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Chitosan/pectin/gum Arabic polyelectrolyte complex: Process-dependent appearance, microstructure analysis and its application

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ABSTRACT

Novel chitosan/pectin/gum Arabic polyelectrolyte complex (PEC) solutions and membranes with various compositions were prepared for biomedical applications. The appearance of the PEC solutions, either clear or turbid, was process-dependent and depended on how the three components were dissolved and mixed. The addition of gum Arabic to the chitosan and pectin significantly decreased the viscosities of the resultant PEC solutions due to the formation of globe-like microstructures that was accompanied by network-like microstructures and other molecular entanglements. The mechanical strength and hydrophilicity of the PEC membranes manufactured from the PEC solutions, especially for a weight ratio of 84/8/8 (chitosan/pectin/gum Arabic), were enhanced compared to pure chitosan membranes. Moreover, the use of the 84/8/8 PEC membranes as a drug carrier exhibited steady and fairly complete release of a drug (insulin) for 6 h. Based on these promising results, the chitosan/pectin/gum Arabic PEC membranes have great potential in controlled drug release applications.

1. Introduction

Blending oppositely charged polycations and polyanions in a solution results in the formation of polyelectrolyte complexes (PECs) by ionic interactions or assembly (Dobrynin & Rubinstein, 2005; Hamman, 2010; Morris, Kok, Harding & Adams, 2010; Wang, Khor, Wee & Lim, 2002; Yan, Khor & Lim, 2001). Many factors influence the formation, structure, and stability of PECs including properties of the polymer (e.g., the mixing ratio, concentration, density of charges, and kinds of functional groups) and the solvent (e.g., pH and ionic strength) (Bhattarai, Gunn & Zhang, 2010; Dobrynin & Rubinstein, 2005; Etrych, Leclereq, Boustta & Vert, 2005; Il'ina and Varlamov, 2005; Park, Saravanakumar, Kim & Kwon, 2010). When the densities of positive charges on the polycations and negative charges on polyanions are not equitable, PECs are usually water-soluble and form homogeneous solutions. Contrary to water-soluble PECs, stoichiometrically charged PECs usually precipitate and form turbid PEC solutions (Kramarenko, Khokhlov & Reineker, 2006; Matralis, Sotiropoulou, Bokias & Staikos, 2006). However, precipitated PECs can become soluble again by increasing the ionic strength of the solution (Trinh & Schnabel, 1993). Polyelectrolytes are used in a variety of biomedical systems such as dental adhesives and for pulp regeneration (Coimbra et al., 2011a), controlled-release devices (Peterson, Mohwald & Shchukin, 2012), and drug delivery (De Geest, Sanders, Sukhorukov, Demeester & De Smedt, 2007).

Chitosan, which consists of glucosamine and N-acetyl glucosamine, is a very popular natural positively charged polysaccharide with nontoxic, biocompatible, and antibacterial properties (Muzzarelli, 1977). The many amino groups on the polysaccharide chain render it positively charged and easily solubilized in an aqueous acetic acid solution with a pH less than 6.5. With these favorable properties, chitosan has long been used as a biomaterial for drug-delivery and hemostatic (wound dressings) applications (Brown, Daya & Worley, 2009; Honarkar & Barikani, 2009; Kang et al., 2011; Klokkevold, Subar, Fukayama & Bertolami, 1992; Peter

Abbreviations: C, chitosan; P, pectin; A, gum Arabic; HAc, acetic acid; C/A, chitosan/gum Arabic; C/P, chitosan/pectin; C/P/A, chitosan/pectin/gum Arabic; PEC, polyelectrolyte complex.

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Table 1
Group abbreviations, compositions, pH values, and zeta potentials of various chitosan/pectin/gum Arabic (C/P/A) solutions (total concentration of C/P/A: 1 wt%) containing 0.2 M acetic acid.

Group abbreviation	C/P/A ratio	Chitosan (C) (wt%)	Pectin (P) (wt%)	Gum Arabic (A) (wt%)	pH	Zeta potential (mV)
C	100/0/0	1	0	0	3.62	94.8
P	0/100/0	0	1	0	2.91	−22.0
A	0/0/100	0	0	1	2.89	−12.9
C/A	99/0/1	0.99	0	0.01	3.75	70.2
	98/0/2	0.98	0	0.02	3.70	64.0
	97/0/3	0.97	0	0.03	3.75	64.3
	95/0/5	0.95	0	0.05	3.66	64.2
	90/0/10	0.9	0	0.1	3.56	65.9
	80/0/20	0.8	0	0.2	3.51	61.0
	70/0/30	0.7	0	0.3	3.44	35.2
C/P	90/10/0	0.9	0.1	0	3.56	92.4
	80/20/0	0.8	0.2	0	3.46	
	70/30/0	0.7	0.3	0	3.35	
C/P/A	98/1/1	0.98	0.01	0.01	3.74	77.0
	96/2/2	0.96	0.02	0.02	3.60	75.1
	92/4/4	0.92	0.04	0.04	3.57	72.3
	84/8/8	0.84	0.08	0.08	3.52	68.7
	78/11/11	0.78	0.11	0.11	3.47	73.9
	70/15/15	0.7	0.15	0.15	3.43	76.3

et al., 2010; Vilanova, Barril & Carrera, 1993). In addition, the structure of chitosan is similar to glycosaminoglycan in the extracellular matrix (ECM), so chitosan can also be blended with other biopolymers to fabricate scaffolds for tissue-engineering applications (Klokkevold, Subar, Fukayama & Bertolami, 1992; Muzzarelli et al., 1993; Shirakura et al., 2010).

Pectin and gum Arabic are negatively charged, hydrophilic, nontoxic biopolymers. Both are used as additives to improve and diversify the properties of chitosan. Pectin is comprised of galacturonic acid, and a portion of the carboxyl groups are methoxylated. The abundant carboxyl groups on the pectin chains can ionically cross-link with calcium ions to form the so-called "egg-box" structure. A similar structure also appears in alginate gels crosslinked with calcium ions (Braccini & Perez, 2001). Furthermore, the concentration of calcium in calcium pectinate matrixes strictly controls the drug-release rate (Ashford, Fell, Attwood, Sharma & Woodhead, 1994; Liu, Fishman, Kost & Hicks, 2003). Our previous study showed that when chitosan was mixed with pectin, the mechanical properties and hydrophilicity of the resulting chitosan/pectin PEC membranes increased (Chen et al., 2010). However, the addition of pectin also greatly increased the viscosity of the chitosan/pectin PEC solution, making it more difficult to prepare homogeneous solutions. To reduce the viscosity of the solution, gum Arabic was also added because of its low viscosity and high water solubility. Gum Arabic is mainly composed of polysaccharides, including a major galactan chain with highly branched galactose/arabinose side chains (Espinosa-Andrews, Baez-Gonzalez, Cruz-Sosa & Vernon-Carter, 2007). Compared to linear biopolymers, this highly branched structure has a smaller hydraulic radius and fewer intermolecular interactions; thus, gum Arabic solutions have very low viscosities (Sanchez, Renard, Robert, Schmitt & Lefebvre, 2002).

In this study, we established a useful process to prepare high-concentration chitosan/pectin/gum Arabic (C/P/A) PEC solutions without precipitation. The physicochemical properties (including the viscosity and turbidity) of the C/P/A PEC solutions were determined to reveal how these three biopolymers (C/P/A) interact. The hydrophilicity, stability, and mechanical strength of the PEC membranes manufactured from the PEC solutions were also characterized. The use of the PEC membranes as a drug carrier exhibited steady and fairly complete release of the proteinaceous drug (insulin), indicating that these membranes have great potential for applications in controlled drug release.

2. Materials and methods

2.1. Materials

Chitosan (with a molecule weight of about 300 kDa and a 90% degree of deacetylation) was purchased from Kiotek (Taipei, Taiwan). Pectin (about 9.4% methoxylation) and gum Arabic (from the acacia tree) were purchased from Sigma (St. Louis, MO, USA). Materials for the controlled-release experiments including insulin were also purchased from Sigma.

2.2. Preparation of various C/P/A solutions

Gum Arabic (A) powder was first dissolved in deionized water, and then pectin (P) powder was added to the solution with strong stirring. After the powders had totally dissolved, chitosan (C) powder was slowly added into the solution with gentle stirring to ensure a uniform distribution. Finally, acetic acid (HAc) was slowly added with proper mixing to dissolve the chitosan powder and thus form various C/P/A PEC solutions (Table 1). The final total concentrations of the C/P/A polyelectrolytes and acetic acid in mixed solution were 1 wt% and 0.2 M, respectively. When studying the process-dependent appearance of the PEC solutions (Section 3.1), two other processes were used to prepare the mixed solutions, as described in Fig. 1. To characterize the PEC solutions, homogeneous C/A and C/P PEC solutions and pure C, P, and A solutions were also prepared for comparative purposes.

2.3. Characterization of various C/P/A PEC solutions

In order to investigate the effects of adding pectin and gum Arabic on the properties of the chitosan solutions, the pH values, viscosities and turbidities of various PEC solutions at 25 °C were measured. The pH value was measured with a pH meter (model 420A, Orion, USA). The viscosity was measured with a viscometer (model DV-II+, Brookfield, USA). The turbidity was obtained with a UV spectrophotometer (model U-2001, Hitachi, Japan) at 600 nm. In order to further analyze the microstructures of PECs in the solution, the size distribution of globe-like substances in the PEC solution and the zeta-potentials of the solutions were obtained with a particle size and zeta potential analyzer (model Nano-ZS, Malvern, UK).

2.4. Preparation of various C/P/A PEC membranes

In order to study the mechanical properties of PEC membranes, various solutions with compositions (relative weight ratios) of chitosan, pectin, and gum Arabic being 100:0:0, 84:8:8, and 70:15:15 were prepared, symbolized as 100/0/0, 84/8/8, and 70/15/15, respectively. These prepared solutions were centrifuged at 3500 rpm (1030 × *g*) for 15 min to remove air bubbles and then poured into a mold with a scale of 2.5 cm × 7 cm × 0.8 cm, and then dried in an oven at 40 °C for 3 days to form dense C/P/A PEC membranes. All dense membranes (except those for the controlled drug release experiments) were immersed in a 0.1 M sodium hydroxide (NaOH) solution for 6 h, and then thoroughly washed in deionized water until neutrality. The thickness of dry membranes was about 0.09 mm.

2.5. Analysis of the mechanical properties

The mechanical properties of the dense PEC membranes were measured using a Materials Testing Machine (model LRX, Lloyd, West Sussex, UK). The membranes were immersed in deionized water for 10 min and then cut into a dog-bone shape (6 cm long, 2 cm wide at the ends and 1 cm wide in the middle). The thickness of the membrane was measured individually. The mechanical properties (tensile strength and elongation) of the membranes were determined at a stretching speed of 10 mm/min.

2.6. Measurement of the contact angle

The contact angles of the various PEC membrane surfaces were measured by the water drop method. Chitosan/pectin/gum Arabic PEC solutions were coated onto glass slides and dried for 2 days. A 5-μL drop of water was loaded on the membrane surface using a pipette. The water drop image was recorded with a video camera mounted on a microscope to determine the contact angle. The water contact angle of the dense membrane was calculated using the following equation:

$$\theta = 2\ \tan^{-1}\left(\frac{2h}{b}\right) \quad (1)$$

where θ is the contact angle of the dense membrane, b is the base length of the water drop, and h is the height of the water drop.

2.7. Water uptake of the dense membranes

To determine the water uptake of the dense PEC membrane, the weight of the wet membrane (W_{wet}) was measured. Then, the membrane was dried in an oven for 1 day. After that, the weight of the dry membrane (W_{dry}) was immediately determined. The water uptake was calculated by the following equation:

$$\text{Water uptake}\,(\%) = \frac{W_{wet} - W_{dry}}{W_{dry}} \times 100 \quad (2)$$

2.8. Disintegration of the dense membranes

To investigate the disintegration behavior (expressed by the remaining weight) of the dense PEC membrane, the initial dry weight ($W_{dry,i}$) of a membrane was measured, and then the membrane was immersed in 40 mL phosphate buffered saline (PBS) in a centrifuge tube and incubated for a predetermined period of time (within 14 days). The membrane was washed in deionized water and dried for 1 day. The final dry weight of membrane ($W_{dry,f}$) was measured, and the percentage of the remaining weight was calculated using following formula:

$$\text{Remaining weight percentage}\,(\%) = \frac{W_{dry,f}}{W_{dry,i}} \times 100 \quad (3)$$

2.9. Controlled release of insulin

To determine the controlled release behavior of these dense PEC membranes as drug carriers, insulin (at a final concentration of 0.005 mM) was added to the C/P/A PEC solutions. In addition, calcium chloride (with calcium at 1/20 and 1/10 of the weight of pectin used) was also added to the solutions to alter the drug-release behavior. The symbols, 84/8/8-0.4 and 84/8/8-0.8, represent the compositions (relative weight ratios) of chitosan, pectin, gum Arabic, and calcium ions in the mixed materials being 84:8:8:0.4 and 84:8:8:0.8, respectively. After drying, individual dense membranes were immersed in 10 mL of phosphate-buffered saline (PBS) in a centrifuge tube and incubated at room temperature. At predetermined times, 100 μL of PBS was sampled, and the concentration of insulin was determined using a Micro BCA Protein Assay Kit (Thermo Fisher Scientific, Suwanee, GA, USA).

2.10. Statistical analysis

Results were analyzed by Student's *t*-test. A significant difference between two sets of data was accepted when $p < 0.05$.

3. Results and discussion

3.1. Process-dependent appearance of the PEC solutions

It was shown that during the preparation of various C/P PEC solutions, insoluble PECs often occur due to strong ionic interactions between the polycationic chitosan and polyanionic pectin (Bigucci et al., 2008; Coimbra et al., 2011b). Even without PEC formation, C/P aqueous mixtures are quite viscous, thus it is difficult to prepare homogeneous solutions. To reduce the viscosity of the mixture, gum Arabic was also added because of its low viscosity and high water-solubility. In this study, three processes were designed to prepare chitosan/pectin/gum Arabic (C/P/A) PEC solutions. The protocols and appearance of the resultant solutions are shown in Fig. 1. In the first process (process (a)), chitosan powder was first suspended in a gum Arabic solution, and then acetic acid was slowly added to dissolve the chitosan. Finally, a pectin solution was added drop-wise to the C/A PEC solution with stirring (Fig. 1a). It was obvious that large amounts of PEC precipitates appeared in the turbid mixed solution because of the relatively high pectin concentration at the spot where the pectin solution was dripped into the chitosan-based solution (Fig. 1a). Therefore, this inhomogeneous solution could not be used in subsequent experiments. A modified process (process (b)) was proposed in which chitosan powder was first suspended in a pectin solution. After the chitosan powder had dissolved by adding acetic acid, a gum Arabic solution was added drop-wise. No obvious PEC precipitates appeared, but the resultant solution still looked slightly milky (Fig. 1b). As the chitosan powder gradually dissolved in the pectin solution, the contact of chitosan with pectin was minimal but sustained, unlike the situation in process (a) in which there was extensive contact of the chitosan solution with the pectin solution. The slightly milky appearance possibly indicated the presence of small-sized PEC precipitates. We also designed another modified process (process (c)) in which pectin and gum Arabic were first dissolved in water, and then chitosan powder was suspended in a mixed pectin/gum Arabic solution. Acetic acid was finally added to dissolve the chitosan powder, and a clear, homogeneous C/P/A PEC solution

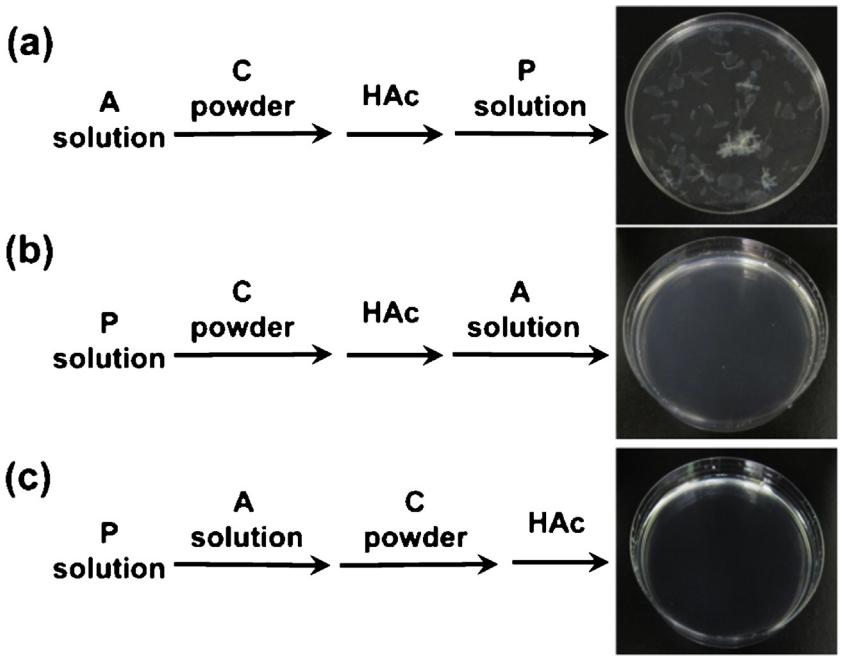

Fig. 1. Process-dependent appearance of the polyelectrolyte complex (PEC) solutions. Three processes (process (a), (b), and (c)) were designed to prepare chitosan/pectin/gum Arabic (C/P/A) PEC solutions with the same composition (84/8/8). The appearance of the resulting solutions was process-dependent, including: (a) turbid with PEC precipitates, (b) slightly milky, and (c) clear. Stirring occurred in all steps of each process. C, chitosan; P, pectin; A, gum Arabic; HAc, acetic acid.

was obtained (Fig. 1c). This process allowed the protonated chitosan (positively charged) to simultaneously react with the pectin and gum Arabic (both negatively charged). As discussed below (Section 3.2), this process may reduce ionic interactions between the chitosan and pectin due to the concomitant presence of gum Arabic, thus generating a clear C/P/A PEC solution. Consequently, in the following experiments all PEC solutions were prepared by the process (c).

3.2. Characteristics of C/A, C/P, and C/P/A PEC solutions and proposed microstructures in these solutions

To characterize the mixed solutions and clarify how the three biopolymers (chitosan, pectin, and gum Arabic) interacted, three groups of PEC solutions including chitosan/gum Arabic (C/A), chitosan/pectin (C/P), and chitosan/pectin/gum Arabic (C/P/A) solutions with varying compositions were prepared (see Table 1 for detailed information). The pH and zeta-potential values of these solutions are listed in Table 1 (the last two columns). Furthermore, the viscosity, particle size, and turbidity of these solutions were analyzed, and results are shown in Fig. 2. Based on the above experimental evidence, schematic diagrams of possible microstructures in these solutions are proposed, as shown in Fig. 3.

Chitosan (C) is a basic polysaccharide and has a positive charge with a zeta-potential of 94.8 mV (Table 1) in an acidic solution. It has a network-like structure formed by molecular entanglements (Fig. 3a). Gum Arabic (A) and pectin (P) are both acidic polysaccharides and have negative charges (with zeta-potentials of −22.0 mV and −12.9 mV (Table 1), respectively) when dissolved in an acidic solution. It was expected that gum Arabic and pectin would interact with chitosan to form PECs through opposite charge interactions. Gum Arabic is a branched biopolymer, and chitosan is a long-chain biopolymer. It was found that globe-like microstructures formed in C/A PEC solutions (Coelho et al., 2011). Therefore, we propose that globe-like microstructures accompanied by other molecular entanglements are present in C/A solutions (Fig. 3b). We also consider that the globe-like microstructures decrease the viscosity of the C/A solutions, owing to the following supportive evidence.

Fig. 2a shows the variations in the viscosity of C/A PEC solutions. When the ratio of gum Arabic in the C/A solution increased, the viscosity of the solution dramatically decreased from 33 cP (for the 100/0/0 solution) to 11 cP (for the 99/0/1 solution), and then to 4 cP (for the 70/0/30 solution). Fig. 2d shows that in C/A solutions, globe-like microstructures existed with globe diameters decreasing from 1000 to 300 nm as the ratio of gum Arabic increased. Since the zeta-potentials of the globe-like microstructures in the C/A solutions were positive (Table 1), it was expected that the chitosan associated with the gum Arabic to form globe-like complexes, with the inner core consisting of gum Arabic/chitosan and the outer part mostly consisting of chitosan. Notably, there was a tremendous decline in the zeta-potential from 94.8 mV (for the 100/0/0 solution) to 70.2 mV (99/0/1 solution) by adding a tiny amount of gum Arabic (only 1% of total content), indicating that chitosan molecules surrounded gum Arabic cores with some amino groups of chitosan hidden in the inner regions. When the ratio of gum Arabic increased, it was very likely that the number of gum Arabic cores increased. The amount of chitosan bound to each gum Arabic core decreased, so the outer regions of the globe-like complexes became thinner, and globe diameters decreased (Fig. 2d). Similarly, the zeta-potential of C/A PEC solution continuously decreased from 70.2 to 61.0 mV (for the 80/0/20 solution) when increasing the ratio of gum Arabic from 1% to 20% (Table 1). While the amount of gum Arabic increased to 30%, the zeta-potential dropped substantially to 35.2 mV (70/0/30 solution) perhaps owing to fewer amounts of chitosan molecules around gum Arabic cores and less free chitosan molecules in the solution. Besides, gum Arabic at a high concentration can restrain the aggregation of particles. The strong dispersive ability of gum Arabic and fewer amounts of chitosan molecules around gum Arabic cores thus could trigger the globe diameters to decrease further to 300 nm when the amount of gum Arabic increased to 30% (Fig. 2d).

As for the C/P PEC solutions, however, the increase in viscosity was very significant when the ratio of pectin in the C/P solution increased. The viscosity of the C/P solution increased from 33 (for the 100/0/0 solution) to 120 (90/10/0), 180 (80/20/10), and then to 360 cP (70/30/0), as shown in Fig. 2b. A different structure

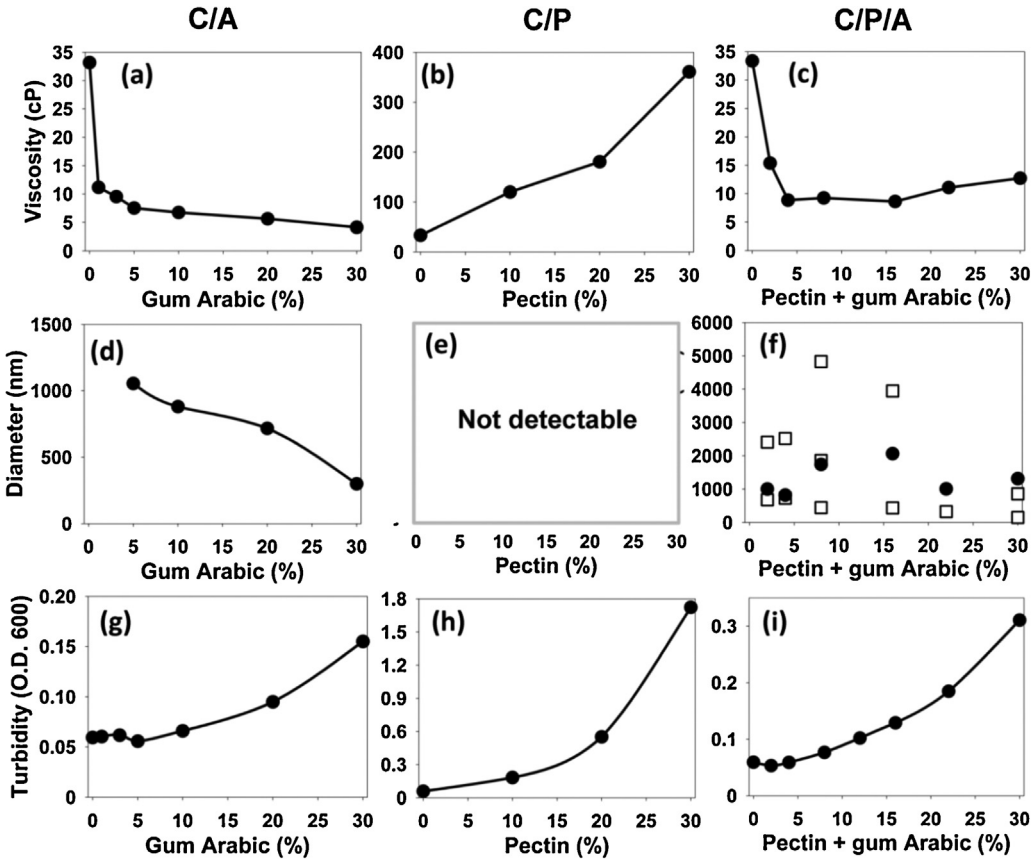

Fig. 2. Characteristics of chitosan/gum Arabic (C/A), chitosan/pectin (C/P), and chitosan/pectin/gum Arabic (C/P/A) PEC solutions with various compositions in terms of: (a–c) viscosity, (d–f) particle diameter, and (g–i) turbidity analyses. The total concentration of each PEC solution was 1 wt%. (e) No particles were detected in the C/P solution. (f) Particle diameter distributions were complicated in the C/P/A solution, with several detectable subpopulations of particles (□) and the mean particle diameters being shown (●).

may have existed in the C/P solutions. As described in Fig. 3c, a network-like structure might have formed by charge interactions between chitosan and pectin (by forming linear PECs) and molecular entanglements (Nordby, Kjoniksen, Nystrom & Roots, 2003). Ionic interactions between chitosan and pectin increased when the ratio of pectin increased, and thus a stronger network-like structure was formed that enhanced the viscosity of C/P solutions and no globe diameter was detected (Fig. 2e). Besides, the p*Ka* value of pectin is about 4. In the C/P PEC solution (90/10/0 solution) with pH 3.56 (Table 1), some of the carboxyl groups of pectin should maintain —COOH form without releasing hydrogen ions. This could be the reason why the zeta-potential of 90/10/0 solution (92.4 mV) was close to that of pure chitosan (100/0/0) solution (94.8 mV) (Table 1). The zeta-potentials of C/P solutions with higher ratios of pectin (80/20/0 and 70/30/0 solutions) could not be determined probably due to the presence of a stronger network-like structure mentioned above.

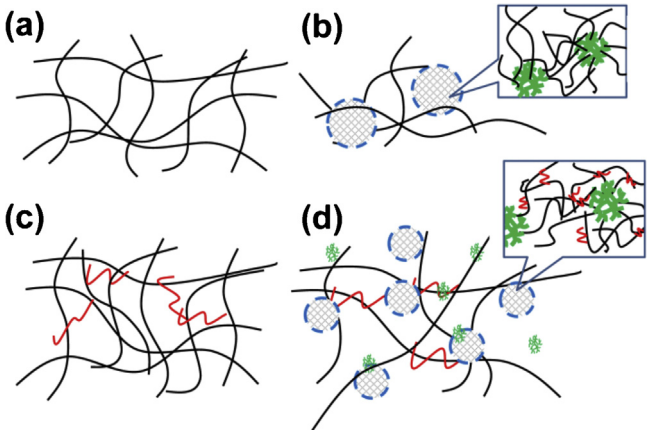

Fig. 3. Schematic diagrams of possible microstructures in various solutions. (a) Network-like microstructures in the chitosan solution, (b) globe-like microstructures accompanied by other molecular entanglements in the chitosan/gum Arabic (C/A) PEC solution, (c) network-like microstructures and other molecular entanglements in the chitosan/pectin (C/P) PEC solution, and (d) globe-like microstructures, network-like microstructures and other molecular entanglements in the chitosan/pectin/gum Arabic (C/P/A) PEC solution. Black, red, and green symbols represent chitosan, pectin, and gum Arabic, respectively. (For interpretation of the references to color in text, the reader is referred to the web version of this article.)

Interestingly, for the C/P/A PEC solutions, when the ratio of pectin and gum Arabic simultaneously increased, the viscosity of the solution first decreased to 15 (for the 98/1/1 solution), 8.8 (96/2/2), 9.2 (92/4/4) and 8.6 cP (84/8/8) and then increased to 11 (78/11/11) and 13 cP (70/15/15), as shown in Fig. 2c. These results might have been due to different interactions of chitosan with gum Arabic and pectin. At a low ratio of pectin and gum Arabic, the effect of gum Arabic was stronger than the effect of pectin, and thus the viscosity profile of the C/P/A solutions (Fig. 2c) was similar to that of the C/A solutions (Fig. 2a). At a higher ratio of pectin and gum Arabic, the effect of pectin became significant. Thus we proposed that both gum Arabic-induced globe-like microstructures and pectin-induced network-like microstructures were important. The latter might have interfered with the detection of globe diameters (Fig. 2f) and zeta-potentials (Table 1), so the results are for reference only. In summary, it is our concept that in the C/P/A PEC solutions, globe-like microstructures,

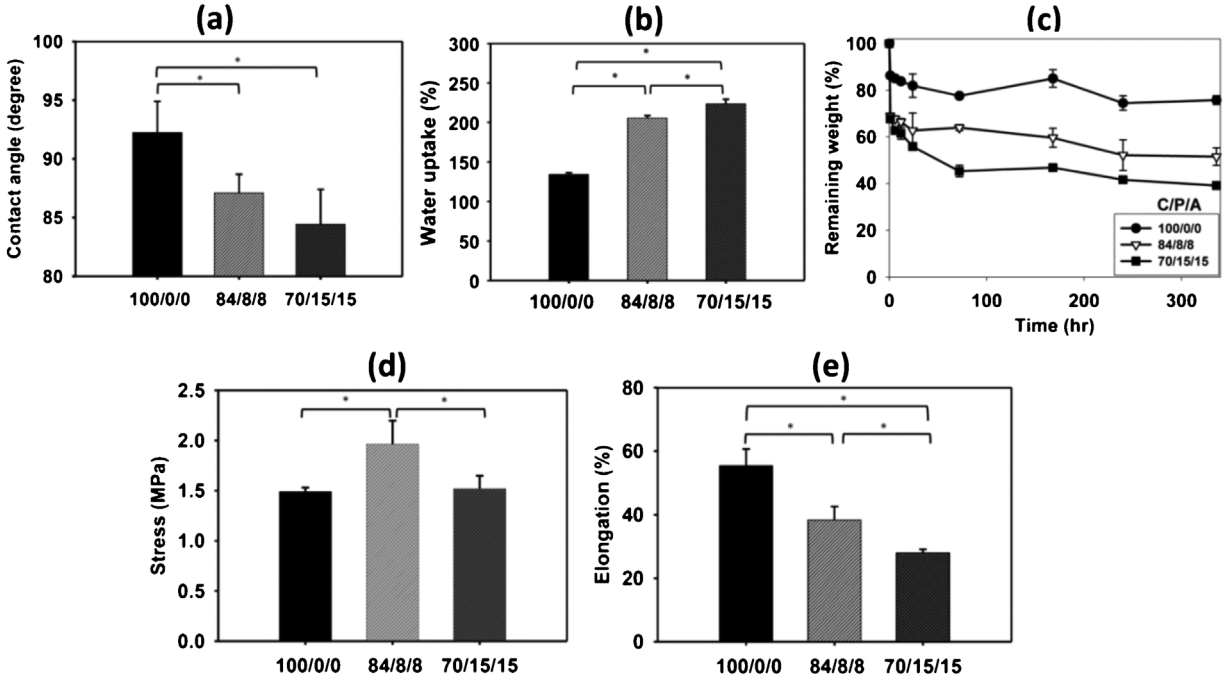

Fig. 4. Properties of chitosan/pectin/gum Arabic (C/P/A) PEC membranes with various compositions in terms of: (a) contact angle, (b) water uptake, (c) disintegration, (d) tensile strength (stress at maximum load), and (e) elongation capability (strain at maximum load). The relative weight ratios of C/P/A were 100/0/0 (pure chitosan), 84/8/8, and 70/15/15. $n \geq 5$, $^*p < 0.05$ by Student's *t*-test.

network-like microstructures and other molecular entanglements might be present, as described in Fig. 3d.

To characterize the formation of PECs, the values of turbidity (O.D. 600 nm) of the various C/A, C/P, and C/P/A PEC solutions were measured. In C/A PEC solutions, the turbidity of the pure chitosan (100/0/0) solution was 0.059. After adding gum Arabic to the solution, the turbidity of the C/A PEC solution slightly increased to 0.095 (80/0/10) and 0.155 (70/0/30), but was still clear (Fig. 2g). In contrast, the turbidity increment of the C/P PEC solution was very significant. After blending pectin with chitosan, the turbidity of the C/P solution increased to 0.183 (90/10/0), 0.551 (80/20/0), and 1.723 (70/30/0) which was quite milky (Fig. 2h), indicating strong ionic interactions and extensive formation of PECs between chitosan and pectin. For the C/P/A PEC solutions, the turbidity of the solution increased to 0.129 (84/8/8), 0.185 (78/11/11), and 0.311 (70/15/15) (Fig. 2i). The turbidity of the C/P/A solution was much less than that of the C/P solution, indicating that the addition of gum Arabic to the solution disturbed interactions between chitosan and pectin, thereby generating relatively clear solutions.

3.3. Physical properties of the C/P/A PEC membranes

In this study, various C/P/A PEC solutions were utilized to prepare the C/P/A PEC membranes for biomedical applications. The hydrophilicity of the dense membranes was determined by measuring the water contact angle. As shown in Fig. 4a, the contact angle decreased from 92.2° to 87° then to 84.3° as the contents of pectin and gum Arabic increased (i.e., from 100/0/0 to 84/8/8 then to 70/15/15), indicating that the addition of pectin and gum Arabic improved the hydrophilicity of the PEC membranes. Moreover, the water uptake increased from 130% to 200% then to 230% with increasing amounts of pectin and gum Arabic due to the hydrophilic nature of these two biopolymers, as shown in Fig. 4b. The addition of more pectin and gum Arabic allowed the PEC membranes to absorb more water.

One of the aims of this study was to alter and diversify the disintegration behavior of membranes by adding pectin and gum Arabic. Considering that many drugs are denatured or deactivated in strongly basic conditions, the PEC membranes for disintegration study were not treated with a sodium hydroxide solution. As shown in Fig. 4c, the disintegration curves exhibited gradual weight loss with time for all membranes. After being immersed in PBS, the major weight loss of the membranes occurred in the first hour. After 14 days of incubation, remaining weights of the 100/0/0, 84/8/8, and 70/15/15 PEC membranes were 75.6%, 51.6%, and 39.1%, respectively. During 1 h to 14 days of immersion in PBS, about 10% of the initial weight was lost from the 100/0/0 membrane. However, the 84/8/8 and 70/15/15 PEC membranes further lost about 17% and 29% during this period. This clearly shows that the disintegration rate increased with the addition of hydrophilic pectin and gum Arabic.

The tensile strength and elongation capability of the C/P/A PEC membranes were also determined. The tensile strength (stress at maximum load) was 1.49 MPa for pure chitosan (100/0/0) membranes. After adding pectin and gum Arabic, the tensile strength increased to 1.90 MPa for the 84/8/8 PEC membranes, and then decreased to 1.51 MPa for the 70/15/15 PEC membranes, as shown in Fig. 4d. Tensile strengths of pure pectin and pure gum Arabic membranes were too low to be measured. Therefore, the tensile strength of PEC membranes with a weight ratio of C/P/A of 84/8/8 was relatively higher than those of the other prepared membranes. As discussed in the previous section, chitosan and pectin molecules can form network-like PECs that may enhance the tensile strength of PEC membranes such as the 84/8/8 membranes. However, gum Arabic is a low-viscosity polysaccharide that interacts weakly with other components and forms globe-like microstructures with chitosan. As the amount of gum Arabic further increased, the globe-like microstructures may have decreased the tensile strengths of the PEC membranes, such as the 70/15/15 membranes. The elongation capability (strain at maximum load) of the PEC membranes decreased from 55% to 38% then to 25% as the contents of pectin and gum Arabic increased (i.e., from 100/0/0 to 84/8/8 then to

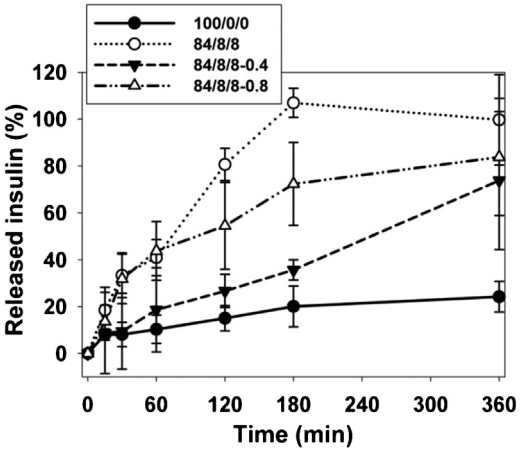

Fig. 5. Insulin release behavior of chitosan/pectin/gum Arabic (C/P/A) PEC membranes with various compositions and calcium concentrations. The symbols, 100/0/0, 84/8/8, 84/8/8-0.4 and 84/8/8-0.8, represent the compositions (relative weight ratios) of chitosan, pectin, gum Arabic, and calcium ions in the membranes being 100:0:0:0 (pure chitosan), 84:8:8:0, 84:8:8:0.4 and 84:8:8:0.8, respectively (please see Section 2.9 for a detailed explanation). $n \geq 3$.

70/15/15), suggesting that the globe-like microstructures may also reduce the extension capability of the C/P/A PEC membranes, as shown in Fig. 4e.

3.4. Application of the C/P/A PEC membranes in controlled release of insulin

Controlled drug-release experiments were carried out to demonstrate a potential application of the prepared C/P/A PEC membranes. In this study, insulin was used as a model proteinaceous drug and the 84/8/8 PEC membrane was selected as a drug carrier. When insulin was added to chitosan-based solutions, ionotropic complexation of the positively charged chitosan and negatively charged insulin occurred. Various insulin-loaded PEC membranes were fabricated, and the insulin-release behaviors of these membranes were determined, as shown in Fig. 5. Because the pure chitosan membrane (100/0/0) showed the least disintegration in PBS (Fig. 4c), the release of insulin from the 100/0/0 membrane was the slowest, and only about 20% of the insulin was released during the first 6 h (Fig. 5). In contrast, almost all of the insulin was released from the 84/8/8 membrane in 3 h (Fig. 5) because the 84/8/8 membrane exhibited higher hydrophilicity and more disintegration (Fig. 4a and c). In addition, it has been reported that calcium can be used as a cross-linker of pectin and consequently alters the drug-release behavior (Braccini & Perez, 2001). Furthermore, Si et al. reported that the release of insulin from pectin-based microbeads was significantly decreased by adding calcium ions because of the crosslinks between calcium ions and pectin molecules (Si et al., 2009). Wang et al. reported that the use of elevated concentration of calcium chloride in preparing alginate-based beads resulted in more insulin release from the beads (Wang & He, 2002).

In this study, we also investigated the influence of calcium and found that the addition of calcium to the 84/8/8 membrane significantly retarded insulin release, because only about 35% and 72% of the insulin were respectively released from the 84/8/8-0.4 and 84/8/8-0.8 membranes (both containing calcium but different amounts) in 3 h (Fig. 5). A comparison of these two membranes also indicated that adding a small amount of calcium improved the stability of the membrane (84/8/8-0.4) because calcium ions acted as a cross-linker of pectin, leading to less swelling of the membrane and a slower release of insulin. As the amount of calcium ions increased, the membrane (84/8/8-0.8) became more fragile because more crosslinks were formed. This phenomenon caused insulin to be released from the 84/8/8-0.8 membrane faster than from the 84/8/8-0.4 membrane. Therefore, the use of the 84/8/8 membranes as a drug carrier exhibited steady and fairly complete release of the drug (insulin), and the release rate could be adjusted by altering the amount of calcium added, which was consistent with the above-mentioned studies (Si et al., 2009; Wang & He, 2002).

4. Conclusions

In this study, we developed a process to prepare homogeneous chitosan/pectin/gum Arabic (C/P/A) polyelectrolyte complex (PEC) solutions. This process requires that protonated chitosan molecules gradually interact with pectin and gum Arabic. The addition of gum Arabic to the chitosan/pectin solution significantly decreased the viscosities and zeta-potentials of the resultant solutions due to the formation of gum Arabic-induced globe-like microstructures. We propose that in the C/P/A PEC solutions, globe-like microstructures, network-like microstructures and other molecular entanglements might be present. The mechanical strength and hydrophilicity of the PEC membranes manufactured from the PEC solutions, especially for a weight ratio of 84/8/8 (C/P/A), were enhanced compared to pure chitosan membranes. The use of the 84/8/8 membranes as a drug carrier exhibited steady and fairly complete release of a proteinaceous drug (insulin), and the release rate could be adjusted by altering the amount of calcium added. Based on these promising results, the novel C/P/A PEC membranes have great potential in controlled drug release and other related applications.

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