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Influence of low methoxyl pectin gel textures and *in vitro* release of rutin from calcium pectinate beads

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ABSTRACT

This study described the preparation and characterization of low methoxyl pectin (LMP) gels and beads for controlled release applications. The rheological characterization of the various formulations was proposed. Then the mechanical and morphological characterizations of beads were determined. Finally, the controlled release studies taking rutin as a model drug was evaluated. The results showed that Young's modulus values of non-amidated LMP gels decrease when adding up to 15% sorbitol. Calcium pectinate beads loaded with rutin are about 600 μm, oblong shaped with dense matrix. Beads containing sodium bicarbonate showed about 80% lower rutin encapsulation efficiency by increasing the pH of the cross-linking solution. The rutin loaded in non-amidated pectinate beads containing 15% sorbitol showed the best release efficiency and swelling behavior. Therefore, the gel texture affects the release rate of the active compounds encapsulated in calcium pectinate beads and can be used as a parameter to modulate drug release.

1. Introduction

Microencapsulation technology has developed significantly and is applied in various fields such as pharmaceuticals, cosmetics, and food. This allows for controlled release of various drugs, enhanced stability of formulations and flavor masking (Madene, Jacquot, Scher, & Desobry, 2006). Moreover, microencapsulation provides a useful technique to protect active molecules from environmental conditions (*e.g.* sensitivity to air/oxygen, heat and light, enzyme, acidic pH in stomach), to extend shelf-life (Favaro-Trindade, Pinho, & Rocha Revisão, 2008; Gouin, 2004). Microencapsulation can also be employed to modulate drug release. The ability of pectin to form rigid gels with divalent cations has also been exploited in the manufacture of microparticles or beads by ionotropic gelation and emulsification technique (Assifaoui, Chambin, & Cayot, 2011; Hagesaether, Bye, & Arnesande, 2008; Munjeri, Collet, & Fell, 1997; Sriamornsak, 1998; Wong, Chan, Lee, & Heng, 2002).

Pectin, one of the main structural water-soluble polysaccharides of plant cell walls, is composed of linear chains of (1 → 4)-linked α-D-galacturonic acid residues. These uronic units have carboxyl groups, some of which naturally present as methyl ester and others which react with ammonia to produce amide groups. The composition of pectin depends on the plant source and conditions employed during pectin isolation and purification. The degree of esterification (DE) and degree of amidation (DA), which are both as a percentage with respect to total carboxyl groups content are important in pectin classification. Pectins, in which the degree of esterification (DE) of the galacturonic acid residues is higher than 50%, are known as high methoxyl pectin (HMP) and those less than 50% are regarded as low methoxyl pectin (LMP) (Rolin, 1993; Ridley et al., 2001). Amidated and non-amidated LMP are used in preparation of gel by cross-linking with divalent cations such as calcium. The gel formation process involves the simultaneous bonding of calcium ions to carbonyl groups of two adjacent pectin molecules and two hydroxyl groups from one of the molecules (Smidsrod, Haug, & Whittington, 1972). An eggbox-like model was proposed to explain the structure of pectin molecules bound by the calcium ions (Grant, Morris, Rees, Smith, & Thom, 1973). This model is correct for alginate, but for pectin it can be an approximation of the gelation mechanism. Moreover, the texture of gel can be changed by adding various compounds such as fructose or sorbitol. When sugars are added, pectin and sugar molecules compete for cations (Ca^{2+}). This interaction is unfavorable to the formation of the gel and therefore decreases the gel rigidity (Grosso, Bobbio, & Airoldi, 2000). Incorporation of carbonate salts such as sodium bicarbonate, a gas-forming agent, into pectin solution prior to gel formation resulted in porous and fragile gel structure (Sriamornsak, Sungthongjeen, & Puttipipatkhachorn, 2007). Few studies have focused on the

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influence of theses compounds on the release profile of the encapsulated molecules when they are added to produce the beads.

Rutin (quercetin-3-*O*-rutinoside), the flavonol glycoside of quercetin, is abundantly found and distributed in plants such as buckwheat seed, fruits, and fruit rinds, especially citrus fruits (orange, grapefruit, lemon). It presents important properties in human health like its significant scavenging properties on oxidizing species such as hydroxyl radical, superoxide radical, and peroxyl radical (Calabrò et al., 2005), as shown by many *in vitro* and *in vivo* experiments (Itagaki et al., 2010; Verma, Vijayakumar, Mathela Ch, & Rao Ch, 2009). Furthermore, rutin has several pharmacological activities including anti-allergic, anti-inflammatory, and vasoactive properties (Mauludin, Müller, & Keck, 2009a; Mauludin, Müller, & Keck, 2009b). However, phenolic compounds as well as rutin are generally not chemically stable when exposed to light and oxidation (Cheynier, Rigaud, Souquet, Duprat, & Moutounet, 1990; Liazid, Palma, Brigui, & Barroso, 2007). Thus, microencapsulation techniques using LMP as wall material may be applied for improving the oxidative stability and release of rutin. The aims of this work were to prepare LMP gel formulations, investigate their texture and prepare rutin encapsulated in LMP beads by ionotropic gelation method as well as evaluate the influence of the texture upon *in vitro* release of rutin beads.

2. Materials and methods

2.1. Materials

Amidated and non-amidated low methoxy pectin (LMP) (Unipectine OF305C; DE = 25% and DA = 21%, and OF300 C; DE = 30% and DA = 0%) were purchased from Cargill™ (Saint Germain, France). Rutin hydrate, D-(+)-gluconic acid δ-lactone (GDL) and sodium bicarbonate ($NaHCO_3$) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Calcium carbonate ($CaCO_3$) and Tris (hydroxymethyl) aminomethane were purchased from Merck (Damstadt, Germany). Sorbitol pure anhydrate was purchased from Cooperation Pharmaceutique Francaise (Melun, France). Hydrochloric acid (HCl) 1 N and sodium hydroxide (NaOH) 1 N were purchased from Prolabo® (Fontenay-sous-bois, France). All reagents were analytical grade.

2.2. Rheological behavior of low methoxyl pectin (LMP) solution

The rheological behavior of 4% (w/v) amidated LMP and 2.5–4% (w/v) non-amidated LMP were investigated using the HAAKE Viscotester 550 (Thermo Scientific, France) with spindle and cup sensor at controlled temperature ($T = 25.0 \pm 0.5\,^{\circ}C$). Data analysis was done with HAAKE RheoWin Job Manager Software. The percentage of non-amidated LMP which give the similar rheologic profile with 4% amidated LMP will be selected for further experiments.

2.3. Gel preparation

The various LMP gel formulations describing the percentages (w/v) and compositions are shown in Table 1. Sorbitol and $NaHCO_3$ were selected to reduce the rigidity and increase the porosity of the gels, respectively (Grosso et al., 2000; Sriamornsak et al., 2007). The gels were prepared by dissolving LMP with and without sorbitol and $NaHCO_3$ in de-ionised water using high magnetic stirring. $CaCO_3$ (1.5 g/L) was then added within 1 h of stirring, followed by GDL (4 g/L). The GDL which slowly hydrolyzes in aqueous solution forming gluconic acid resulted in a slow release of calcium ions inducing and forming gels. Five replicates of each gel were prepared.

2.4. Rigidity measurements

Rigidity of each gel was estimated using a TA-XT2*i*® Texture analyzer (Stable Micro Systems, Ltd. Godalming, Surrey, UK). Approximately 25 mL of the gel formulations were filled in plastic bottles and tightly sealed in order to avoid air contamination and assuring generation of a smooth upper surface. A 10-mm (diameter) cylinder was compressed into the gel and redrawn. The method settings, including pre-test, test and post-test speeds were 2.0, 1.0 and 2.0 mm/s, respectively, and distance (depth of the insertion) was set as 30% of deformation. Five replicate analysis were performed at $25.0 \pm 0.5\,^{\circ}C$, providing the same conditions for each measurement. Young's modulus values (Pa) of each gel were calculated from the slope of the resultant force (N) and percentage of deformation curves.

2.5. Preparation of LMP beads by encapsulator

The ionotropic gelation technique of using drug encapsulated in the beads (Aydin & Akbuga, 1996; Bourgeois et al., 2002; El-Gibaly, 2002) was modified as following: LMP aqueous formulations including 3NA, 3NA15Sor, 3NA1Bica and 3NA15Sor1Bica were prepared followed by 2% (w/v) of rutin dispersed in the solution and stirred until a uniform dispersion was obtained. The pH (SevenEasy™, pH meter S20, Schwerzenbach, Switzerland) and viscosity (HAAKE Viscotester 550, Thermo Scientific, France) of the solution were measured. The beads were made using Encapsulator UNIT VAR1 (Nisco engineering Inc., Zurich, Swichzerland) with a nozzle of 0.7 mm inner diameter. The slurry was dropped into 50 mL of a gently agitated solution of the crosslinking agent (2%, w/v $CaCl_2$) at flow rate 100 mL/h with falling distance of 4 cm. The gelled beads were formed immediately and allowed to stand in the cross-linking solution for 10 min. Then beads were separated by filtration, washed with deionized water and dried at $37 \pm 2\,^{\circ}C$ for 24 h in a drying room.

2.6. Microparticles characterization

2.6.1. Morphological studied

Morphological examination of the LMP microparticles were conducted by scanning electron microscopy (SEM) using a FEI LaB6 (Eindhoven, Netherlands) at 14 kV. LMP beads were coated with gold, under vacuum by sputter coating unit (Polaron, Thermo VGScientific, East Grinstead, Sussex). The experiments were performed at magnifications ×150. Size, shape and surface of microparticles were evaluated (Dupuis et al., 2004).

2.6.2. Encapsulation efficiency

The amount of rutin encapsulated into the microparticles was determined by adding 100 mg to each bead into 1 L of phosphate buffer (PB) pH 7.4 for 3 h until disintegrated. The solution was filtered and the absorbance measured at 267 nm by UV spectroscopy (Biochrom Libra S22, Cambridge, England). Rutin contents were determined from the standard curve of rutin in PB, which demonstrated linearly with high correlation ($r^2 = 0.9989$). The following regression equation was obtained: $y = 0.0259x + 0.0108$, where y was the absorbance and x was the concentration of rutin (mg/L). The experiment was done in triplicate. The encapsulation efficiencies (%) were calculated according to the following equation: Encapsulation efficiency (%) = $AQ/TQ \times 100$. Where AQ is the actual quantity of rutin present in the matrices and TQ is the theoretical quantity of rutin.

Table 1
Percentages (w/v) and compositions of LMP gel formulations.

Formulation	% Amidated LMP	% Non-amidated LMP	% Sorbitol	% Bicarbonate
4A	4	–	–	–
3NA	–	3	–	–
3NA5Sor	–	3	5	–
3NA10Sor	–	3	10	–
3NA15Sor	–	3	15	–
3NA20Sor	–	3	20	–
3NA25Sor	–	3	25	–
3NA1Bica	–	3	–	1
3NA15Sor1Bica	–	3	15	1

2.6.3. Water content determination

The water content was determined using a moisture analyzer at 160 °C until a stabilization of weight was achieved (OHAUS MB35, Greifensee, Switzerland).

2.7. In vitro rutin release

The release of rutin from various bead formulations was investigated using an *in vitro* rotating paddle dissolution apparatus (Sotax AT7, Binningerstrasse, Allschwil). The dissolution studies were performed in PB (KH_2PO_4/NaOH 1 N) and Tris (Tris/HCl 1 N) buffers, both at pH 7.4, with a rotation speed of 50 rpm and a temperature of 37 ± 0.2 °C. The precise weight of beads (100 mg) was added to 1 L of dissolution medium. Samples were withdrawn at various time intervals up to 900 min by automatic pump and analyzed spectrophotometrically (Cary 50Bio UV–visible Spectrophotometer, France) at 267 nm. All dissolution runs were performed in triplicate.

2.8. Swelling test

Swelling tests were performed using an optical stereo microscope (magnification ×10) equipped with a micrometric device (CETI, Medline, Sainte Luce Sur Loire, France). The beads were observed in the dry state and after swelling in Tris solutions pH 7.4 at 1, 5, 10, 15, 20, 25 and 30 min, respectively. The percentage of swelling was calculated as follows: percentage of swelling = $D_{swollen}/D_{dried}$ where $D_{swollen}$ and D_{dried} are the longest diameter of the bead in the swollen and dry states, respectively.

2.9. Statistical analysis

The results were presented as the mean of three independent experiments and analyzed by SPSS (version 16.0). ANOVA was used for the analysis of the test results (LSD test) at the significance level of p-value <0.05.

3. Results and discussion

3.1. Rheological behavior of low methoxyl pectin (LMP) solution

The rheological behavior of LMP solution is shown in Fig. 1. All LMP solutions exhibited non-newtonian fluid flow. The viscosities of 4% (w/v) amidated (4A), 2.5, 3 and 4% (w/v) non-amidated (2.5-4NA) LMP were 0.098, 0.080, 0.132 and 2.267 Pa s, respectively. Amidated LMP at concentration 4% (w/v) has been used for producing beads by several authors (Assifaoui et al., 2011; Dhalleine et al., 2011; Dupuis et al., 2004). For the formation of the beads from non-amidated LMP and comparing other behaviors, the similar rheological profile of its solution with amidated LMP was required in order to reduce the influence of the viscosity. In this study, 2.5 and 3NA exhibited the similar viscosity and rheological behavior to 4A. However, 3NA was selected for further experiments.

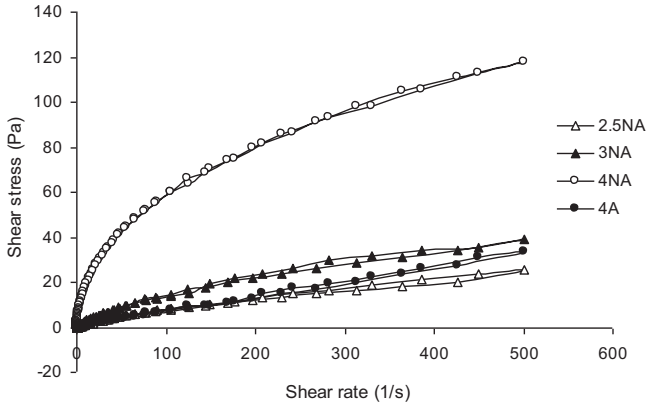

Fig. 1. Rheological behavior of 4% (w/v) amidated LMP (4A) and 2.5–4% (w/v) non-amidated LMP (2.5-4NA).

From our preliminary test, 2.5% (w/v) non-amidated LMP (2.5NA) did not produce beads because of the lower viscosity of its solution.

3.2. Gel preparation and rigidity measurements

When GDL was added into the LMP solution containing $CaCO_3$, calcium ions were released and the gels were obtained by ionotropic gelation mechanism in which intramolecular crosslinks were formed between the negatively charged carboxyl groups of LMP and the positively charged counter ion (Ca^{2+}). The GDL and $CaCO_3$ were selected for use of the gel preparation method in order to slow down the formation and achieve a homogeneous gel. The rigidity of various gel formulations were evaluated as Young's modulus value (Fig. 2). The 4A which was amidated LMP gel exhibited the highest Young's modulus value about 2217 Pa and higher than 3NA (Young's modulus value about 1124 Pa) gel of about 1.97 times. The rigidity of the gel prepared with non-amidated LMP was

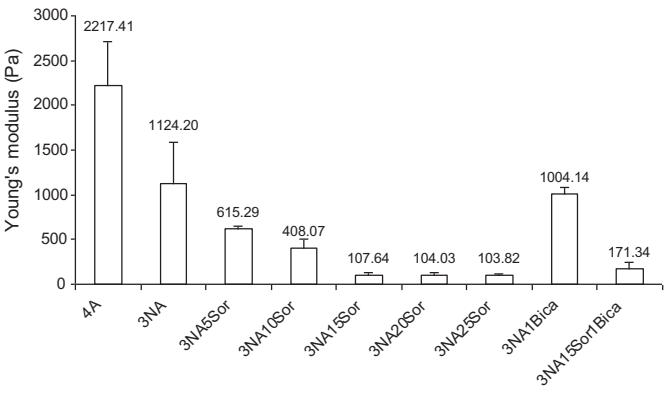

Fig. 2. Young's modulus (Pa) of various gel formulations.

Table 2
Viscosity, pH, encapsulation efficiency and water content of the selected LMP formulations with and without 2% (w/v) of rutin.

Sample	3NA		3NA15Sor1Bica		3NA15Sor		3NA1Bica	
	2%rutin		2%rutin		2%rutin		2%rutin	
	–	+	–	+	–	+	–	+
Viscosity (mPa s)	137.86 ± 47.71	124.44 ± 103.25	47.16 ± 11.23	46.34 ± 6.08	157.71 ± 64.30	159.05 ± 68.45	33.04 ± 7.64	37.87 ± 5.54
pH	4.89 ± 0.02	4.07 ± 0.04	6.88 ± 0.03	6.90 ± 0.02	4.90 ± 0.03	4.82 ± 0.03	7.11 ± 0.09	7.03 ± 0.10
Encapsulation efficiency (%)	–	95.58 ± 1.36	–	79.77 ± 1.45	–	94.43 ± 0.79	–	80.40 ± 0.84
Water content (%)	–	6.11 ± 0.61	–	7.94 ± 0.56	–	9.01 ± 0.35	–	3.07 ± 0.32

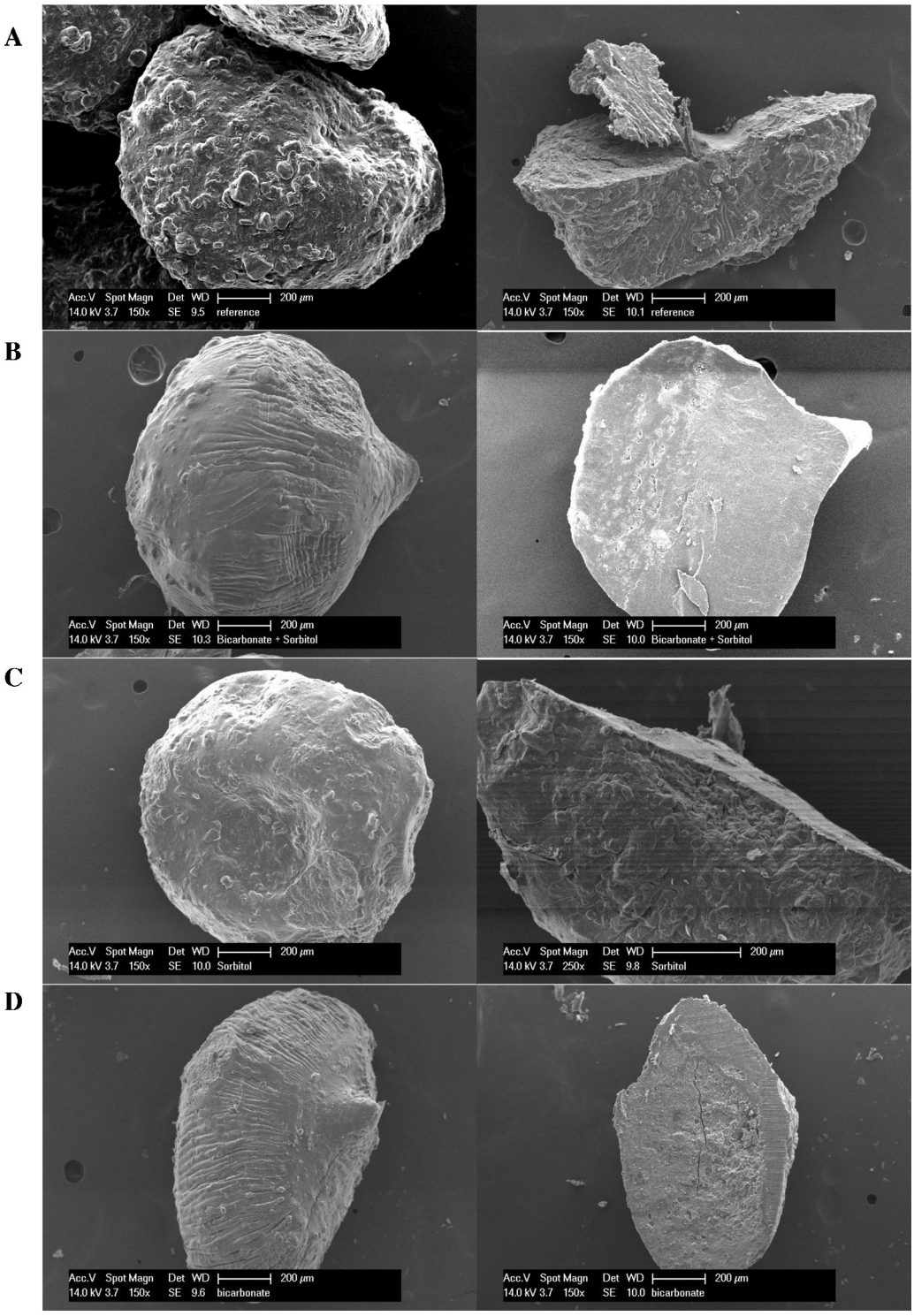

Fig. 3. Scanning electron micrographs of 3NA (A), 3NA15Sor1Bica (B), 3NA15Sor (C) and 3NA1Bica beads (D) (left) and their cross-sections of bead (right) at magnification ×150.

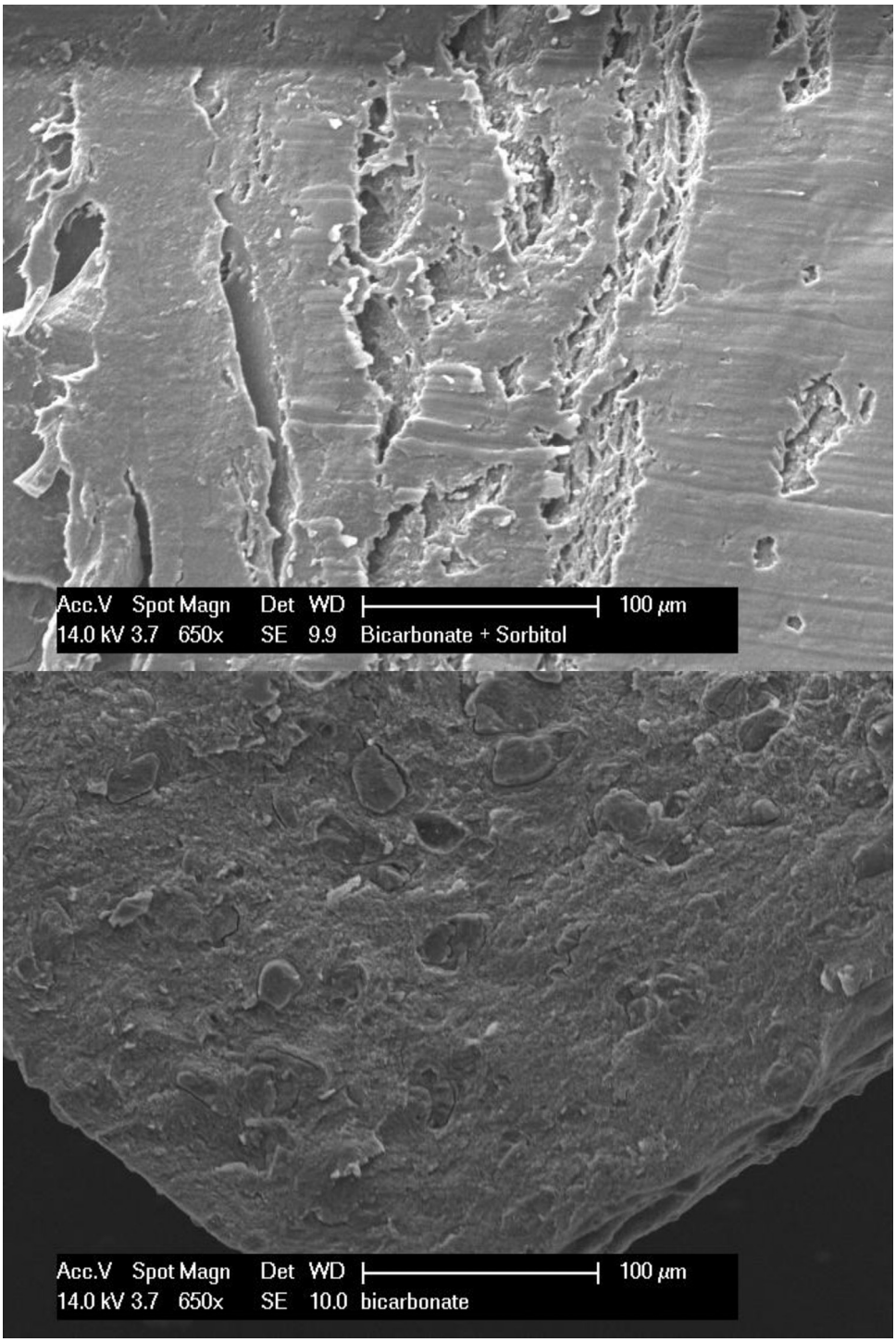

Fig. 4. Scanning electron micrographs of small hollow pockets in 3NA15Sor1Bica (up) and 3NA1Bica (down) matrices at magnification ×650.

decreased by added sorbitol but not $NaHCO_3$ (Young's modulus value about 1004 Pa). The higher percentage of sorbitol showed the lower Young's modulus value. However, for more than 15% of sorbitol, the Young's modulus value of the gels was not significantly different ($p < 0.05$). The gel containing 15% sorbitol combined with 1% $NaHCO_3$ also showed the lower rigidity than the gel containing only 1% $NaHCO_3$ but higher than the gel containing only 15% sorbitol. Amidated LMPs are more prone to form a rigid gel structure with divalent cations than non-amidated LMPs due to more compact Ca- or Zn-pectinate network (Thakur, Singh, & Handa, 1997; Wakerly, Fell, Attwood, & Parkins, 1997). The possibility of bonding between sugar molecules including sorbitol and a metallic ion (Ca^{2+}) was established by Angyal (1972). Pectin and sorbitol molecules could compete for cations. Depending on the sorbitol structure a stable complex can be formed between sorbitol and Ca^{2+}. This interaction can be unfavorable to the gel formation, due to the decrease of Ca^{2+} available to associate with pectin molecules and therefore inducing the decrease of the gel rigidity (Grosso et al., 2000). For $NaHCO_3$, carbon dioxide was generated when in contact with acidic environment of pectin solution increasing porosity and decrease of gel density (Badve, Praveen, Aruna, & Atmaram, 2007). In this study, rigidity of gels containing $NaHCO_3$ was higher than

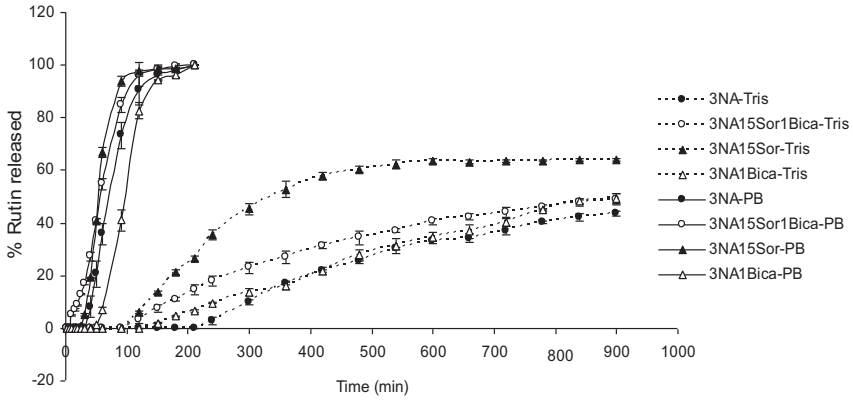

Fig. 5. Dissolution profiles of beads in comparison of phosphates (PB) *versus* Tris buffers.

the gel containing sorbitol determined by Young's modulus value (Fig. 2).

3.3. Microparticle preparation and characterization

The viscosity and pH of selected LMP formulations are shown in Table 2. The viscosity and pH of 3NA were not significantly different ($p < 0.05$) when 15% sorbitol was added, however the viscosity decreased as pH increased significantly, ($p < 0.05$) when $NaHCO_3$ was added as in 3NA1Bica and 3NA15Sor1Bica formulations. When the pectin droplets came in contact with the calcium chloride solution, ionic interaction occurred and formed gelled spheres. These interactions in non-amidated LMP allowed the formation of a compact pectinate network, in which rutin particles were encapsulated. All beads were oblong or spherical shape with around 600 μm size. Scanning electron micrographs of the beads are presented in Fig. 3. The surface of the sphere of 3NA rutin-loaded bead showed a rough and globulous morphology (Fig. 3A), while 3NA15Sor rutin-loaded bead presented a much smoother surface (Fig. 3C). The rutin-loaded with sodium carbonate ($NaHCO_3$) beads (3NA15Sor1Bica and 3NA1Bica) showed oblong shape with wrinkled circumference (Fig. 3B and D). The cross-section of all types of beads exhibited dense and small hollow pockets in their matrices especially 3NA15Sor1Bica and 3NA1Bica beads. This can be due to the gas bubbles formed by the gas-forming agent in the formulations ($NaHCO_3$) increasing porosity of beads (Badve et al., 2007; Gadad, Reddy, Dandagi, & Masthiholimath, 2012; Sriamornsak et al., 2007). The high encapsulation efficiency was observed in 3NA and 3NA15Sor beads (about 95 and 94%, respectively). Low solubility in cross-link solution of rutin was related to its high encapsulation in the beads. Adding sorbitol into 3NA solution did not change the pH of the cross-link solution and had no effect to the encapsulation efficiency. However, the formulation composed with $NaHCO_3$ (3NA15Sor1Bica) and without $NaHCO_3$ (3NA1Bica) revealed lower rutin encapsulation efficiency (about 80%) than 3NA and 3NA15Sor beads. This is due to $NaHCO_3$ in the formulations increasing the pH of $CaCl_2$ solution, the cross-linking solution, and increasing the subsequent diffusion and solubility of rutin into $CaCl_2$ solution during curing time. In fact, rutin is freely soluble in alkaline solutions (Chebil et al., 2007; Miyake et al., 2000; Tommasini et al., 2004). The water content of beads increased when adding sorbitol due to the water binding ability of sorbitol (Cheng, Abd-Karim, & Seow, 2006; Grosso et al., 2000; Labuza, 1985).

3.4. In vitro rutin release

Rutin release of four formulations including 3NA, 3NA15Sor, 3NA1Bica and 3NA15Sor1Bica was assessed *in vitro* in two different dissolution media (pH 7.4); phosphates (PB) and Tris buffers. The dissolution profiles into different dissolution media are presented in Fig. 4. The release profiles are affected by the dissolution media. Rutin was released into Tris-buffer slower than in PB. More than 80% of rutin was released after 120 min and 100% after 210 min in PB dissolution media. Using Tris-buffer as a dissolution media, only 3 and 6% of rutin were released from 3NA15Sor and 3NA15Sor1Bica at 120 min. Less than 65% of rutin release with the remained insoluble beads at the end of experiment (900 min). When PB was used as dissolution media, the release of rutin from Ca-pectinate beads occurred by hydration, swelling and erosion of the pectin matrix. Beads in contact with PB containing K^+, exchanged Ca^{2+} and K^+. A partially soluble pectin region is formed due to displacement of K^+ ions into Ca-pectinate network, which are more permeable. Pores are created in the matrix due to the release of undissolved rutin particles, which allow more PB fluid media penetration into the matrix. The subsequent release of additional rutin particles resulted in the disintegration of the pectin matrix (Assifaoui et al., 2011; Das, Ng, & Ho, 2010; Sriamornsak, 1998). Thus, using PB as a dissolution media does not provide the best release profile of pectinate beads as it induces disintegration of the beads.

However, the orders of rutin released from beads were similar in both dissolution media: 3NA15Sor > 3NA15Sor1Bica > 3NA > 3NA1Bica in PB and 3NA15Sor > 3NA15Sor1Bica > 3NA = 3NA1Bica in Tris-buffer (Fig. 4). Adding 15% of sorbitol into the pectinate bead matrix showed higher and faster release of rutin. This could be linked to the lower Young's modulus value of the 15% sorbitol gel and the water adsorption ability of sorbitol, resulting in the softness of the beads followed by the high release efficiency of rutin.

3.5. Swelling behavior

Phosphates buffer caused the pectinate beads disintegration and therefore cannot be used to study their swelling behavior. Thus, swelling tests were performed using Tris buffer as the swelling medium. The 3NA and 3NA1Bica rutin beads displayed a small percentage of swelling at the final time (30 min) were 20.41 ± 2.69 and 19.68 ± 3.51%, respectively, compared with 3NA15Sor beads which reached the highest percentage of swelling (34.51 ± 5.21%), but not significantly higher than 3NA15Sor1Bica (percentage of swelling was 31.99 ± 3.89%). The erosion of the surface of 3NA15Sor-rutin beads were observed and could change the Tris-buffer (swelling media) to yellow as same as the color of rutin during 5 min, this observation was not found in 3NA, 3NA15Sor or 3NA1Bica formulation. The swelling of all bead formulations were stopped after 10 min (Fig. 5). The difference of the rigidity of gels which were used for preparing the beads could affect the percentage of swelling

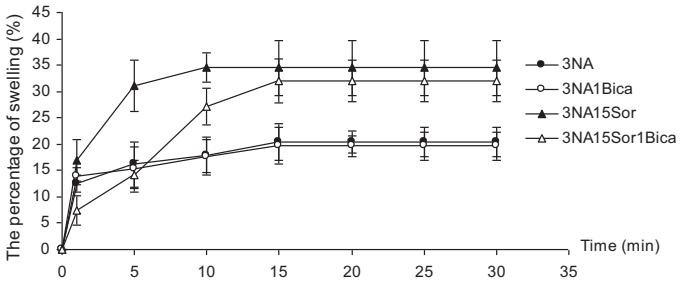

Fig. 6. Swelling behaviors of four different calcium pectinate beads including 3NA (A), 3NA15Sor1Bica (B), 3NA15Sor (C) and 3NA1Bica (D) in Tris-buffer with magnification ×10.

of the beads. 3NA15Sor1Bica and 3NA15Sor exhibited high percentages of swelling due to the water retention ability of sorbitol (Fig. 6).

4. Conclusion

LMP gel formulations were prepared and examined for texture. Calcium pectinate beads containing rutin as a model drug were prepared using ionotropic gelation. Then the influences of textures on drug release and swelling behavior were investigated. Two dissolution media were used in order to imitate the simulated intestinal fluid (pH 7.4), with PB and Tris-buffer. Differences in rutin release and swelling behavior for all formulations were observed. These differences can be attributed to the rigidity of texture of different compositions in LMP formulation. The pectinate beads containing sorbitol (3NA15Sor) showed the best release efficiency and swelling behavior where as the beads containing sorbitol with $NaHCO_3$ (3NA15Sor1Bica) showed burst release with less lag time. 3NA15Sor showed the soft texture from added sorbitol resulting in more rutin release. In addition, adding $NaHCO_3$ into 3NA15Sor could increase the rutin release which could show less lag time but $NaHCO_3$ also increased the hardness of the beads as shown in Young's modulus value. In addition, 3NA15Sor1Bica exhibited less release efficiency. Therefore, the difference in appeared released profiles could be explained by the difference in the composition and rigidity of gel used for preparing the beads as well as the percentages of swelling of four bead types. These results can be applied to increase the release as well as decrease the lag time of the active compounds encapsulated in calcium pectinate beads by changing the texture of the gel.

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