Carbohydrate Polymers 103 (2014) 550–557

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Swelling and morphology of calcium pectinate gel beads obtained from *Silene vulgaris* callus modified pectins

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ARTICLE INFO

Article history:
Received 16 July 2013
Received in revised form
23 December 2013
Accepted 25 December 2013
Available online 3 January 2014

Keywords:
Callus
Gel beads
Medium nutrients
Pectin
Swelling
Silene vulgaris

ABSTRACT

The aim of this research is to investigate the swelling properties and morphology of the calcium pectinate gel (CaPG) beads made from pectins of campion callus cultured using various medium nutrients (carbon sources, concentration of sucrose, calcium and 2,4-dichlorophenoxyacetic acid (2,4-D)). Gelled spheres were prepared by ionotropic gelation. The mean diameter, total surface area and volume of the dried beads varied depending on the plant cell culture conditions. The swelling of dried CaPG beads in solutions with pH 2 and pH 4 was demonstrated to occur more slowly (within 4 or 24 h) with increasing sucrose and calcium concentrations or in the absence of auxin. All beads swelled less when placed in acidic media (pH 2 and pH 4) and swelled most extensively in NaCl (pH 6). The surface morphology of the CaPG beads was demonstrated to depend on the presence of sugars, calcium and auxin in the plant cell culture medium used. The slow swelling of dried CaPG beads was apparently related to their grooved surfaces. An applied strategy involving changing the composition and concentration of media components altered the swelling behavior of the CaPG beads and enhanced the acid and water resistance of the resultant pectinate hydrogels in physiological environments. In particular, the swelling of Ca 4.5, 2,4-D0, Suc30 and Suc100 CaPG beads occurred more slowly.

1. Introduction

Pectin and their derivatives are widely used in the pharmaceutical and food industries as non-toxic, biodegradable and biocompatible polymers for a large number of applications such as binding, thickening, emulsifying and gelling agent, among others (Sharma & Ahuja, 2011). As an additive in pharmaceutical applications, researchers have developed pectin as a drug delivery system (DDS) for controlled drug release (Liu, Fishman, & Hicks, 2007). Pectin formulations are easily tailored into gels, 3-D matrices, films, micro- or nano-particles. Various drugs can be incorporated into pectin formulations with high loading efficiency using simple procedures. However, the commercial potential pectin in DDS technology has yet to be realized. This is partially because of the lack of reproducible performance of pectin formulations. The obstacles include the large diversity in pectin's molecular characteristics, which generates difficulty in quality control and quality assurance during the intermediate preparation of pectin derivatives and in the final products. The solutions to resolve these problems fall into two categories: the development of new technologies for pectin isolation, purification, and characterization and the modification of pectin macromolecules (Liu, Fishman, & Hicks, 2007).

Chemical modifications of pectin have improved the polysaccharide for several specific applications. Pectin derivatives carrying a higher charge density (either positive primary amine or negative carboxyl groups) were shown to enhance penetration of incorporated drugs in tissues. A high degree of methyl esterification (DE) allows pectin derivatives to better retain incorporated drugs (Liu, Fishman, & Hicks, 2007). One obstacle in applying pectin gels for colon-specific drug delivery is their high swelling behavior in physiological environments. To overcome this problem, pectin has been used in combination with polyacrylate derivatives or with hydroxypropylmethyl cellulose and chitosan (Liu, Fishman, Hicks, Kende, & Ruthel, 2005). These strategies enhance the water resistance of the resultant pectin complexes to some degree. However, improved pectin-based drug delivery systems are still desired.

Pectins are galacturonans and are considered to be polymers of D-galacturonic acid. They have linear areas of galacturonan and rhamnogalacturonan (smooth regions) and branched areas (hairy or ramified regions) represented by rhamnogalacturonan I and rhamnogalacturonan II (RGI and RGII) with lateral chains of galactan, arabinan, arabinogalactan, and other more complicated

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http://dx.doi.org/10.1016/j.carbpol.2013.12.071

fragments. The galacturonic acid of the backbone is partially methyl esterified (Albersheim et al., 1994). Low methyl-esterified pectin with a DE less than 50% can form rigid gels via the action of calcium ions or multivalent cations that cross-link the galacturonic acid chains. The diffusion of a molecule through a three-dimensional polymer matrix requires cooperative movement of several segments of the polymer chain. The molecular flexibility (which depends on the type and composition of the polysaccharide), the type and extent of cross-linking and the degree of hydration of the polysaccharide matrix significantly influence the effective mesh size of the gel and, therefore, molecular diffusion in the gel. Alginate and pectins can be gelled at low pH or crosslinked by calcium. Calcium pectinate hydrogels are stable in low pH solutions and have been investigated as a carrier material in different controlled release systems (Sriamornsak & Kennedy, 2010).

The characteristics of the final gel and its functional properties depend on many factors, especially the chemical fine structure of pectin and the system composition (e.g., pH, soluble solids, and pectin content) (Fraeye, Duvetter, Doungla, Loey, & Hendrickx, 2010). In this connection, the controllable swellability and degradability of the gel may be realized through alteration of the sugar composition of pectin.

The conditions of plant cell cultivation (phytohormones, carbohydrates, nitrogen, and calcium concentration) are known to affect the composition of the cell wall (Blaschek & Franz, 1983; Konno, Nakashima, Maitani, & Katoh, 1999; Masuda, 1990; Nevins, English, & Albersheim, 1967). As a result, influencing polysaccharide biosynthesis by altering the various components of the nutrient medium would allow pectins to be obtained with a modified structure and produce certain characteristics and functional properties in the final pectinate gel. Previously, we demonstrated that hormonal factors (Günter & Ovodov, 2001), carbohydrates (Günter & Ovodov, 2002; Günter & Ovodov, 2003), calcium, phosphate and nitrogen (Günter & Ovodov, 2005) influence the sugar composition of the pectin silenan from campion callus *Silene vulgaris* (M.) G. (*Oberna behen* (L.) I.). The silenan macromolecule consists of linear and ramified regions (Ovodova, Bushneva, Shashkov, & Ovodov, 2000; Bushneva et al., 2006). The linear region consists of α-1,4-D-galacturonan and α-1,2-rhamno-α-1,4-D-galacturonan, which is also the backbone of the silenan ramified region consisting of rhamnogalacturonan I. The side chains of the ramified region consist of terminal- and α-1,5-linked arabinofuranose and β-1,3-, β-1,4-, and β-1,6-linked galactopyranose residues (Bushneva, Ovodova, Shashkov, Chizhov, & Ovodov, 2003). The preliminary studies of *S. vulgaris* callus indicated the synthesis of polysaccharides possessing immunomodulatory activity (Popov, Popova, Ovodova, Bushneva, & Ovodov, 1999). *S. vulgaris* cultures are considered to be useful as an alternative raw material source for obtaining new valuable pectins and calcium pectinate hydrogels. In the present study, modified pectins obtained from campion callus cultured using various medium nutrients (carbon sources, concentration of sucrose, calcium and 2,4-D) were used to form calcium pectinate gel (CaPG) beads.

The aim of this research is to investigate the swelling properties and morphology of the CaPG beads made from pectins of campion callus cultured using various medium components.

2. Materials and methods

2.1. Materials

Low methyl-esterified pectins with a DE of 7–8% and the specific viscosity 1.2–1.4 dl/g were obtained from the cell walls of *Silene vulgaris* (M.) G. callus cells cultured using various medium nutrients. The pectins obtained from the callus cells cultured using sucrose concentrations of 20, 30 and 100 g/L are referred to as Suc20, Suc30 and Suc100, respectively. The pectins isolated from the callus cells cultured using sucrose (30 g/L), galactose (30 g/L) or sucrose (15 g/L) + glucose (15 g/L) are referred to as Suc30, Gal30 and SucGlc30, respectively. The chemical characteristics of these pectins were previously described (Günter & Ovodov, 2003) (Table 1). The pectins obtained from the callus cells cultured with calcium concentrations of 1.5, 3.0 and 4.5 mM are referred to as Ca1.5, Ca3.0 (corresponding to SucGlc30) and Ca4.5, respectively. The chemical characteristics of these pectins were previously described (Günter & Ovodov, 2005) (Table 1). The pectins isolated from the callus cells cultured with 2,4-dichlorophenoxyacetic acid (2,4-D) concentrations of 0, 1.0 and 2.0 mg/L are referred to as 2,4-D0, 2,4-D1.0 (correspond to SucGlc30) and 2,4-D2.0, respectively. The chemical characteristics of these pectins were previously described (Günter & Ovodov, 2001) (Table 1). Commercial apple pectin (AP) (Classic AU 701) with a DE of 36–44% and the specific viscosity 6.6 dl/g was purchased from Herbstreith & Fox, Germany. All other chemicals were of analytical grade.

The calcium content in extracted pectin samples was determined using roentgen fluorescent analyser (Rigaku Corporation, Japan) and was equal to 0.003–0.259 μM/mg pectin.

2.2. Preparation of conventional calcium pectinate gel (CaPG) beads

Conventional CaPG beads were prepared using the ionotropic gelation method, which was previously described (Sriamornsak & Nunthanid, 1998; Sriamornsak, 1999). Briefly, 30 mg of each pectin was dissolved in water (1 mL). The solutions were extruded using a nozzle with a 0.6-mm inner diameter into a 0.34 M calcium chloride solution with gentle agitation at 24 °C. The distance from the nozzle to the calcium chloride solution was 5 cm. The gel beads formed were allowed to be in the solution for 20 min, followed by separation, and the beads were washed twice with distilled water. The prepared beads were dried at 37 °C for 12 h. The moisture content of 15 individual particles of each type was determined gravimetrically by drying at 130 °C until constant weight.

2.3. Study of the size and morphology of the gel beads

Fifteen individual dried hydrogel beads of each pectin were characterized according to projected equivalent diameter (the diameter of a circle with the equivalent area), aspect ratio (the longest Feret's diameter (length)/the shortest Feret's diameter (width)), total surface area and volume using a optical microscope (Altami, Russia) fitted with a camera and an image analysis system (ImageJ 1.46r program, National Institutes of Health, USA). Under the same optical conditions, an image of a linear scale was used for calibration. One pixel corresponds to 0.024 mm. Standard error of measurement was 0.012 mm.

Surface morphology was observed under a scanning electron microscope (SEM) (JEOL, JSM6510LV, USA) at 15 kV. The samples were coated with platinum (ion sputter, 30 s) for the SEM observations. Digital images were collected at magnifications of 50× and 5000×. The elemental analysis of the cross-sections was done by energy-dispersive X-ray spectroscopy (EDX).

2.4. Swelling characterization of the gel beads

Fifteen individual dried CaPG beads of each pectin were swelled in solutions of HCl at pH 2.0 or 4.0 and in 0.9% NaCl at pH 6.0 for 0.5, 1.0, 2.0, 4.0, and 24 h. The beads soaked in the solutions were shaken (100 rpm) in an orbital shaker incubator (Titramax 1000, Heidolph, Germany) at 37 °C. Thereafter, the projected equivalent diameter was measured as described in Section 2.3. The swelling degree (SD,

Table 1
Characterization of pectins of *S. vulgaris* callus cells cultured with various medium nutrients.

Pectin	Contents (%)								Mw, kDa	Mn, kDa	Mw/Mn
	GalA	Gal	Ara	Rha	Glc	Xyl	Man	Protein			
SucGlc30 (=Ca3.0; 2,4-D1.0)	68.3	1.9	1.2	1.0	1.7	0.5	0.7	11.8	263.0	68.9	3.82
Suc20	80.5	2.9	2.8	1.6	1.6	1.1	1.2	15.7	168.1	45.9	3.67
Suc30	70.0	1.6	0.9	0.6	1.5	0.5	0.7	12.9	179.0	38.1	4.70
Suc100	57.8	2.6	3.7	1.4	2.2	0.9	1.2	21.5	317.3	56.9	5.58
Gal30	79.0	3.2	1.8	1.2	0.9	1.0	0.7	13.0	178.0	34.4	5.18
Ca1.5	68.2	4.4	2.8	1.4	2.3	0.8	1.0	16.0	175.5	30.5	5.76
Ca4.5	78.1	2.3	1.4	1.0	1.2	0.4	0.7	20.6	136.8	41.5	3.30
2,4-D0	65.7	3.6	1.9	1.0	1.4	0.3	1.1	12.3	80.1	20.3	3.95
2,4-D2.0	62.3	2.8	2.4	1.1	3.4	1.1	1.5	15.8	159.4	24.2	6.60

Note: SucGlc30 – sucrose 15 g/L + glucose 15 g/L; Suc20 – sucrose 20 g/L; Suc30 – sucrose 30 g/L; Suc100 – sucrose 100 g/L; Gal30 – galactose 30 g/L; Ca1.5 – calcium 1.5 mM; Ca3.0 – calcium 3.0 mM; Ca4.5 – calcium 4.5 mM; 2,4-D0 – 2,4-D 0 mg/L; 2,4-D1.0 – 2,4-D 1.0 mg/L; 2,4-D2.0 – 2,4-D 2.0 mg/L.

in %) was calculated using the following equation (Oliveira, Ferrari, Carvalho, & Evangelista, 2010):

$$SD = \frac{D_1 - D_0}{D_0} \times 100\%$$

where D_1 is the diameter of the particle after a determined contact time with the liquid and D_0 is the initial diameter.

2.5. Statistical analysis

The results are given as the mean ± standard deviation. The statistical significance of the differences between two means was evaluated by Student's *t*-test and a value of $p < 0.05$ was considered significant. Statistical calculation was performed with the Excel Microsoft software (version Windows 2002).

3. Results and discussion

3.1. Bead formation and size of the CaPG beads

The CaPG beads were prepared by dropping the pectin solution into calcium chloride solution. 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 pectin molecules as previously described (Chambin, Dupuis, Champion, Voilley, & Pourcelot, 2006; Sriamornsak, Thirawong, Cheewatanakornkool, Burapapadh, & Sae-Ngow, 2008). The pectins obtained from the cell walls of the callus cells cultured with various medium nutrients (carbon sources, concentration of sucrose, calcium and 2,4-D) were used for preparation of the CaPG beads.

The moisture content of the beads varied from 2% to 4% and (data not shown), and no correlation could be observed between type of pectin and moisture content. Upon air drying, pectin beads became small and dense microspheres (density was 0.69–1.48 mg/mm^3). The projected equivalent diameter, aspect ratio, total surface area and volume of the dried CaPG beads obtained are shown in Table 2. The mean diameter (0.73–1.54 mm), total surface area (1.68–8.21 mm^2) and volume (0.17–1.76 mm^3) of the dried beads varied depending on the plant cell culture conditions. In particular, the diameter (1.54 ± 0.14 mm), total surface area (8.21 ± 0.76 mm^2) and volume (1.76 ± 0.33 mm^3) of the 2,4-D0 beads were the largest. The diameter (1.50 ± 0.15 mm), total surface area (8.36 ± 1.23 mm^2) and volume (1.92 ± 0.46 mm^3) of the dried beads obtained from apple pectin AP were reliable larger than those of the dried beads formed from different pectins obtained from plant cells cultured using various nutrient media conditions with the exception of the 2,4-D0 CaPG beads. The aspect ratio for the tested beads was 1.15–1.39 indicating acceptable roundness.

3.2. Morphological analysis of the CaPG beads by SEM

SEM images illustrating the external structure of the dry CaPG beads are shown in Figs. 1 and 2. The dry CaPG beads formed from various pectins of plant cells cultured using different nutrition media differed in shape and surface macrorelief (Fig. 1) as well as surface microrelief (Fig. 2).

The concentrations of sucrose in media and carbon sources (sucrose, sucrose + glucose, galactose) influenced the surface morphology of the CaPG beads. Increasing sucrose contents in the plant cell culture media led to the modification of the microrelief of the CaPG bead surfaces. In particular, the CaPG beads had a more rough and grooved surface with increasing sucrose concentration from 20 to 100 g/L (Fig. 2b–d). The Suc100 CaPG beads displayed the most heterogeneous surface (Fig. 2d). The SucGlc30 and Gal30 CaPG beads were observed to have similar microrelief, which was characterized as a smooth surface (Fig. 2a and e).

Although the surface macrorelief appearance of the Ca1.5 CaPG beads (Fig. 1f) was different from the Ca3.0 CaPG beads (Fig. 1a), including a grooved surface, the microrelief appearance was similar (smooth surface) (Fig. 2a and f). The CaPG beads had a more rough and grooved surface with increasing of calcium concentration up to

Table 2
Characterization of the dried CaPG beads.

Pectin	Diameter (mm ± SD)	Aspect ratio ± SD	Total surface area (mm^2 ± SD)	Volume of the CaPG beads (mm^3 ± SD)
SucGlc30 (=Ca3.0; 2,4-D1.0)	0.81 ± 0.06	1.21 ± 0.17	2.27 ± 0.48	0.28 ± 0.09
Suc20	0.73 ± 0.09	1.39 ± 0.29	1.68 ± 0.34	0.17 ± 0.05
Suc30	0.82 ± 0.05	1.32 ± 0.25	2.65 ± 0.43	0.35 ± 0.08
Suc100	0.87 ± 0.08	1.25 ± 0.17	2.91 ± 0.35	0.40 ± 0.09
Gal30	0.74 ± 0.11	1.32 ± 0.20	2.71 ± 0.27	0.35 ± 0.06
Ca1.5	0.96 ± 0.08	1.28 ± 0.16	3.39 ± 0.64	0.51 ± 0.17
Ca4.5	1.05 ± 0.07	1.15 ± 0.15	4.18 ± 0.47	0.69 ± 0.11
2,4-D0	1.54 ± 0.14	1.18 ± 0.12	8.21 ± 0.76	1.76 ± 0.33
2,4-D2.0	0.98 ± 0.04	1.30 ± 0.09	3.68 ± 0.49	0.56 ± 0.13
AP	1.50 ± 0.15	1.29 ± 0.22	8.36 ± 1.23	1.92 ± 0.46

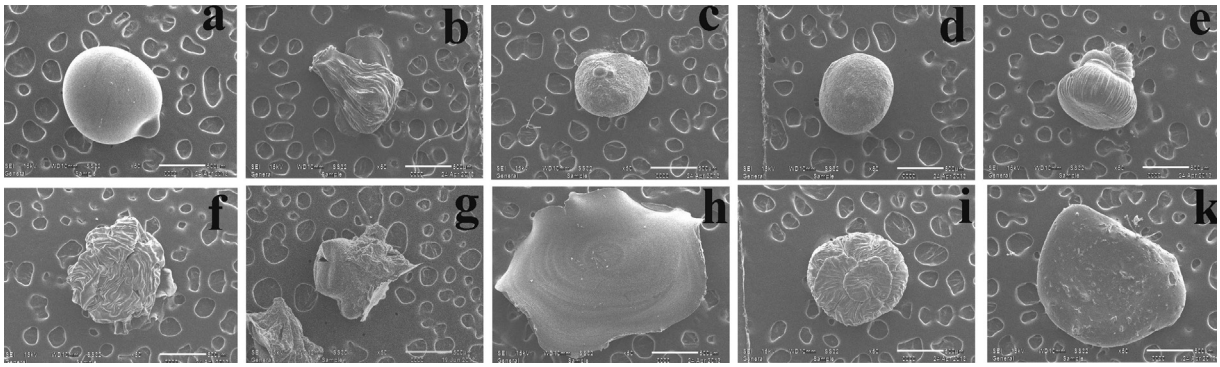

Fig. 1. Scanning electron micrographs of the CaPG beads: (a) SucGlc30, (b) Suc20, (c) Suc30, (d) Suc100, (e) Gal30, (f) Ca1.5, (g) Ca4.5, (h) 2,4-D0, (i) 2,4-D2.0, (k) AP; magnification 50×, scale bar 500 μm.

4.5 mM (Figs. 1g and 2g). The microrelief appearance of the Ca4.5 CaPG beads was the most heterogeneous (Fig. 2g).

The surface morphology of the CaPG beads was demonstrated to depend on the presence of auxin 2,4-D in the plant cell culture media. Pectins obtained from the cell walls of callus cells cultured in medium without auxin 2,4-D (2,4-D0) formed flat CaPG beads with no spherical shape (Fig. 1h). Microrelief modification of the CaPG beads surface was observed. In particular, microforms in the form of grains (size 0.5–2.0 μm) were observed on the surface of the beads (Fig. 2h). Increasing the 2,4-D concentration in the media for plant cell culture up to 2.0 mg/L led to groove modification of the macrorelief of the CaPG bead surfaces (Fig. 1i). However, the microrelief appearance was similar (smooth surface) for both the 2,4-D1.0 and 2,4-D2.0 beads (Fig. 2a and i).

The CaPG beads obtained from apple pectin AP possessed a spherical shape and a smooth-surface microrelief (Fig. 2k).

The elemental analysis obtained by EDX showed that external layer of the beads is composed of oxygen, carbon, chlorine and calcium. The content of Ca was found to be identical irrespective of pectin type and ranging from 11% to 14%.

3.3. Swelling behavior of the CaPG beads

The concentration of sucrose in media and carbon sources (sucrose, sucrose + glucose, galactose) was observed to influence the properties of the CaPG beads. The swelling of dried Suc20 CaPG beads occurred quite rapidly and reached a maximum within 4, 0.5 and 1 h at pH 2, pH 4 and pH 6, respectively (Fig. 3). The swelling degree of the Suc20 CaPG beads soaked for 4, 0.5 and 1 h in HCl (pH 2), HCl (pH 4) or NaCl (pH 6) was 40%, 36% and 58%.

The swelling of dried Suc30 and Suc100 CaPG beads occurred slowly and reached a maximum at 4–24 h at pH 2, pH 4 and pH 6 (Fig. 3). The swelling degree of Suc30 and Suc100 CaPG beads soaked for 24 h in HCl (pH 2) was 44% and 40% (Fig. 3a). The swelling degree of Suc30 and Suc100 CaPG beads soaked in HCl (pH 4) was changed slightly (10% and 13%) and reached a maximum within 4 and 24 h, respectively (Fig. 3b). The swelling degree of Suc30 and Suc100 CaPG beads soaked in NaCl (pH 6) was 35 and 40% and reached a maximum within 4 and 24 h, respectively (Fig. 3c).

Suc20, Suc30 and Suc100 CaPG beads swelled less when placed in acidic media (HCl (pH 2) and HCl (pH 4) (Fig. 3a and b) and swelled most extensively in NaCl (pH6) (Fig. 3c). These findings are in agreement with the results of Sriamornsak and Kennedy (2008). Immersion of the CaPG films in electrolyte solutions allowed ion-exchange reactions to be established and during exposure for up to 4 h, there was extensive displacement of calcium from the pectic films. In water, the greatest calcium loss was approximately 20%. However, the addition of 0.1 M NaCl caused significantly greater losses of calcium (70–90%) irrespective of the type of CaPG. The exchange of the bound calcium ions with the sodium ions in solution leads to the partial formation of hydrophilic and poorly cross-linked pectinate films, followed by expansion of the gel network as a consequence of extensive water uptake into the gel films (Sriamornsak & Kennedy, 2008).

Thus, an increase of the sucrose concentration in the plant cell culture media affect the chemical characteristics of pectin and subsequently, the pectins with different characteristics result in modification of the swelling properties of the CaPG beads. In particular, the quantities of the galacturonic acid residues decreased from 81% to 58% and Mw increased from 168 to 317 kDa in the samples of silenan with increasing sucrose concentration in medium from 20 to 100 g/L (Table 1). The arabinose:galactose ratio was 1:1.0 and 1:1.8 at sucrose concentrations of 20 and 30 g/L, respectively, and this ratio was 1:0.7 in the presence of 100 g/L of sucrose (Günter

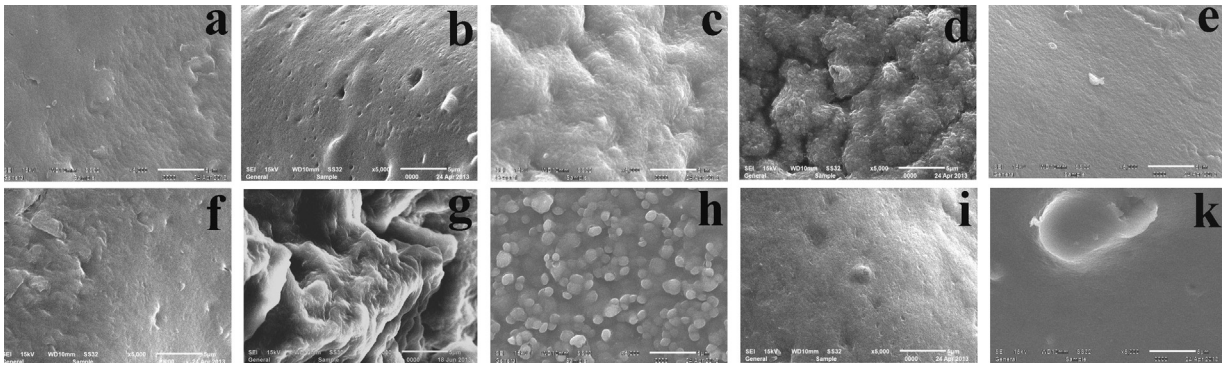

Fig. 2. Microrelief appearances of the CaPG bead surfaces: (a) SucGlc30, (b) Suc20, (c) Suc30, (d) Suc100, (e) Gal30, (f) Ca1.5, (g) Ca4.5, (h) 2,4-D0, (i) 2,4-D2.0, (k) AP; SEM images, magnification 5000×, scale bar 5 μm.

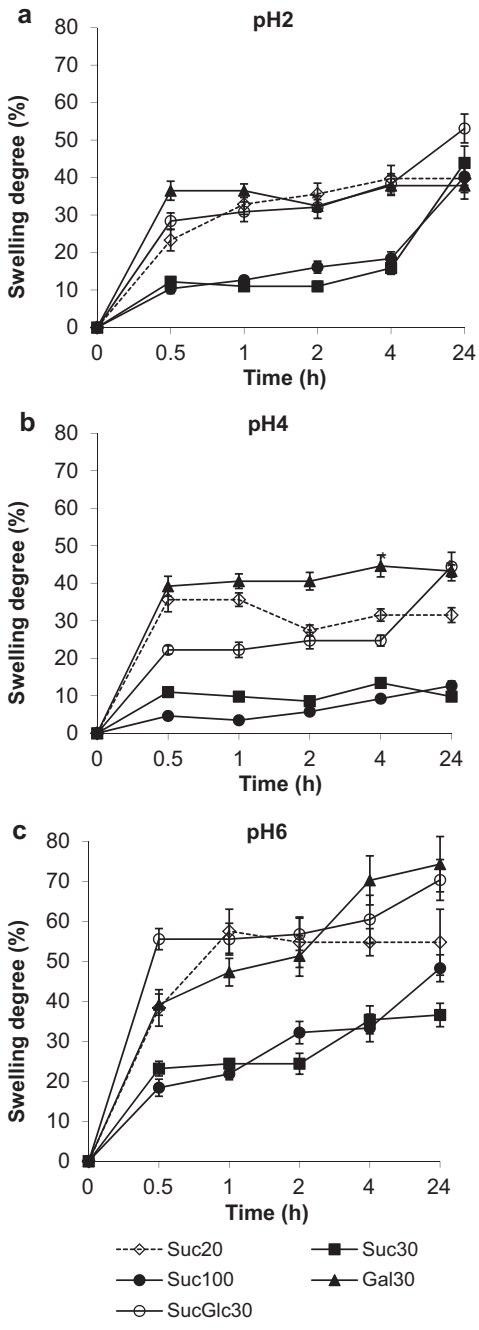

Fig. 3. Swelling measurement of the calcium pectinate gel beads in pH 2 (a), pH 4 (b) and pH 6 (c) solutions obtained from the pectins of *S. vulgaris* callus cells cultured with different medium concentrations of sucrose and carbon sources.

& Ovodov, 2003). The swelling of dried CaPG beads (Suc30 and Suc100) occurred more slowly (within 4 and 24 h) with increasing sucrose concentration. The slow swelling of the dried Suc30 and Suc100 CaPG beads appeared to be related to refer above chemical characteristics of pectins and their grooved surfaces, which is in agreement with the data of Sharma and Ahuja (2011) who demonstrated that beads of thiolated pectin had a rough and grooved surface but failed to display a large amount of swelling.

In a comparison with the Suc30 CaPG beads, the Gal30 and SucGlc30 CaPG beads swelled the most extensively. The swelling of dried Gal30 CaPG beads reached a maximum within 0.5 h at pH 2 and pH 4. The swelling degree of the beads soaked for 0.5 h in HCl (pH 2) and HCl (pH 4) was 37 and 39%, respectively (Fig. 3a and b). The Gal30 CaPG beads soaked in NaCl (pH 6) swelled gradually (Fig. 3c). Although the Gal30 CaPG beads swelled most extensively in NaCl (pH 6), they required 24 h to reach equilibrium. The swelling degree of the Gal30 CaPG beads soaked for 24 h in NaCl was 74%.

The swelling of the dried SucGlc30 CaPG beads reached a maximum within 0.5 and 24 h at pH 2, pH 4 and pH 6 (Fig. 3). Our data on a rapid swelling of the CaPG beads within 0.5 h and then a gradual swelling ones are in agreement with the results of other researches (Liu et al., 2005; Liu, Fishman, & Hicks, 2007; Oliveira et al., 2010). The swelling degree of the SucGlc30 CaPG beads soaked for 0.5 h in HCl (pH 2), HCl (pH 4) and NaCl (pH 6) was 28, 22 and 56%, respectively (Fig. 3). The swelling degree of the SucGlc30 CaPG beads immersed for 24 h in HCl (pH 2), HCl (pH 4) and NaCl (pH 6) was 53%, 44% and 70%, respectively (Fig. 3). The SucGlc30 CaPG beads swelled most extensively in NaCl (pH 6). The SucGlc30 and Gal30 CaPG beads had a smooth surface (Fig. 2a and e) and swelled more extensively compared with the Suc30 CaPG beads that had a rough and grooved surface.

Previously, we demonstrated that pectins from the campion callus cells cultured using different sucrose concentrations and carbon sources had similar qualitative sugar compositions and were distinguished in terms of the ratio of sugar residues and galacturonic acid contents (Günter & Ovodov, 2003). The neutral sugar:galacturonic acid ratio was 1:12, 1:9 and 1:10 when using sucrose, galactose and sucrose + glucose, respectively, as carbon sources. The slow swelling of the Suc30 CaPG beads in a comparison with the SucGlc30 and Gal30 beads appeared to be related to the high ratio of the neutral sugar:galacturonic acid in the Suc30 pectin. It appears that the functional properties of the studied gels are determined by pectin structural characteristics. It is likely that differences between the pectins obtained from plant cells cultured using various concentrations of sucrose and carbon sources in the medium contribute to the different responses of the CaPG beads to the electrolytes used.

The swelling of the dried Ca1.5 CaPG beads as well as the Ca3.0 (=SucGlc30 CaPG) beads occurred similar and reached a maximum within 0.5 and 24 h at pH 2, pH 4 and pH 6 (Fig. 4). The swelling degree of the Ca1.5 CaPG beads soaked for 0.5 h in HCl (pH 2), HCl (pH 4) and NaCl (pH 6) was 15, 12 and 26%, respectively (Fig. 4). The swelling degree of the Ca1.5 CaPG beads immersed for 24 h in HCl (pH 2), HCl (pH 4) and NaCl (pH 6) was 30%, 19% and 49%, respectively (Fig. 4).

The swelling of the dried Ca4.5 CaPG beads occurred slowly and reached a maximum within 24 h at pH 2 and within 2 h at pH 6 (Fig. 4). The swelling degree of the Ca4.5 CaPG beads soaked for 24 h in HCl (pH 2) and for 2 h in NaCl (pH 6) was 31 and 29%, respectively (Fig. 4a and c). The swelling degree of the Ca4.5 CaPG beads immersed in HCl (pH4) was changed slightly (2–5%) (Fig. 4b). Thus, the Ca4.5 CaPG beads were demonstrated to swell to a lesser extent, especially in HCl (pH 4), compared with the Ca1.5 and Ca3.0 beads.

In a comparison with the Ca1.5 and Ca4.5 CaPG beads, the Ca3.0 beads swelled the most extensively. The intensive swelling of the Ca3.0 CaPG beads appeared to be related to the high Mw (263 kDa) of the Ca3.0 pectin in a comparison with the Ca1.5 (Mw 176 kDa) and Ca4.5 (Mw 137 kDa) pectins (Table 1).

Previously, we demonstrated that the galacturonic acid residues contents in silenan increase with enhancement of the calcium concentration from 3.0 to 4.5 mM in the culture medium (Günter & Ovodov, 2005). The galacturonic acid residues contents in silenan increased up to 78% with 4.5 mM calcium (Table 1). The molecular weight distribution of silenan was demonstrated to shift with 4.5 mM calcium. In a medium containing 4.5 mM calcium, the yield of the dominating fraction with a molecular weight more than 300 kDa against the control increased 25% (data not published). The galacturonic acid residue content in this fraction was high (75%). Evidently, the polymerization of pectin occurred with an increase in

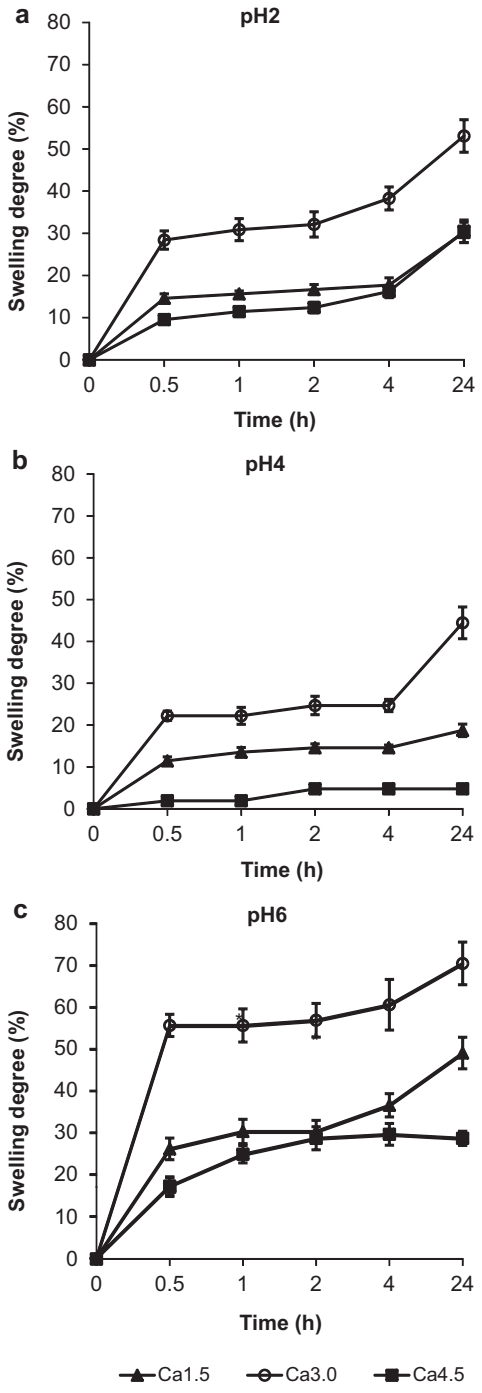

Fig. 4. Swelling measurement of the calcium pectinate gel beads in pH 2 (a), pH 4 (b) and pH 6 (c) solutions obtained from the pectins of S. vulgaris callus cells cultured with different medium concentrations of calcium.

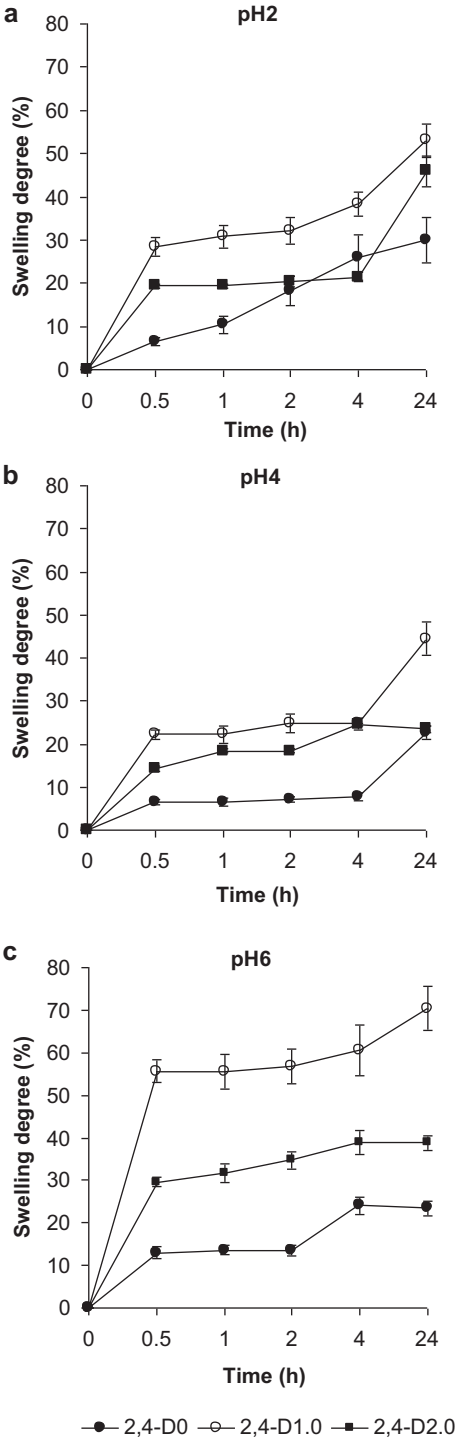

Fig. 5. Swelling measurement of the calcium pectinate gel beads in pH 2 (a), pH 4 (b) and pH 6 (c) solutions obtained from pectins of S. vulgaris callus cells cultured with different medium concentrations of 2,4-dichlorophenoxyacetic acid.

the extent of calcium cross-linking, and an increase in the quantity of the galacturonic acid residues are joined by these linkages.

The expansion of the gel film due to penetration of water is limited by the extent of entanglement and the retractive force within the gelled network (Sriamornsak & Kennedy, 2008). The latter is influenced by the rigidity of the polysaccharide, the extent of calcium cross-linking and any additional inter- or intra-molecular associations.

Enhancement of the extent of cross-linking would be expected to lead to an increased retractive force and allow less HCl and NaCl to be absorbed. Therefore, the reduced swelling of the Ca4.5 CaPG beads in HCl and NaCl, compared with the Ca1.5 and Ca3.0 beads, could be due to a higher number of calcium cross-linking. Moreover, the slow swelling of the dried Ca4.5 CaPG beads appeared to be related to their grooved surfaces (Fig. 2g).

Fig. 5 shows the effect of the concentration of auxin 2,4-D in the plant cell culture media on the properties of the CaPG beads obtained from the cell wall pectins. The swelling of dried 2,4-D2.0 CaPG beads as well as 2,4-D1.0 (=SucGlc30 CaPG) occurred similar and reached a maximum within 0.5 and 24 h at pH 2 (Fig. 5a). The

swelling degree of the 2,4-D2.0 CaPG beads soaked for 0.5 and 24 h in HCl (pH 2) was 19 and 46%, respectively. At pH 4 and pH 6, the 2,4-D2.0 CaPG beads swelled rapidly within 0.5 h and then swelled gradually and reached a maximum within 4 h (Fig. 5b and c). The swelling degree of the 2,4-D2.0 CaPG beads soaked for 0.5 h and 24 h in HCl (pH4) was 14 and 24%, respectively (Fig. 5b). The swelling degree of the 2,4-D2.0 CaPG beads soaked for 0.5 h and 24 h in NaCl (pH 6) was 30 and 39%, respectively (Fig. 5c).

The swelling of dried 2,4-D0 CaPG beads occurred slowly and reached a maximum within 24 h at pH 2 and pH 4 and within 4 h at pH 6 (Fig. 5). The swelling degree of the 2,4-D0 CaPG beads soaked for 24 h in HCl (pH2 and pH4) was 30% and 23%, respectively (Fig. 5a and b). The swelling degree of the 2,4-D0 CaPG beads immersed for 4 h in NaCl (pH6) was 24% (Fig. 5c).

Thus, the 2,4-D1.0 and 2,4-D2.0 CaPG beads swelled most extensively in comparison with the 2,4-D0 beads. Most likely, the similar swelling of both the dried 2,4-D1.0 and 2,4-D2.0 CaPG beads was connected to its similar surface microrelief appearance (Fig. 2a and i). The slow swelling of the dried 2,4-D0 CaPG beads appeared to be related to their heterogeneous surfaces (Fig. 2h) and low Mw (80.1 kDa) of the 2,4-D0 pectin (Table 1). The highest swelling of the 2,4-D1.0 CaPG beads (Fig. 5) appeared to be related to the higher Mw of the 2,4-D1.0 pectin in a comparison with the 2,4-D2.0 and 2,4-D0 pectins (Table 1).

Previously, we demonstrated that the a 2-fold increase in arabinose residue content was observed in 2,4-D2.0 pectin with enhancement of the 2,4-D concentration up to 2.0 mg/L in the culture medium (Günter & Ovodov, 2001). The data obtained demonstrated that the higher concentration of 2,4-D supported the biosynthesis of pectin with higher arabinose residue content.

A study of the 2,4-D effect on the polysaccharide biosynthesis in sycamore cell culture has demonstrated that 2,4-D contributes to the transfer of arabinose from UDP-arabinose to arabinogalactan and, thus, the amount of branched arabinose groups, attached to the galactan core, increased (Rubery & Northcote, 1970). Arabinogalactan is the origin of the neutral sugar side chains of pectin. Large amounts of side chains are likely to cause steric hindrance, which may hamper pectin–pectin interaction (Fraeye et al., 2010). The neutral sugar side chains increase solubility and reduce intrinsic viscosity but contribute to polymer entanglement, resulting in higher viscosity at a high polymer concentration followed by further implication in the water-binding capacity of pectic polymers (Ulvskov et al., 2005). The higher arabinose residue content in the side chains of silenan likely causes an increase of the water-binding capacity of pectin and swelling of the CaPG beads.

All beads swelled less when placed in acidic media (HCl (pH 2) and HCl (pH 4)) (Figs. 4a, b and 5a and b) and swelled most extensively in NaCl (pH 6) (Figs. 4c and 5c).

The dried beads obtained from apple pectin AP swelled gradually and reached a maximum within 24 h (Fig. 6). The swelling degree of the AP CaPG beads immersed for 24 h in HCl (pH 2), HCl (pH 4) and NaCl (pH 6) was 150%, 53% and 111%, respectively. Thus, the apple pectin AP CaPG beads swelled the most compared with the CaPG beads obtained from the plant cells pectins. The rapid swelling of the dried AP CaPG beads appeared to be related to their smooth-surface microrelief (Fig. 2k).

The characteristics of the final gel and its functional properties depend on many factors, especially the chemical fine structure of pectin and the system composition (e.g., pH, soluble solids, and pectin content) (Fraeye et al., 2010). The gelling mechanisms of pectins are known to depend on various factors, such as distribution of charge on the pectin backbone (Löfgren, Guillotin, & Hermansson, 2006), which are related to the insertion of the α-L-rhamnose and other neutral sugars into the backbone (Sriamornsak & Kennedy, 2008). It is likely that these differences between the pectins obtained from plant cells cultured using various nutrient

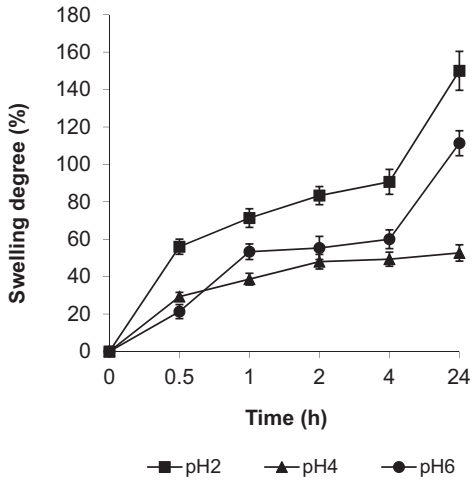

Fig. 6. Swelling measurement of the calcium pectinate gel beads obtained from apple pectin AP in pH 2, pH 4 and pH 6 solutions.

media compositions and AP contribute to the different responses of the pectins to the electrolytes used in this study.

4. Conclusion

The plant cell culture conditions affect the chemical characteristics of pectin and subsequently, the pectins with different characteristics result in different CaPG bead properties. In particular, the swelling properties as well as the macro- and microrelief appearance of the surface of the gelled beds obtained from different pectins were demonstrated to alter. An applied strategy altered the swelling behavior of the CaPG beads and enhanced the acid and water resistance of the resultant pectinate hydrogels in physiological environments. In particular, the swelling of the Ca4.5, 2,4-D0, Suc30 and Suc100 CaPG beads occurred more slowly. The slow swelling of the dried CaPG beads appeared to be related to their grooved surfaces. These pectinate gels have potential application as a carrier material in different controlled release systems, especially in the use of gels for colon-specific drug delivery.

Acknowledgement

The study is supported by Program of UD RAS, projects No 12-C-4-1006 and No 12-P-4-1033).

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