Novel chitosan–pectin composite membranes with enhanced strength, hydrophilicity and controllable disintegration

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ABSTRACT

Novel composite porous membranes made of basic and acidic polysaccharides (i.e. chitosan and pectin) were developed. Three types of chitosan/pectin composite membranes were fabricated: PEC-type (by polyelectrolyte complex (PEC) formation between chitosan and pectin); PEC/Ca-type (by PEC and calcium ion-mediated ionic crosslinking toward pectin); and PEC/covalent-type membranes (by PEC and EDC/NHS-mediated covalent bond). Tensile strengths (about fourfold that of pure chitosan membranes), hydrophilicity and water uptake capability of the composite membranes were significantly greater than membranes composed of chitosan or pectin only. Such composite membranes had faster disintegration rates which could be controlled by the content of pectin. Addition of calcium ions or EDC/NHS further increased the tensile strengths of the composite membranes (by about 1.5-fold) and also changed the profile of disintegration. Such chitosan/pectin composite membranes, due to their enhanced strength, hydrophilicity and controllable disintegration behavior, have potential as edible sponges or disintegrant carrying food or drug.

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1. Introduction

Natural and nontoxic polysaccharide materials (chitosan and pectin) were used to fabricate novel composite porous membranes via effective intermolecular interactions. Chitosan is an abundant natural polysaccharide constituted by *N*-glucosamine and a small amount of *N*-acetyl-glucosamine (Chen et al., 2008). Because the amino groups on the *N*-glucosamine repeating units can be positively charged in an acidic environment, chitosan can dissolve in acidic solutions, thus being a basic polysaccharide (polycation). Chitosan is widely used for various applications (Han, Guenier, Salmieri, & Lacroix, 2008). However, its unsatisfactory hydrophilicity and low degradability have limited its use in further applications.

Pectin is an edible and water-soluble polysaccharide which consists primarily of D-galacturonic acid with a part of the carboxyl groups being methoxylated. It is widely used in food industry (Reginald, 1991), and its potential for biomedical applications has also been investigated (Nishijima, Iwai, Saito, Takida, & Matsue, 2009). Pectin has carboxyl groups, making it an acidic polysac-

charide (polyanion). However, the major obstacle limiting the use of pectin is its water-soluble nature in aqueous environments, which causes it to dissolve quickly. To overcome this drawback, several researchers tried to mix pectin with other materials to develop composite materials (Lam, Paulsen, & Corredig, 2008; Liu, Liu, Fishman, & Hicks, 2007). The abundant carboxyl groups on acidic polysaccharides can be ionically crosslinked by calcium ions (Ca^{2+}) because the two positive charges of the divalent cation can bind with different carboxyl groups (Fang et al., 2007, 2008). Via this mechanism, gelation or crosslinking of pectin can be achieved due to strong intra- or intermolecular interactions.

Ionic interactions occur between polyanions and polycations, leading to the formation of a polyelectrolyte complex (PEC). As a positively charged polysaccharide, chitosan has been incorporated with pectin to fabricate various composite materials (Elsabee, Abdou, Nagy, & Eweis, 2008; Hiorth, Versland, Heikkila, Tho, & Sande, 2006). In addition to ionic interactions, there are other types of interactions that can form between amino groups and carboxyl groups, such as hydrogen bonds and covalent bonds formed using specific conjugating chemicals, like 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) (Richert et al., 2004). Traditionally, in order to crosslink PEC composite materials, the materials were treated by crosslinking reagents after the formation of the PEC or even after the fabrication of the materials (Peng, Yu, Mi, & Shyu, 2006). However, the effect of crosslinking would be not so significant because the crosslinking

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treatment was carried out after the formation of PEC. The crosslinking took place at the same functional groups as those used for PEC formation, so the crosslinking reaction had to compete with PEC formation for the functional groups since the total amounts of amino groups and carboxyl groups were limited. Utilizing the traditional process, most of the amino groups and carboxyl groups had formed PEC and thus lost the ability to form other types of interactions. The process was modified in this study by adding the crosslinking reagents into the pectin solution before the addition of chitosan (i.e., before the formation of the PEC of chitosan/pectin). With this process, the effect of crosslinking could be quantified because all the reagents were present in the solution. In addition, since the crosslinking could occur before or together with the formation of the PEC, the crosslinking effect would not be blocked by PEC formation and thereby could be more significant.

In the present study, by utilizing the aforementioned fabrication process, chitosan/pectin PEC-type composite membranes were successfully fabricated. Additional conjugating reagents, including calcium ions or EDC/NHS were introduced to the chitosan/pectin composite to fabricate PEC/Ca-type and PEC–covalent-type composite materials. For the PEC/Ca-type material, an additional ionic crosslinking effect toward pectin was introduced by calcium ions. For the PEC/covalent-type material, both ionic interactions (PEC formation) and covalent bonds (EDC/NHS-mediated amide bond formation) formed between chitosan and pectin. Characterizations of the composite materials indicated that the tensile strength, hydrophilicity, and water uptake of the chitosan/pectin membranes were significantly improved by increasing the content of pectin. Disintegration of the composite membranes speeded up as the content of pectin increased. The material properties were further diversified by calcium ions or EDC/NHS.

2. Experimental

2.1. Materials

Chitosan (with a M.W. of about 3.1×10^5 and degree of deacetylation of about 90%) was purchased from Kiotek (Taipei, Taiwan). Pectin (extracted from citrus fruits with a M.W. of about 3×10^4 to 8×10^4 and a degree of methoxylation of about 8%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). All other reagents used were of reagent grades.

2.2. Preparation of chitosan/pectin mixture solutions

An acetic acid solution (at a final concentration of 0.2 M) was used as a solvent to dissolve the chitosan. For the PEC-type mixture, a homogeneous solution was obtained as described previously (Hsieh, Tsai, Wang, Chang, & Hsieh, 2005). Six solutions with different chitosan/pectin ratios were prepared. The symbols and compositions of the solutions used in this study are shown in Table 1, in which total amount of chitosan and pectin was fixed at 3 wt%.

To prepare the PEC/Ca-type and PEC/covalent-type mixture solutions, the weight ratio of chitosan to pectin was fixed at 70/30. The reason why 70/30 was chosen was mainly due to the extremely high viscosity of the 50/50 mixture solution (data not shown), which made the subsequent fabrication and characterization of materials difficult. Calcium chloride (20 mM) or EDC/NHS (20 mM/10 mM) was added to the pectin solution prior to the addition of the chitosan powder. After stirring for 30 min, the chitosan powder was added to the solution, followed by the addition of acetic acid. As a result, a homogenous PEC/Ca-type or PEC/covalent-type mixture solution was obtained. Their symbols are shown in Table 1.

In order to investigate the interaction between chitosan and pectin, pH values of the solutions were measured with a pH-meter (model 420A, Orion Research Inc., Boston, MA). Besides, the amount of free amino groups remaining in the mixture solutions was determined to reveal the extent of the chitosan–pectin interaction. The method utilized was reported in a previous research (Toei & Kohara, 1976). First, each solution was diluted to 0.5 wt%. Second, two drops of a 0.1% toluidine solution were added to the solution. After adequate mixing, each solution was titrated with an N/400 potassium poly(vinyl sulfate) solution (PVSK). The concentrations of free amino groups in the solutions were then calculated (Toei & Kohara, 1976).

2.3. Fabrication of the chitosan/pectin composite materials

The above mixture solutions were used to fabricate both dense films and porous membranes. To prepare the dense films, the solution was poured into dishes and dried in an oven for 24 h with the temperature maintained at 60 °C. The dehydrated films were then immersed in a 3 M NaOH solution for 3 h, followed by rinsing with water and a phosphate-buffered saline (PBS) solution. The fabricated films were stored at 4 °C for further analyses.

To fabricate the porous membranes, a freeze-gelation method was utilized (Ho et al., 2004; Hsieh et al., 2005). The prepared porous membranes were stored at 4 °C for subsequent analyses. Scanning electron microscopy (SEM) was used to observe the pore structures of the membranes. In order to identify the existence of the polysaccharides in the fabricated porous membranes, Fourier-transformed infrared (FTIR) spectra of various membranes were obtained by an FTIR spectrometer (Spectrum One, Perkin-Elmer). The range of the wave number was from 600 cm^{-1} to 4000 cm^{-1}, and each sample was scanned for 32 times.

2.4. Mechanical properties of the porous membranes

The mechanical properties of the porous membranes were determined in a tensile strength instrument (model LRX, Lloyd, Hampshire, UK). The method of measuring the mechanical properties was described in a previous work (Chen et al., 2008). Considering the actual environments during application, wet membranes were used for the test.

2.5. Contact angles of dense films and water uptake capabilities of porous membranes

The dense films were used to measure the contact angles of the composite materials in order to avoid the effect of pores on the contact angles, while the porous membranes were used to measure the water uptake capabilities of the composite materials.

To measure the contact angles of the composite films with water, a fabricated dense film was immersed in water and a video camera mounted on a microscope was used to record the image of air bubbles in contact with the film. According to the photograph of the bubble taken, the contact angle (θ) was then calculated by the following equations:

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

where b is the length of interface between the material and the attaching air bubble, and h is the height of the attaching air bubble.

To measure the water uptake capability of the porous membranes, the wet weight (W_{wet}) of a membrane was measured, and then the wet membrane was dried in a 60 °C oven for 24 h, followed by vacuum drying for 24 h. The dry weight (W_{dry}) of the membrane was immediately measured. The water uptake was calculated using

Table 1
pH values and the concentrations of free amino groups of the various solutions.

Symbol Chitosan/pectin (weight ratio)	Type	Composition		pH of the solution[a]	Determined conc. of free amino groups (mM)	Calculated conc. of amino groups (mM)[b]
		Chitosan (wt%)	Pectin (wt%)			
100/0	–	3.0	0	4.67	141.2	141.2
85/15	PEC	2.55	0.45	4.51	114.0	120.0
70/30	PEC	2.1	0.9	4.48	82.9	98.9
50/50	PEC	1.5	1.5	4.12	52.5	70.6
30/70	PEC	0.9	2.1	3.84	11.5	42.3
0/100	–	0.0	3.0	3.43	0.0	0.0
70/30/Ca[c]	PEC/Ca	2.1	0.9	4.35	77.9	98.9
70/30/covalent[d]	PEC/covalent	2.1	0.9	4.38	68.2	98.9

EDC, 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide; NHS, *N*-hydroxysuccinimide.

[a] All the solutions contained 0.2 M acetic acid.

[b] Calculated concentration of amino groups in the solution was calculated from the determined concentration in the 100/0 solution (i.e. without the influence of pectin).

[c] The concentration of $CaCl_2$ was 20 mM.

[d] The concentrations of EDC and NHS used for forming covalent bonds between amino groups and carboxyl groups were 20 mM and 10 mM, respectively.

the following formula:

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

2.6. Disintegration of the porous membranes

To measure the disintegration of the porous membranes, samples were cut into small pieces, dried in a 60 °C oven for 24 h, followed by vacuum drying for 24 h to remove the water in the membranes. The initial dry weight ($W_{dry,i}$) of a membrane was immediately measured. Then the dry membrane was immersed in PBS and incubated at 37 °C with shaking. At specific time points, the membrane was dried again and weighed to measure the final dry weight ($W_{dry,f}$). The remaining weight (%) was calculated using the following formula:

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

3. Results and discussion

3.1. Characterization of the chitosan/pectin mixture solutions

PEC was expected to form between chitosan and pectin in the solutions. The solution pH values and the amounts of free amino groups were measured (Table 1). First, the pure chitosan solution (100/0) showed the highest pH, and the pH decreased as the addition of pectin (column 5 in Table 1). In addition, as the content of the acid (pectin) increased and the base (chitosan) decreased, the pH value of the solution was lowered. These results were consistent with the concept of base/acid neutralization. The pH values of 70/30/Ca and 70/30/covalent mixture solutions were similar to the pH value of the 70/30 solution, indicating that the content of base/acid primarily dominated the pH value of the mixture solution.

It is expected that negatively charged carboxyl groups on pectin would ionically interact with amino groups of chitosan to form PEC, and reducing the amount of free amino groups remaining in the solution. In Table 1, the determined concentrations of free amino groups were presented in the column 6. The column 7 shows the concentrations of amino groups calculated from that in 100/0 (pure chitosan) solution. For example, the concentration of amino groups in 85/15 solution equals $141.2 \times 0.85 = 120.0$ (mM). Such values can be deemed as the calculated amount of amino groups in the solutions without considering the influence of pectin.

First, comparing the values in columns 6 and 7, the determined amounts of free amino groups were less than the calculated amounts of amino groups in each solution. This result can be an evidence of the PEC formation between chitosan and pectin. Moreover, if comparing the 70/30 solution with 70/30/Ca and 70/30/covalent solutions, the amounts of free amino groups decreased further. For 70/30/Ca mixture solution, the major effect of calcium ion was toward pectin, thus the addition of calcium ion might cause no significant effect on the amount of free amino groups. The decrease in the amount of free amino groups may be caused by the chlorine ion (Cl^-) added together with calcium ion into the solution, because chlorine ion could also form ionic interactions with amino groups, and reduced the detectable amount of free amino groups. For 70/30/covalent mixture solution, there should be additional covalent bond formation between chitosan and pectin mediated by the EDC/NHS, causing the detectable amount of free amino groups to decrease. According to such results, the covalent bond formation between chitosan and pectin was verified.

3.2. Characterization of the porous membranes

According to the SEM micrographs, it was first found that porous membranes made of pure pectin could be successfully fabricated by the freeze-gelation method (Fig. 1a, 0/100), implying that it would be a novel and suitable method for fabricating pectin-based porous membranes. In a previous study the porous membranes made of chitosan, alginate, PLLA, and PLGA were fabricated by the freeze-gelation method (Ho et al., 2004). In this study, we demonstrated that this method is also suitable for pectin. Second, after mixing pectin with chitosan (Fig. 1a, 70/30), the fabricated composite porous membranes were still uniform and had interconnected pore structures similar to the pure chitosan or pure pectin membranes. Finally, after adding crosslinking reagents such as calcium chloride or EDC/NHS, the porous membranes could still be fabricated successfully (Fig. 1a, 70/30/Ca, 70/30/covalent).

The FTIR spectra of the various chitosan/pectin composite membranes were shown in Fig. 1b. Chitosan showed a peak at 1657 cm^{-1} which could be the C=O bond (acetyl group), and another peak at 1564 cm^{-1} being the N–H bond (amino group). On the other hand, pectin had a peak at 1735 cm^{-1} which could be the C=O bond of the methoxylated carboxyl groups and 1611 cm^{-1} being the carboxyl groups. However, in the 70/30 composite material, the amino groups of chitosan would ionically interact with the carboxyl groups of pectin, making the two peaks at 1564 cm^{-1} (chitosan) and 1611 cm^{-1} (pectin) shift to 1598 cm^{-1} of 70/30 and 1589 cm^{-1} of 70/30/Ca composite material. Besides, due to the strong ionic interaction, the peaks of C=O bonds on both chitosan and pectin were
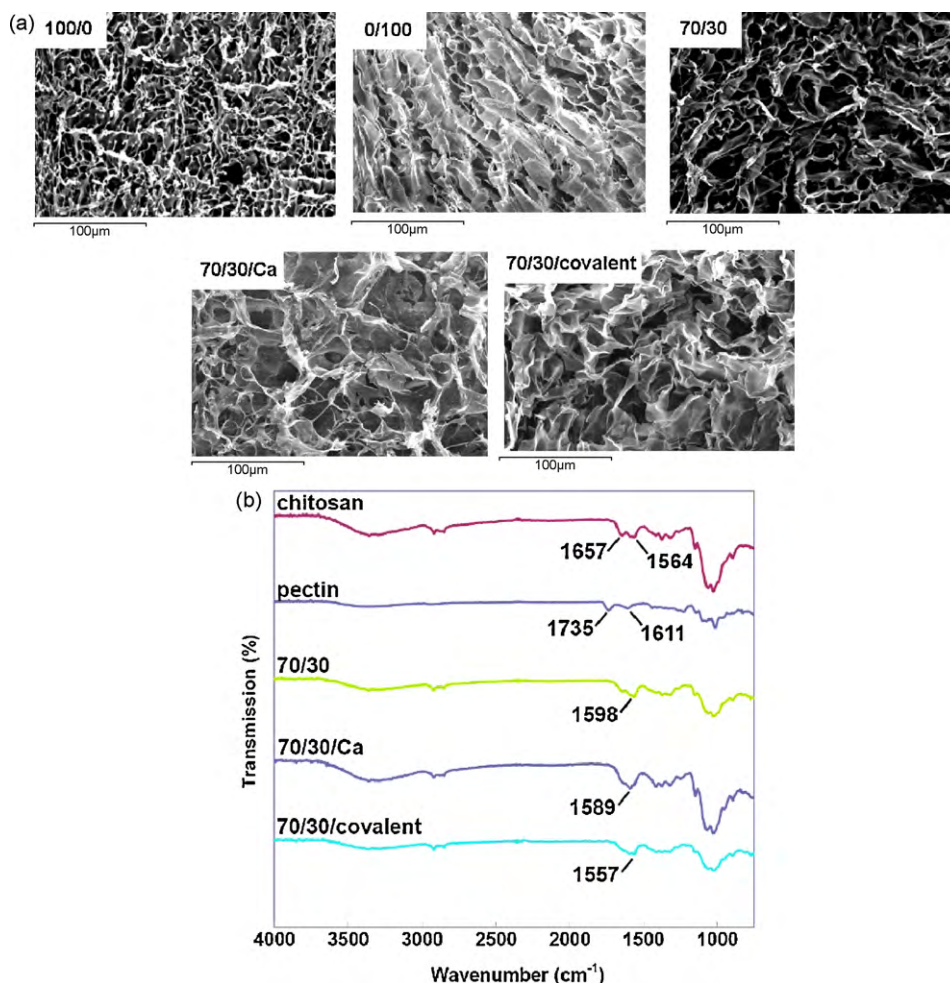
Fig. 1. (a) SEM micrographs of the various chitosan/pectin porous membranes fabricated by the freeze-gelation method (bar = 100 μm). Compositions are listed in Table 1. One-component: 100/0 (chitosan), 0/100 (pectin). PEC-type: 70/30. PEC/Ca-type: 70/30/Ca. PEC/covalent-type: 70/30/covalent. (b) FTIR spectra of the various chitosan/pectin composite membranes.

covered. The 70/30/covalent composite material showed a peak at 1557 cm^{-1} which could be the amide bond that formed between the amino groups of chitosan and carboxyl groups of pectin. Taken together, our results provided evidence that strong ionic interactions between chitosan and pectin were present in the fabricated materials.

3.3. Analysis of the mechanical properties

Because the membranes were porous, the use of the maximum load divided by the cross-sectional area (i.e., the stress at maximum load) might not be a suitable index to express the tensile strength of the membranes. Besides, the thicknesses of all the membranes were almost the same (0.2 cm), and thus the effect of cross-sectional area could be neglected. Therefore we used the maximum load (N) of each membrane divided by its dry weight (g), not by the cross-sectional area, to reflect the tensile strength of each unit amount of material. We also measured the elongation capability to investigate the ductility of the composite membranes.

The influences of the composition (for PEC-type) and the addition of calcium ions (for PEC/Ca-type) or EDC/NHS (for PEC/covalent-type) on the mechanical properties of the membranes were investigated. For the PEC-type membranes (Fig. 2a), it was found that with an increasing ratio of pectin up to 50% in the composite membranes, the strength of the membrane significantly increased. Compared to the pure chitosan membrane

(100/0), the tensile strength of the chitosan/pectin membrane (50/50) was about 4-times higher (32 N/g vs. 8 N/g). Since ionic interactions formed between chitosan and pectin, increasing the content of pectin would increase the amount of ionic interactions formed in the composite membranes, thus improving their tensile strengths. When the weights of chitosan and pectin were equal, the molar ratio of the carboxyl groups of pectin to the amino groups of chitosan was about 0.94 which was higher than other PEC-type membranes. Therefore, the strength of ionic interactions in 50/50 membranes might be the highest among all the PEC-type membranes fabricated in this study, thus maybe leading to the greatest tensile strength. Higher tensile strength of the wet membranes means that they have greater workability at the initial stage when in use. On the other hand, although the pure pectin membranes, as well as 30/70 membranes, could be successfully fabricated, the structures of the wet membranes were loose and easily collapsed. Therefore, the tensile strength of the 0/100 and 30/70 membrane was too low to be determined. Only with the addition of adequate amount of chitosan which acted as a stabilizer, the solid status of pectin can be maintained in an aqueous solution. For the same reason, some of the subsequent characterization of the 0/100 membranes and 30/70 membranes could not be carried out. According to these results, it was clear that the improvement in the strength of the composite membranes was caused by the ionic interactions between chitosan and pectin, not due to chitosan or pectin individually. As for the elongation capability, pure chitosan membranes
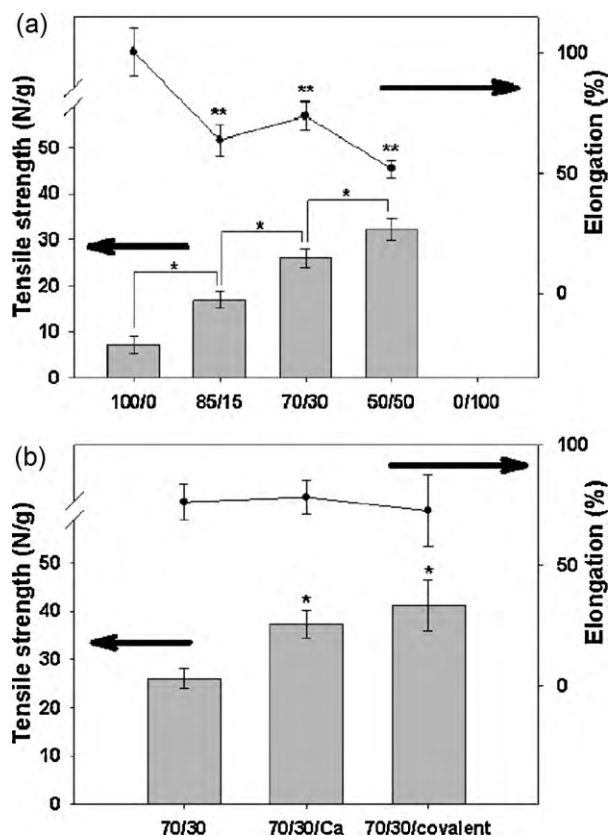
Fig. 2. Tensile strengths and elongations of the various chitosan/pectin composite porous membranes. (a) PEC-type ($n = 6$, mean ± S.D., *$p < 0.05$, **$p < 0.05$ vs. 100/0), and (b) PEC/Ca-type and PEC/covalent-type membranes ($n = 6$, mean ± S.D., *$p < 0.05$ vs. 70/30).

had a greater elongation capability (Fig. 2a). After the addition of pectin into the membranes, the elongation capability decreased. We propose that the decreasing ductility of the composite membranes resulted from an increasing amount of ionic interactions. Therefore the structure of the composite membranes was rigidly fixed, and the ductility of the membranes significantly decreased. Based on these results, we may say that in this study a new chitosan/pectin composite material with greater tensile strength and stiffness has been fabricated.

It was previously mentioned that ionic interactions can be partially replaced by amide bonds with the addition of EDC/NHS. This proposition was supported by the enhanced tensile strength of the PEC/covalent-type composite membranes (Fig. 2b). The tensile strength of the 70/30/covalent membranes was about 40 N/g, significantly greater than the 26 N/g of 70/30 membranes. However, the elongation capability of 70/30/covalent membranes did not differ from that of the 70/30 membranes (Fig. 2b), suggesting that EDC/NHS only strengthened the interactions formed between chitosan and pectin (i.e. replacing ionic interactions by stronger covalent bonds), but did not affect other properties of the fabricated composite membranes. As a result, EDC/NHS can be deemed as a "strength enhancer" for chitosan/pectin composite membranes. The same improvement effect was also observed in the 70/30/Ca membranes (Fig. 2b). Although the mechanism of crosslinking by calcium ions differed from that of EDC/NHS, the total intermolecular interactions (interactions between chitosan and pectin plus interactions between pectin and pectin mediated by calcium ions) formed in the chitosan/pectin mixture may increase, thus resulting in the higher tensile strength of 70/30/Ca membranes than 70/30 membranes.

3.4. Hydrophilicity of dense films and water uptake capabilities of porous membranes

Hydrophilicity can be exhibited by the contact angle of a material with water. The smaller the contact angle, the more hydrophilic the material would be. To measure the contact angles of the composite materials, dense films instead of porous membranes were used in order to avoid the effect of pores on the contact angles. In previous researches, pectin was usually deemed as a hydrophilic polysaccharide (Parris, Coffin, Joubran, & Pessen, 1995). In this study, we created a way to exhibit the hydrophilicity of pectin. Considering the PEC-type composite films, one can see from Fig. 3a that the contact angle of pure chitosan films was about 86°. As the ratio of pectin increased, the contact angle decreased to about 45° for the 50/50 composite films. So we proposed that the increased hydrophilicity of the composite films was caused by the presence of pectin in the composite films. This improvement in the hydrophilicity might also lead to the increased water uptake capability (Fig. 3c). Because the porosities (above 95%) and pore sizes (about 10–20 μm) of various membranes were similar (data not shown), the difference in the water uptake capabilities might result mainly from the different amounts of water absorbed by the material itself. The pure chitosan porous membrane (100/0) absorbed water about 15 times the dry weight of the membrane. After being incorporated with pectin, the water uptake increased with the rising content of pectin in the composite membranes to a maximum of about 24 times the weight of the membranes (50/50). Therefore, we may propose that the hydrophilic property of pectin further improved the water uptake capability of the membranes. However, as mentioned above, the pure pectin membranes could not maintain its solid status in water so the water uptake could not be measured. Also, the contact angles of the 0/100 and 30/70 films could not be determined due to their high water-solubility. Therefore, chitosan was required for pectin to maintain its stability in water.

The contact angles of the PEC/Ca-type (70/30/Ca) are shown in Fig. 3b. There was a slight change in the contact angles of the composite films containing calcium ions. The slight drop in the contact angles might result from the calcium ions. Due to the existence of hydrophilic calcium ions bound in the fabricated composite material, its contact angle with water was decreased. For the PEC/covalent-type (70/30/covalent) composite films (Fig. 3b), there was no significant change in the contact angles of the composite films containing EDC/NHS. The major effect of EDC/NHS was considered to replace some of the ionic interactions between chitosan and pectin by covalent bonds. Since there was no change in the polysaccharide composition compared to the 70/30 films, the hydrophilicity showed no significant difference between the 70/30/covalent films and the 70/30 films. But adding calcium chloride or EDC/NHS to prepare the PEC/Ca-type (70/30/Ca) or PEC/covalent-type (70/30/covalent) composite membranes resulted in the decrease of the water uptake of the membranes (Fig. 3d). For the 70/30/covalent composite membranes, the ionic interactions forming between the chitosan and the pectin were partially replaced by covalent bonds (amide bonds). Due to the stronger binding force of covalent bonds than ionic interactions, the intermolecular space might be reduced (Richert et al., 2004), making it more difficult for water to enter the membranes. Calcium ions might have a similar water uptake-reducing effect (70/30/Ca) due to the stronger intermolecular interactions, although the mechanism differs from that of EDC/NHS. The opposite effect of calcium ions or EDC/NHS (negative) vs. pectin content (positive) on the water uptake means that the properties of the composite membranes can be widely adjusted, thus making it easier to prepare membranes suitable for various applications.
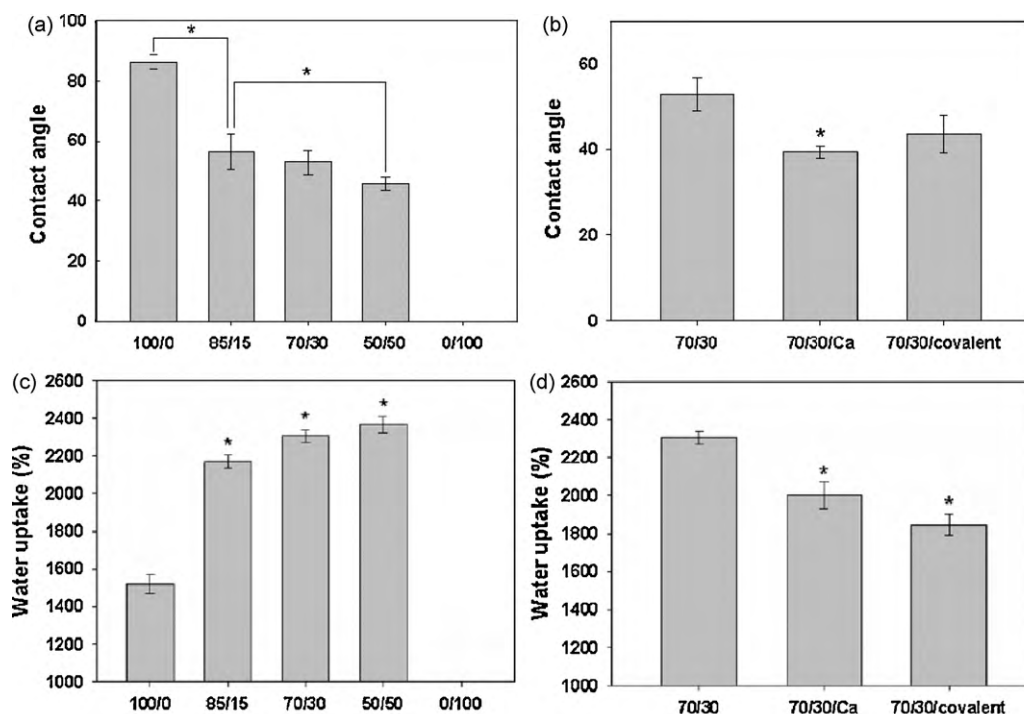
Fig. 3. Contact angles (of dense films) and water uptakes (of porous membranes) for the various chitosan/pectin composite materials. Contact angles of (a) PEC-type dense films ($n = 5$, mean $\pm$ S.D., $*p < 0.05$), (b) PEC/Ca-type and PEC/covalent-type dense films ($n = 5$, mean $\pm$ S.D., $*p < 0.05$ vs. 70/30). A smaller contact angle indicates that the material is more hydrophilic. Water uptakes of (c) PEC-type ($n = 6$, mean $\pm$ S.D., $*p < 0.05$ vs. 100/0), (d) PEC/Ca-type and PEC/covalent-type porous membranes ($n = 6$, mean $\pm$ S.D., $*p < 0.05$ vs. 70/30).

3.5. Disintegration of the porous membranes

In many situations, a material used as an edible sponge or disintegrant carrying food or drug is expected to gradually disintegrate as time goes by. In the previous research (Zhu et al., 2009), the weight loss of a material was also used as an index of degradation of a composite material. From the results shown in Fig. 4a, the pure chitosan membranes (100/0) had about a 13% weight loss after immersion for 14 days, while weight loss of the pure pectin membranes (0/100) was extremely quick (data not shown). For the PEC-type composite membranes, as the content of pectin increased, the weight loss increased. The weight losses of 85/15, 70/30 and 50/50 composite membranes were about 40%, 55% and 75%, respectively, after immersion for 14 days. Considering the components of the membranes, the weight loss was not merely caused by the loss of pectin, since the total weight loss (40%, 55% and 75%) exceeded the content of pectin (15%, 30% and 50%) in the composite membranes. During the immersion period, a part of chitosan might be carried away from the membranes with the pectin because they were ionically bound with the pectin, thus making the composite membranes more easily disintegrate. The disintegration of the composite membranes was notable from several aspects. For pectin, the extremely high solubility in aqueous environment limits its applicability. Chitosan can stabilize pectin via ionic interactions, improving the sustainability of pectin in an aqueous environment. For chitosan, incorporating with pectin could be a feasible way to speed up the disintegration. Chitosan was sometimes criticized as a material with too slow disintegration rate. Therefore, additional enzymes such as lysozyme or chitosanase, were usually required to speed up the degradation or the disintegration. In this study, we have provided a more economic way to speed up the disintegration of the chitosan-based materials without the help of additional enzymes (Zhu et al., 2009). Finally, the disintegration of such composite membranes could be controlled by the content of pectin included. Reviewing our results about the tensile strength
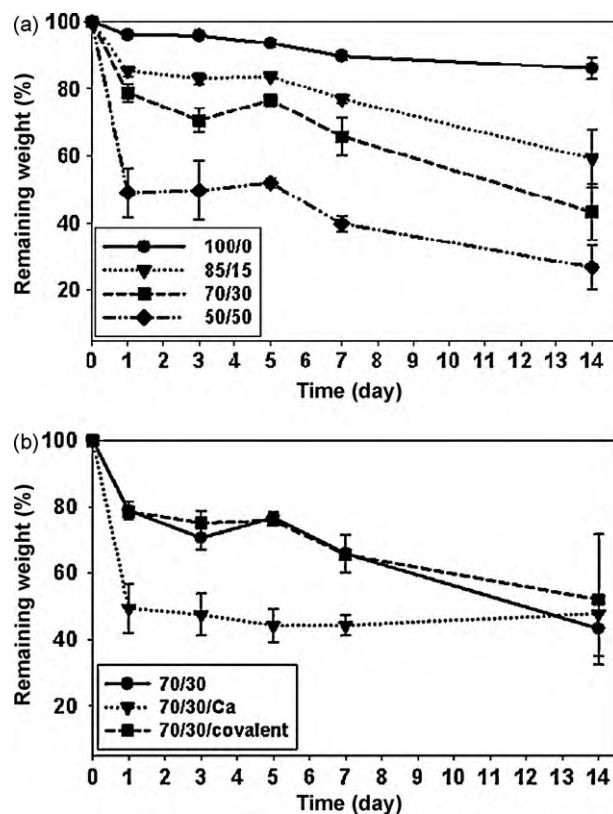

Fig. 4. Disintegration (weight loss) profiles of the various chitosan/pectin composite porous membranes in phosphate-buffered saline (PBS). (a) PEC-type, (b) PEC/Ca-type and PEC/covalent-type membranes ($n = 4$).

(Fig. 2a), the chitosan/pectin composite membranes had greater tensile strength. Combined with the results of disintegration behavior, the chitosan/pectin composite membranes have both improved workability at the initial stage when in use and controllable disintegration rate, making it useful as a edible sponge or disintegrant carrying food or drug (Oshima, Sonoda, Ohkuma, & Sunada, 2007).

The disintegration behaviors of PEC/covalent-type and PEC/Ca-type composite membranes are shown in Fig. 4b. The 70/30/covalent composite membranes lost its weight by about 50% after 14 days, similar to the profile of the 70/30 composite membrane. It was implied by this result that the disintegration profile of chitosan/pectin composite membranes might not be affected by the bonding type between chitosan and pectin. The covalently bound chitosan by pectin might also be carried away with the pectin, just like the ionically bound chitosan. This result may be useful for future development of chitosan/pectin composite materials, because one can improve the strength of a chitosan/pectin composite membrane via EDC/NHS without affecting its disintegration behavior. On the other hand, the disintegration profile of the PEC/Ca-type composite membranes significantly differed from those of the other types of composite membranes. The 70/30/Ca membranes quickly lost its weight in the initial period (by 50% in the first day) and then remained relatively stable in the subsequent period. After 14 days, however, the weight loss of 70/30/Ca membranes (about 53%) was similar to those of the 70/30 (about 55%) and 70/30/covalent (about 50%) membranes. Intrinsically, the polysaccharide compositions of these composite membranes were similar; the only difference among them was the interactions forming in the composite membranes. In the 70/30/Ca composite membranes, a part of the carboxyl groups of pectin did not ionically interact with chitosan, but was ionically crosslinked by calcium ions with other pectin. However, chitosan was aforementioned that it could be a stabilizer for pectin to maintain its solid status in an aqueous environment. Without the stabilizing effect provided by chitosan, calcium ion-crosslinked pectin might not be so sustainable in water and would be more easily released from the composite membranes, resulting in the greater weight loss in the initial period of immersion. Besides, the weight loss in day 1 (about 50%) had exceeded the amount of pectin in the composite membranes (30%), meaning that not only pectin but also some of the chitosan was released from the composite membranes. In other words, the function of chitosan to be a stabilizer for pectin via ionic interactions was partially "blocked" by the added calcium ions. This blocking effect by a salt, such as NaCl, between polycation and polyanion was also reported in a previous research (Espinosa-Andrews, Baez-Gonzalez, Cruz-Sosa, & Vernon-Carter, 2007). According to our results, we may suggest that the addition of calcium ions could be used to alter the disintegration profile of chitosan/pectin composite membranes. The altered disintegration profile could be helpful to meet the various requirements for food- or drug-carrying applications.

4. Conclusions

Novel chitosan/pectin composite membranes of various compositions with several types of interaction were successfully fabricated using a freeze-gelation method. There are several aspects of significance in this study. First, we have verified that freeze-gelation method could be a novel and suitable method for pectin or pectin-based materials. Second, from the standpoint of chitosan, pectin could be an ideal additive that could significantly improve the membrane properties such as tensile strength, hydrophilicity, and water uptake. These improvements can be adjusted by the content of pectin in the composite membranes. Moreover, such composite membranes had higher disintegration rate which could be controlled by the content of pectin without the help of additional

enzymes. The diversity of the disintegration behavior makes the composite membranes useful as an edible sponge or disintegrant carrying food or drug. Finally, considering the use of crosslinking reagents, we have demonstrated a way to enhance the function of calcium ions or EDC/NHS by adding them before the two polysaccharides (chitosan and pectin) come in contact with each other. The tensile strength of the composite membranes can be increased by EDC/NHS without affecting the disintegration profile, while the tensile strength and the disintegration profile of the composite membranes were both changed by calcium ions. With the addition of different crosslinking reagents, the variety of chitosan/pectin composite materials could be further expanded.

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