

PEC Films Prepared from Chitosan–Alginate Coacervates

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Chitosan–alginate polyelectrolyte complex (PEC) have been prepared *in situ* in beads and microspheres. This study examines the preparation of suitable chitosan–alginate coacervates for casting into homogeneous PEC films for potential applications in packaging, controlled release systems and wound dressings. Coacervation between chitosan and alginate was rapid, but the rate may be controlled with the addition of water miscible organic solvents. Compared with ethanol and PEG200, acetone was the more promising solvent moderator. Suspensions of fine, uniformly dispersed coacervates were produced by a dropwise addition of 0.25% w/v chitosan solution (solvent: 1 : 1 v/v of 2% acetic acid and acetone) into 0.25% w/v sodium alginate solution in water under rapid agitation. The PEC films were transparent and flexible. They exhibited high permeability to water vapor, but resisted complete dissolution in 0.1 M HCl, distilled water and pH 7.4 phosphate buffer solution. Microscopic heterogeneity in the films could be reduced by immersion in aqueous media, but this was accompanied by modifications in the thickness, permeability and mechanical property of the films.

Key words chitosan–alginate PEC films; coacervation; morphology; permeability; water sorption; mechanical properties

Chitosan is the deacetylated product of chitin, a polysaccharide found in abundance in nature, primarily in crustacean shells. It is water soluble at low pH due to the protonation of the glucosamine moieties. Alginate is a polysaccharide derived from brown seaweed and comprises blocks of guluronic and mannuronic acids. Some salts of alginates, e.g. sodium alginate, are soluble in water. Both polymers are biocompatible and edible, and have been widely studied in the formulation of biomedical^{1–3} and agricultural products.^{4,5}

Whilst the water solubility of the polymers is an advantage in eliminating the use of noxious solvents during processing, it limits the use of the polymers as packaging materials, carriers in controlled release drug delivery systems and as biomaterials. In recent years, the development of polyelectrolyte complexes (PEC) involving chitosan and alginate has gained impetus.^{1,5–12} The PEC has been shown to be water insoluble and proven more effective in limiting the release of encapsulated materials compared to either polymer alone.^{1,7} Such complex formation is expected to result mainly from electrostatic interactions between the amino groups in chitosan and the carboxyl groups in alginate. However, the reaction between chitosan and alginate has been reported to be independent of the pH and ionic strength of the media,^{11,12} leading Mireles *et al.*¹² to postulate that hydrogen bonding and other intramolecular interactions may predominate in the complex formation process.

The preparation of chitosan–alginate PEC to date has involved mainly beads and microsphere systems.^{1,5–10} These systems were heterogeneous, comprising the PEC membrane encapsulating a chitosan or alginate core. In most cases, the extent of reaction is not known or controlled. PEC involving chitosan with other polymers have been reported,^{13–17} and some have been cast into films.^{15–17} The demonstration of film forming capability is important as it widens the applications of the PEC to include membrane products that can be used for packaging, dialysis, coating and wound dressings. The objectives of this study were to examine the feasibility of preparing homogeneous PEC films from chitosan–alginate coacervates, and to characterize the films as a function of processing parameters.

Experimental

Materials Chitosan (Tokyo Kasei Kogyo Company Ltd., Tokyo, Japan) was purified and further deacetylated by refluxing twice with 40% w/v sodium hydroxide in the presence of NaBH₄, intermitted by washing with copious amounts of water. The polymer was then dissolved in 3% v/v acetic acid, regenerated with 1 M sodium hydroxide, washed with distilled water and freeze-dried. Viscosity average molecular weight, M_v , of the purified chitosan, measured by solution viscometry (Cannon Ubbelohde, 30 °C, 0.2 M CH₃COOH/0.1 M CH₃COONa solvent, $K=6.6\times 10^{-3}$, $a=0.88$),¹⁸ was $8.60 (\pm 0.64)\times 10^5$ ($n=5$) while its degree of deacetylation, determined by the first derivative UV spectrophotometric method,¹⁹ was $95.7\pm 0.2\%$ ($n=5$). Sodium alginate (Lot #2445310L, BDH Ltd., Poole, England) was used as received. It has an intrinsic viscosity of 3.23, which may be approximated to a M_v of 1.04×10^5 (Cannon Ubbelohde, 25 °C, 0.1 M NaCl solvent, $K=6.9\times 10^{-6}$, $a=1.13$).²⁰ All other chemicals and reagents were of analytical grade.

Preparation of Films Chitosan solutions (0.0625 to 0.5% w/v) were prepared by adding acetone, ethanol or PEG 200 (0 to 50% v/v) to filtered solutions of the polymer in 2% v/v aqueous acetic acid. Twenty-five milliliters of the solution were added dropwise to 25 ml of sodium alginate (0.0625 to 0.5% w/v) in water. Coacervation was effected under vigorous manual agitation for 20 min. Selected suspensions were cast into polyethylene petri dishes (Sterilin, UK, internal ϕ 85 mm) and allowed to dry overnight at ambient temperature (25 °C).

Processing of Films Dried chitosan–alginate PEC films were immersed for 1 h in distilled water, 0.1 M NaOH, 0.1 M HCl or by sequential immersion for 1 h in 0.1 M NaOH followed by 1 h in 0.1 M HCl. The films were then retrieved with care, washed with distilled water until the washings were neutral to litmus, and dried for 10 h in an oven at 60 °C.

Characterization of Films The PEC films were stored in desiccators at ambient temperature, and measured for weight (Mettler-Toledo) and thickness (Mitutoyo thickness gauge). Surface morphology was observed under a light microscope (LM) (Leica 520804 microscope, Nikon FM2 camera, 200 \times magnification) and a scanning electron microscope (SEM) (JEOL JSM-T220A, 15 kV and 200 \times magnification, Mamiya camera). Film samples were coated with gold-platinum (Ion sputter JFC-1100, 5 min) for the SEM observations.

To determine film permeability to water vapor, film samples of 40 mm diameter were mounted on plastic cups with the help of water impermeable sealant to give test areas of 7.07×10^{-4} m². Each plastic cup contained weighed amounts (5–6 g) of silica gel previously activated at 200 °C for 1 h. At prescribed time intervals, the cups were weighed to determine the amount of water vapor transmitted through the films. The experiments were conducted in triplicates at controlled temperature and relative humidity (Contherm environmental chamber). Control experiments comprised using empty cups sealed with the PEC films, as well as using aluminum foil to seal cups containing the silica gel. In both controls, there were negligible changes in cup weight with time, suggesting that the extent of water vapor sorption by

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the PEC films, and water permeability through the plastic cup *per se*, were negligible.

The water sorption capabilities of the PEC films in distilled water, 0.1 M HCl and pH 7.4 phosphate buffer solutions were measured. Triplicate film samples of 30 mm diameter were stored in desiccators to constant weight before immersion in 100 ml of test media at $37.0 \pm 0.1^\circ\text{C}$. At periodic intervals, the films were retrieved from the media, blotted with filter paper and weighed immediately. The extent of water sorption is given as the ratio of weight at time t (W_t) and original weight (W_0) of the films.

Mechanical properties of the films ($n=5$) were determined at 25°C and 50% relative humidity using a tensile tester (Instron 4502). Film samples (70×10 mm) were held at a grip distance of 50 mm and subjected to a strain rate of 1.0 mm/min.

Two milligrams of film were mixed with 200 mg KBr and pressed at 13 tons for 3 min to give sample disks for IR spectroscopic (Jasco FTIR) measurements. Disks were similarly prepared using isolated chitosan–alginate coacervates, alginic acid, chitosan acetate films and purified chitosan instead of the PEC films. The coacervates were obtained from mixing 0.25% w/v chitosan and alginate solutions, and were retrieved by filtration, washed with water, processed as described for the PEC films and freeze-dried. Alginic acid was precipitated by adding 30 ml of 0.1 M HCl to 25 ml of the 0.25% w/v sodium alginate solution, and was recovered by filtration, washed with water and freeze-dried. The chitosan acetate film was prepared by casting 50 ml of 0.25% w/v chitosan solution in 2% v/v acetic acid.

Results and Discussion

Coacervation between Chitosan and Alginate Chitosan has pK_a of 6.5,²¹⁾ while alginate has pK_a values of 3.4 to 4.4.²²⁾ Chitosan and alginate solutions in this study had initial pHs of about 4.15 and 6.84 respectively. Under these circumstances, the amino groups in chitosan and the carboxyl groups in alginate are largely ionized, and coacervation would arise primarily from electrostatic attraction, although hydrogen bonding and hydrophobic interactions have been implicated.¹²⁾

When the chitosan and alginate solutions were mixed by dropwise addition of one solution into the other, capsules were obtained. Rapid coacervation induced the formation of a dense interphasic PEC membrane that separated the polymer solutions and prevented further reaction. Although this technology was commonly employed to prepare beads and microspheres^{5–10)} of chitosan (or alginate) encapsulated by chitosan–alginate PEC membrane, it was not suitable for the production of coacervates for casting into films. Suspensions of the capsules gave rise to heterogeneous films comprising demarcated capsules dispersed in sodium alginate matrix. To prepare coacervates for casting into homogeneous films, the rate of reaction between the two polymers must be sufficiently slow to prevent the formation of interphasic membranes, so that the reaction may be allowed to proceed to completion.

Addition of a water miscible organic solvent (acetone, ethanol or PEG200) into the chitosan solution was effective in slowing the rate of coacervation. The efficiency of the solvent moderators in producing homogeneous precipitation was in the order of acetone > ethanol > PEG200. In solvents of lower polarity, chitosan assumes less extended conformations, which restricts the reaction between the two polyelectrolytes. The rate and extent of coacervation would be determined by the accessibility of reactive groups as the polymers undergo conformational changes in solution.

It was more effective to add the solvent moderator to the chitosan, rather than the alginate, solution because of the latter's poor solubility in the mixed solvents. Other formulation parameters investigated were the concentrations of polymers

(0.0625 to 0.5% w/v) and solvent moderators (10 to 50% v/v), the rate of addition of one polymer solution to the other and the rate of stirring during mixing. Optimal suspensions of fine, uniformly dispersed coacervates were prepared by dropwise addition of 25 ml chitosan solution (0.25% w/v in a 1:1 v/v solvent mixture of 2% v/v water acetic acid and acetone) to 25 ml sodium alginate solution (0.25% w/v in water) under vigorous agitation. These suspensions were used to cast the chitosan–alginate PEC films for this study.

The IR spectrum of the isolated chitosan–alginate coacervates showed a broad band at 1607 cm^{-1} , a shoulder at 1520 cm^{-1} ($\delta_{\text{N-H}}$), and another band at 1400 cm^{-1} ($\delta_{\text{O-H}}$) (Fig. 1a). It had a fingerprint region, 1000 to 1400 cm^{-1} , typical of cellulosic polymers. Coacervates that had been processed with aqueous HCl, whether alone or sequential to NaOH immersion, gave rise to 3 prominent bands at 1750 cm^{-1} , 1635 cm^{-1} and 1520 cm^{-1} . These bands may be attributed to the protonation of residual amino groups (1520 cm^{-1}), and the conversion of the PEC to alginic acid, the 1750 cm^{-1} and 1635 cm^{-1} bands corresponding to the carboxyl and carboxylate carbonyl groups, respectively, of alginic acid (Fig. 1c). On the other hand, when the coacervates were processed with aqueous NaOH, the IR spectrum became poorly resolved, and resembled that for purified chitosan (Fig. 1c). The basic medium may cause the PEC to dissociate into soluble sodium alginate and chitosan precipitate. The latter was retained by filtration and therefore detected in the IR spectrum.

Chitosan–Alginate PEC Films Figures 2 and 3 show the LM and SEM photographs of PEC films prepared from suspensions of the chitosan–alginate coacervates. The surface of unprocessed film (Fig. 2) was undulating, comprising uniformly distributed nodule-like elevations. Mean film thickness was $48.2\text{ }\mu\text{m}$. Processing the PEC films in aqueous NaOH and HCl removed most of the nodules. The films were intact upon retrieval but, due to loss of film matrix ($25.08 \pm 2.45\%$ and $26.42 \pm 1.33\%$ ($n=8$), respectively), became thinner on drying. Film thickness is given in Table 1. Sequential immersion of the PEC films in NaOH followed by HCl also removed the nodules. These films were the thinnest (Table 1), although the weight loss ($29.22 \pm 1.02\%$) was only slightly higher than those processed in NaOH or HCl alone. Extensive film expansion in the aqueous HCl may have given rise to the thinner films.

IR spectra for the chitosan–alginate PEC films are shown in Fig. 1b. Unprocessed films gave a spectrum that was poorly resolved, showing a broad band spanning from 1500 to 1700 cm^{-1} . Immersion of the films in aqueous media reduced their thickness, which improved the resolution of the IR spectra. Processing with aqueous NaOH alone did not significantly change the IR spectrum of the PEC films. Processing the films with HCl, whether alone or sequential to NaOH treatment, produced an additional band at 1520 cm^{-1} , which may be attributed to the protonation of residual amino groups in the films. Unlike the coacervates, acid processing of the films did not produce a prominent IR band at 1750 cm^{-1} . The apparent absence of alginic acid in the PEC films may be explained by its removal during repeated washings of the films during processing. On the other hand, alginic acid would be retained in the coacervates because they were retrieved by filtration after processing.

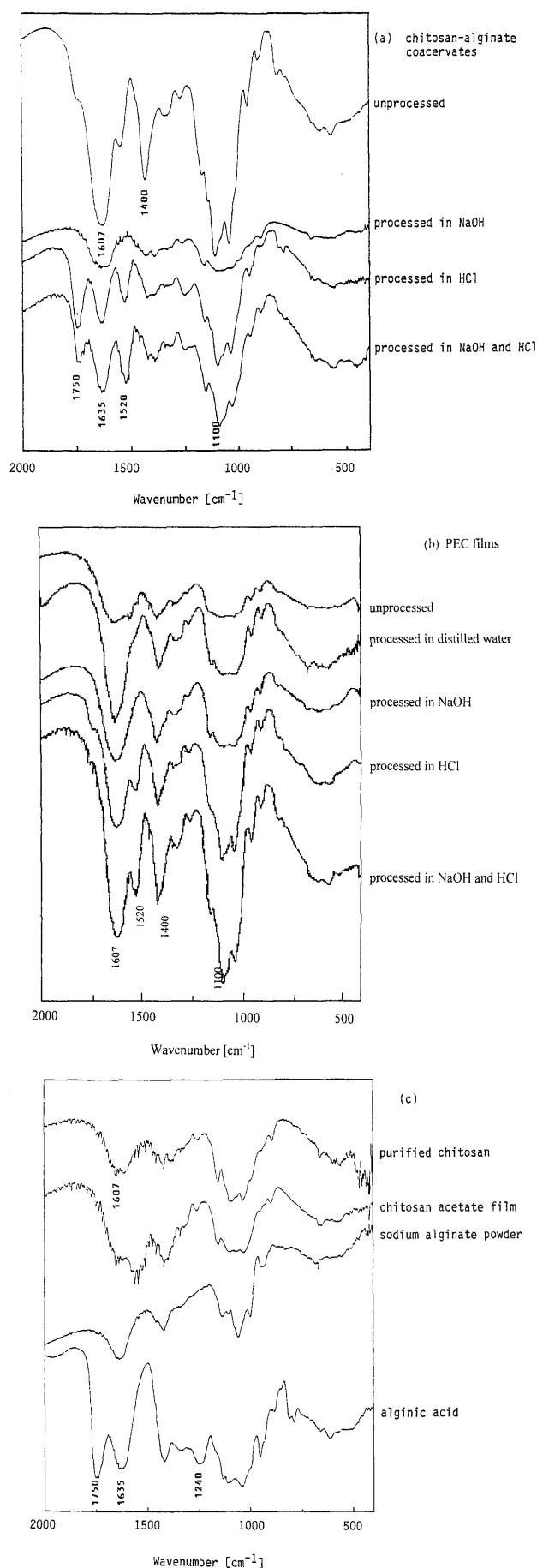


Fig. 1. IR Spectra of (a) Isolated Chitosan-Alginate Coacervates; (b) Chitosan-Alginate PEC Films; (c) Alginate Acid, Sodium Alginate, Chitosan Acetate Film and Purified Chitosan

As mass loss was similar regardless of the nature of processing medium used, we conducted further experiments by processing the PEC films in a similar manner using 0.1 M aqueous acetic acid, distilled water and pH 7.4 phosphate buffer solutions. Weight losses recorded for the films were $25.48 \pm 0.46\%$ ($n=3$), $22.93 \pm 0.75\%$ ($n=3$) and 20.1% ($n=1$) respectively. Compared to the unprocessed film, the film processed with distilled water had a similar but better resolved IR spectrum (Fig. 1b) because it was thinner (Table 1). These results suggest that the loss of film matrix was relatively independent of the nature of the aqueous media, and may be attributed to the removal of non-reacted molecules and unstable PEC from the films during processing and washings. It is pertinent to recognize that the films comprised significant amounts of stable PEC that were not solubilized by distilled water, aqueous acids or bases.

The stability of the PEC is a function of the number of binding sites between adjacent chitosan and alginate molecules. Chitosan molecules in this study had high molecular weight, about 8 times higher than that for the alginate, and are not expected to have extended conformations in the mixed solvent. Since the coacervation reaction is time-dependent, it would continue in the suspensions even as they were cast and allowed to dry into films. For this reason, the films should contain more stable PEC and less residual reactive groups than the isolated coacervates. Although quantitative analysis of residual reactive groups was not carried out, chitosan and alginate should bind in the ratio of 1:1 monomeric unit, equivalent to 1:1.5 by weight of chitosan:alginate, if reaction were carried out to completion.¹¹⁾

The permeability of the chitosan-alginate PEC films to water vapor (Table 1) was relatively high, comparable to cellophane and polyvinyl alcohol films.²³⁾ Thickness and area of the films available for water vapor transmission were considered when calculating the film permeability. Film permeability was reduced after processing with aqueous media, with HCl having a greater effect than NaOH. The film permeability was in the order of unprocessed films > films processed in aqueous NaOH > films processed in distilled water > films processed in aqueous HCl > films processed in both NaOH and HCl. The poorer permeability of processed films could be associated with greater homogeneity in film composition, which allowed for closer molecular chain packing in the films. Rearrangement of polymer chains was probably more extensive in films processed with aqueous HCl because of film expansion in this medium.

While the permeability of the processed films was relatively unchanged, the unprocessed film had lower permeability when the temperature was increased from 25 °C to 32 °C. Enhanced chain mobility at higher temperature may have reduced the porosity of the unprocessed film through molecular rearrangement; but for the already dense processed films, such molecular arrangement would not significantly affect the film porosity. Raising the RH from 50% to 80% also lowered the permeability of the unprocessed film, probably through a mechanism similar to that seen at elevated temperature, while the acid- and base-processed films showed slightly higher permeability to water vapor. The latter may be attributed to a hydration effect.

The capacity of the PEC films for water sorption in different liquid media is shown in Fig. 4. In many instances, the

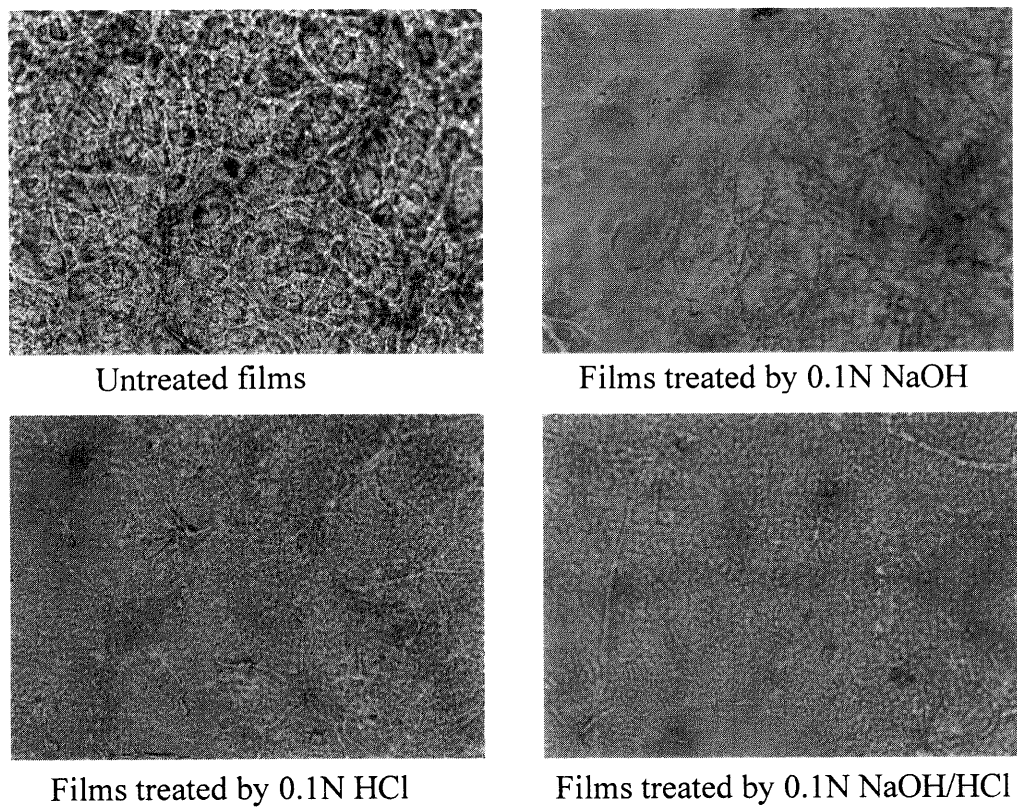


Fig. 2. Morphology of Chitosan-Alginate PEC Films as Observed under a Light Microscope

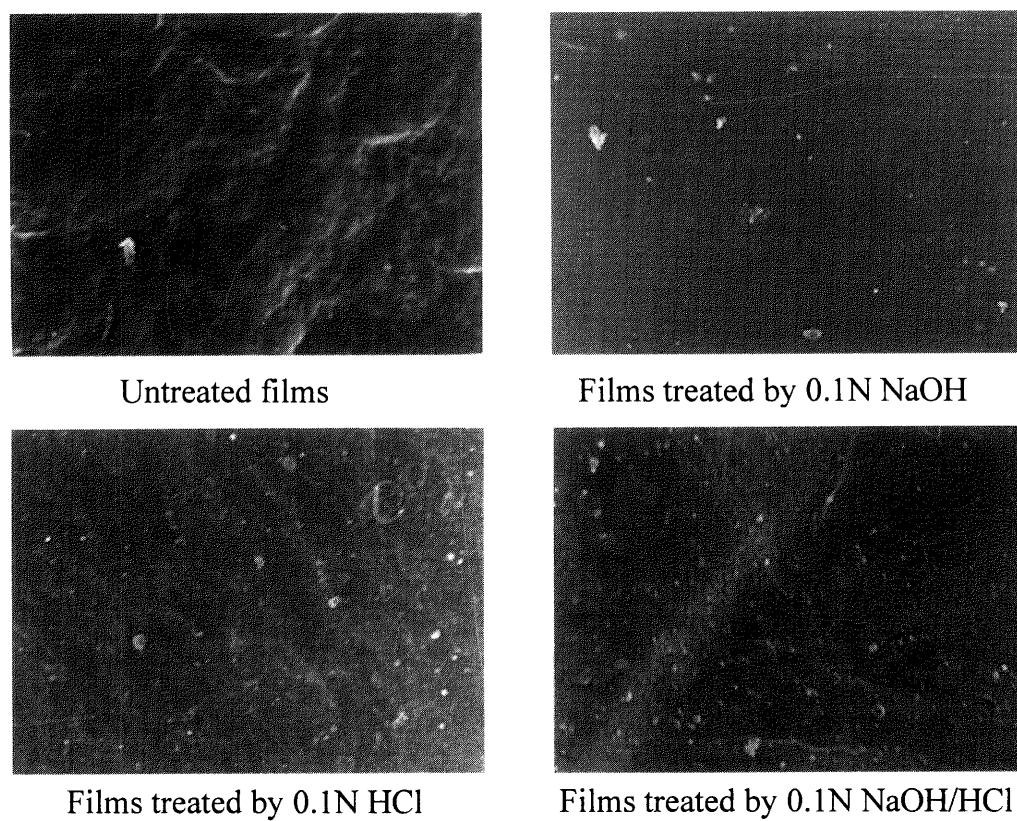


Fig. 3. Surface Morphology of Chitosan-Alginate PEC Films as Observed under a Scanning Electron Microscope

Table 1. Permeability ($\text{g} \cdot \text{mm} \cdot \text{m}^{-2} \cdot 24 \text{ h}^{-1} \cdot \text{mmHg}^{-1}$) and Mechanical Properties of Chitosan–Alginate PEC Films

Processing medium	Thickness (μm) ($n=5$)	Permeability to water vapour ($n=3$)			Mechanical properties ($n=5$)		
		RH 50%, 25 °C	RH 80%, 25 °C	RH 50%, 32 °C	Young's modulus (MPa)	Tensile strength (MPa)	Strain at break (%)
Unprocessed	48.2 ± 7.3	6.21 ± 0.12	5.78 ± 0.32	4.08 ± 0.12	450.91 ± 123.77	4.53 ± 0.83	8.41 ± 1.84
Distilled water	31.9 ± 4.7	3.29 ± 0.06	—	—	—	—	—
0.1 M NaOH	29.6 ± 3.6	3.69 ± 0.14	4.03 ± 0.29	3.89 ± 0.12	1338.22 ± 101.91	12.34 ± 1.20	7.75 ± 1.85
0.1 M HCl	19.1 ± 0.6	2.49 ± 0.05	2.81 ± 0.09	2.37 ± 0.06	918.34 ± 146.38	7.53 ± 1.88	2.46 ± 0.57
0.1 M NaOH, and 0.1 M HCl	17.6 ± 0.9	2.07 ± 0.03	2.19 ± 0.08	2.21 ± 0.10	752.76 ± 48.19	4.49 ± 0.76	2.32 ± 0.20

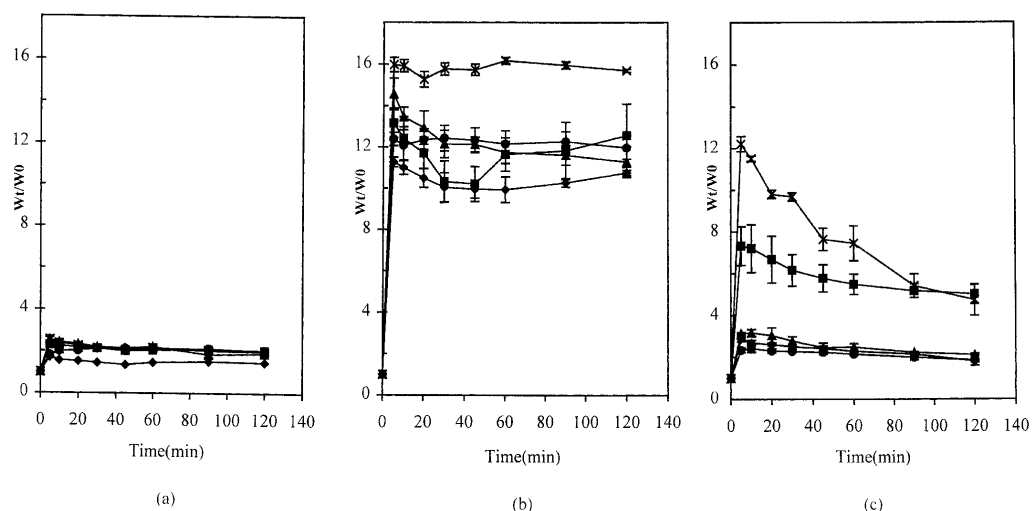


Fig. 4. Water Uptake by PEC Films as Measured by the Extent of Weight Change in (a) pH 7.4 Phosphate Buffer Solutions, (b) 0.1 M HCl and (c) Distilled Water

Symbols: \blacklozenge , unprocessed films; \bullet , processed with distilled water; \blacktriangle , processed with aqueous NaOH; \blacksquare , processed with aqueous HCl; \times , processed with aqueous NaOH and HCl.

films showed rapid weight gain in the first 5 min, followed by gradual weight loss to reach equilibrium level at about 20 min. The weight loss, which was substantial for the thin processed films, may be attributed to solubilization of film matrix. However, it is perplexing that such weight loss was not apparent in the very thin NaOH/HCl processed film when placed in 0.1 M HCl.

All films showed poor water uptakes in the pH 7.4 phosphate buffer solution (Fig. 4a), while relatively large amounts of water were absorbed, particularly for processed films, in 0.1 M HCl (Fig. 4b). This difference is related to medium pH, since the ionic strengths of 0.1 M HCl and the pH 7.4 phosphate buffer solutions were similar (0.1 M and 0.0895 M). In the acid medium, protonation followed by the repelling action of residual amino groups in the PEC films caused film expansion, which facilitated water sorption. PEC films processed in both aqueous NaOH and HCl showed higher water sorption because they were thin films.

Disparate water sorption capabilities were observed in distilled water (Fig. 4c). Unprocessed films and films processed in distilled water or aqueous NaOH had similarly small weight gains, while films processed in aqueous HCl showed higher water uptakes. These results may be accounted for by the presence of protonated amino groups in those films pre-processed in aqueous HCl. In this medium, thin films processed in both NaOH and HCl again showed the highest water uptake in the first few minutes.

The mechanical properties of the PEC films are given in Table 1. The unprocessed film had a moderate Young's modulus and relatively low tensile strength and percent elongation at break point. Processing in NaOH significantly improved the mechanical properties of the films in terms of the Young's modulus and tensile strength, but not the elongation at break point. The enhanced strength may be attributed to the neutralization of charges at residual amino groups, which confer mechanical strength reminiscent of the chitosan film.²⁴ Tensile strength and Young's modulus were also improved, although more modestly, by processing in HCl, or in both NaOH and HCl. In this case, tensile strength may have been acquired from improved chain packing in the relatively more homogeneous films. However, these films were less ductile when compared to the unprocessed film.

Conclusion

Coherent PEC films can be prepared by casting aqueous suspensions of chitosan–alginate coacervates. The PEC films were flexible, porous and hydrophilic, yet resistant to dissolution in water, aqueous acids and bases. Despite gross homogeneity, microscopic heterogeneity was observed in the film morphology. These can be removed by processing with aqueous HCl or NaOH, which also modify the physicochemical properties of the films. Further refinements in the formulation and method of preparation, as well as mechanistic studies, are currently being investigated.

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References

- 1) Filipovic-Grcic J., Maysinger D., Zorc B., Jalsenjak I., *Int. J. Pharm.*, **116**, 39—44 (1995).
- 2) Aiedeh K., Gianasi E., Orienti I., Zecchi V., *J. Microencapsulation*, **14**, 567—576 (1997).
- 3) Lloyd L. L., Kennedy J. F., Methacanon P., Paterson M., Knill C. J., *Carbohydr. Polym.*, **37**, 315—322 (1998).
- 4) Ghaouth A. E., Arul J., Asselin A. "Advances in Chitin and Chitosan," ed. by Brine C. J., Sandford P. A., Zikakis J. P., Elsevier Applied Science, 1992, pp. 440—452.
- 5) Tay L. F., Khoh L. K., Loh C. S., Khor E., *Biotechnol. Bioeng.*, **42**, 449—454 (1993).
- 6) Liu L. S., Liu S. Q., Ng S. Y., Froix M., Ohno T., Heller J., *J. Control. Release*, **43**, 65—74 (1997).
- 7) Alexakis T., Boadi D. K., Quong D., Groboillot A., O'Neill I., Poncelet D., Neufeld R. J., *Appl. Biochem. Biotechnol.*, **50**, 93—106 (1995).
- 8) Murata Y., Maeda T., Miyamoto E., Kawashima S., *Int. J. Pharm.*, **96**, 139—145 (1993).
- 9) Knorr D., Daly M., *Process Biochem.*, **23**, 48—50 (1988).
- 10) Lee K. Y., Park W. H., Ha W. S., *J. Appl. Polym. Sci.*, **63**, 425—432 (1997).
- 11) Takahashi T., Takayama K., Machida Y., Nagai T., *Int. J. Pharm.*, **61**, 35—41 (1990).
- 12) Mireles C., Martino M., Bouzas J., Torres J. A., "Advances in Chitin and Chitosan," ed. by Brine C. J., Sandford P. A., Zikakis J. P., Elsevier Applied Science, 1992, pp. 506—515.
- 13) Hugerth A., Caram-Lelham N., Sundelof L. O., *Carbohydr. Polym.*, **34**, 149—156 (1997).
- 14) RemunianLopez C., Bodmeier R., *Int. J. Pharm.*, **135**, 63—72 (1996).
- 15) Thacharodi D., Rao K. P., *Int. J. Pharm.*, **120**, 115—118 (1995).
- 16) Yao K. D., Liu J., Cheng G. X., Lu X. D., Tu H. L., Da Silva J. A. L., *J. Appl. Polym. Sci.*, **60**, 279—283 (1996).
- 17) Nam S. Y., Lee Y. M., *J. Memb. Sci.*, **135**, 161—171 (1997).
- 18) Wang W., Bo S., Li S., Qin W., *Int. J. Biol. Macromol.*, **13**, 281—285 (1991).
- 19) Tan S. C., Khor E., Tan T. K., Wong S. K., *Talanta*, **45**, 713—719 (1998).
- 20) Martinsen A., Skjåk-Bræk G., Smidsrød O., *Carbohydr. Polym.*, **15**, 171—193 (1991).
- 21) Roberts G. A. F., *Chitin Chemistry*, Macmillan: London, 1992, Chapter 5.
- 22) King A. H., "Food Hydrocolloids," Vol. II, ed. by Glicksman M., CRC Press, Boca Raton, 1983, pp. 134.
- 23) *Plastics Design Library*, "Permeability and Other Film Properties of Plastics and Elastomers," William Andrew Inc., 1995, pp. iii.
- 24) Lim L. Y., Khor E., Koo O., *J. Biomed. Mater. Res.*, **43**, 282—290 (1998).