardu Britanske Farmakopeje. Utvrđeno je da zmca napravljena od mase koja je imala veću koncentraciju pektina imaju manje propuštajuću strukturu matriksa, a veću mehaničku čvrstoću. Ova zmca su takođe više bubrila nakon hidratacije. Međutim, ovaj efekat nije značajan kada je koncentracija pektina bila veća od 8%. Primenom metode mikromanipulacije pokazano je da postoji značajan efekat sile relaksacije kod jednog hidratišanog zmca, što se nije opazilo sa osušenim zmcom. To ukazuje da je sila relaksacije posledica gubitka vode iz hidratišanog zmca. Određena je brzina relaksacije i korelirana sa brzinom kojom se lek kontrolisano otpušta iz zmca kalcijum pektinata.

Ključne reči: Kalcijum pektinat • Hidrogel • Kontrolisano otpuštanje leka • Mehaničke osobine • Permeabilnost • Mikromanipulacija • Key words: Calcium pectinate • Hydrogel • Controlled drug delivery • Mechanical property • Permeability • Micromanipulation •
Simulated
gastric fluid
pH 1.2

Hank's solution
pH 7.4

**Drug Release Rate Constant c**