Interactions between Alginate and Chitosan Biopolymers Characterized Using FTIR and XPS

Gwen Lawrie,[†,‡] Imelda Keen,[‡] Barry Drew,[†] Adrienne Chandler-Temple,[†] Llewellyn Rintoul,[§] Peter Fredericks,[§] and Lisbeth Grøndahl*[,†]

School of Molecular and Microbial Sciences, The University of Queensland, Brisbane, Queensland 4072, Australia, Nanotechnology and Biomaterials Centre, The University of Queensland, Brisbane, Queensland 4072, Australia, and School of Physical and Chemical Sciences, Queensland University of Technology, Brisbane, Queensland 4001, Australia

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This study investigates alginate−chitosan polyelectrolyte complexes (PECs) in the form of a film, a precipitate, as well as a layer-by-layer (LbL) assembly. The focus of this study is to fully characterize, using the complementary techniques of Fourier transform infrared (FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS) in combination with solution stability evaluation, the interactions between alginate and chitosan in the PECs. In the FTIR spectra, no significant change in the band position of the two carbonyl vibrations from alginate occurs upon interaction with different ionic species. However, protonation of the carboxylate group causes a new band to appear at 1710 cm^{-1}, as anticipated. Partial protonation of the amine group of chitosan causes the appearance of one new band (∼1530 cm^{-1}) due to one of the $-NH_3^+$ vibrational modes (the other mode overlaps the amide I band). Importantly, the position of the two main bands in the spectral region of interest in partly protonated chitosan films is not dependent on the extent of protonation. XPS N 1s narrow scans can, however, be used to assess the degree of amine protonation. In our alginate−chitosan film, precipitate, and LbL assembly, the bands observed in the FTIR correspond to the species $-COO^-$ and $-NH_3^+$, but their position is not different from each of the single components. Thus, the conclusion of the study is that FTIR cannot be used directly to identify the presence of PECs. However, in combination with XPS (survey and narrow N 1s scans) and solution stability evaluation, a more complete description of the structure can be obtained. This conclusion challenges the assignment of FTIR spectra in the literature.

Introduction

Polysaccharide biopolymers including alginate and chitosan have been the focus of an expanding number of studies reporting their potential use in biomedical research applications such as cell encapsulation, drug delivery, and tissue engineering. Calcium alginate hydrogels, in particular, have had widespread application as the key component of microcapsules for the protection of cells for reimplantation or encapsulation of molecules for in vivo therapy. The popularity of these biopolymers results from their demonstrated biocompatibility. Matrices formed from alginate have demonstrated longevity within the body and, if highly pure, do not provoke immunogenic responses.[1−4] Chitosan has well-known antibacterial and nonimmunogenic response properties. There have been several excellent reviews of these biopolymers and their applications.[5−8]

Alginate, derived from seaweed, possesses a polysaccharide backbone comprised of two repeating carboxylated monosaccharide units (manuronic acid, M, and guluronic acid, G), the ratio of which influences the physical properties of the biopolymer. These monomers are epimers resulting in different orientation in the polymer chain (Scheme 1a) and only the G units are oriented in a manner that renders the carboxylate moieties accessible for ionic cross-linking. The addition of calcium ions to an aqueous solution of sodium alginate results in the formation of a three-dimensional calcium alginate hydrogel as the divalent calcium cations cross-link adjacent biopolymer chains (Figure 1A).[9] Chitosan (Scheme 1b) is the deacetylated form of chitin,[10] a biopolymer derived from shellfish, and is comprised of a polysaccharide backbone with mainly primary amine functional groups. The deacetylation process results in a residual percentage (typically 10−30%) of amide groups. Chitosan has excellent film forming capabilities; the film properties are affected by many parameters, including solvent systems, molecular weight, and degree of deacetylation.[11]

One of the factors that have enabled the development of tailored biomaterials using alginate and chitosan has been their potential to form a polyelectrolyte complex (PEC) through ionic interaction. It is assumed that the carboxylate moieties on alginate will ionically interact with the protonated amines on chitosan to form a three-dimensional matrix known as a physically cross-linked hydrogel (Figure 1B). The formation of these PECs does not seem to be affected by the type (G/M ratio) of the alginate used,[12] in contrast to the formation of calcium cross-linked alginate hydrogels. The ability to tailor the physicochemical properties of alginate−chitosan PECs by controlling the degree of association between the functional groups (Figure 1 B&C) offers a route toward rationalizing biopolymer scaffold design. Control of the molecules at this level requires a comprehensive understanding of the structure and dynamic properties of this system in a range of environments.

* Corresponding author. Address: School of Molecular and Microbial Sciences, The University of Queensland, Brisbane, QLD 4072, Australia. Tel: +61 7 3365 3671. Fax: +61 7 3365 4299. E-mail: l.grondahl@uq.edu.au.

† School of Molecular and Microbial Sciences, The University of Queensland.

‡ Nanotechnology and Biomaterials Centre, The University of Queensland.

§ Queensland University of Technology.

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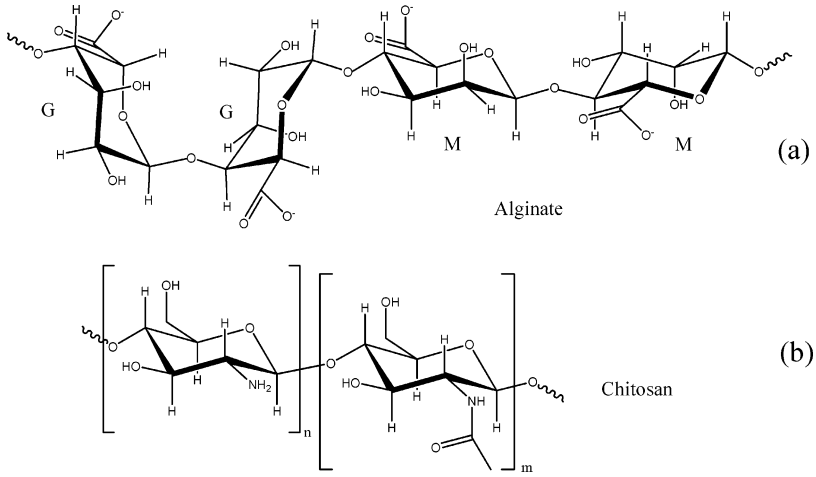
Scheme 1. Chemical Structures of (a) Alginate (Deprotonated Form) and (b) Chitosan[a]



[a] G = guluronic acid; M = manuronic acid; $n = 0.7-0.9$; $m = 0.3-0.1$

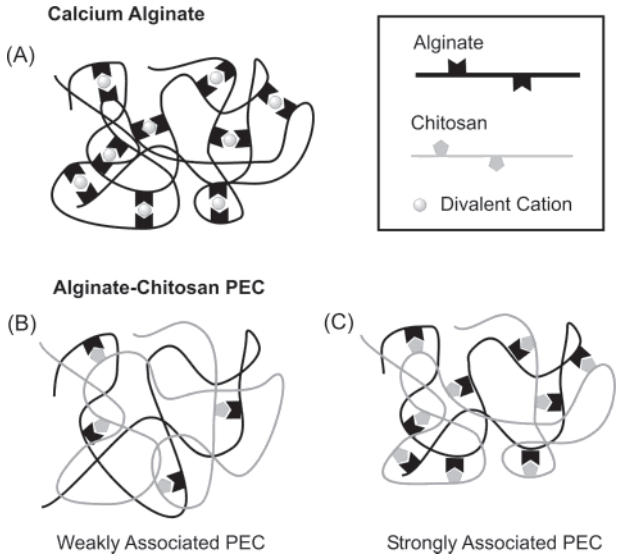


Figure 1. Schematic representation of the structures formed by alginate in association with (A) calcium ions, (B) weakly associated PEC with chitosan, or (C) strongly associated PEC with chitosan. In the PECs, the interassociated groups are shown, but these are separated on the polymer backbone by many repeating units that are not involved in ionic associations.

In this study, alginate−chitosan PECs in the form of a film, a precipitate, as well as a layer-by-layer (LbL) assembly have been prepared from various solution environments. The focus of this study is to fully characterize, using the complementary techniques of Fourier transform infrared (FTIR) and X-ray photoelectron spectroscopy (XPS) in combination with solution stability evaluation, the interactions between alginate and chitosan in the PECs. In particular, the assignment of FTIR spectra in the literature is challenged, and it is shown that complementary characterization (i.e., XPS) is needed to assess the nature of the PECs.

Experimental

Materials. Two types of chitosan were used in this study. One chitosan product was obtained from Sigma and had a reported degree of deacetylation of 85%, which was verified by XPS (see Results and Discussion section). The other chitosan product was a chitosan hydrochloric acid salt (Protasan UP CL 113) from Nova MatriX, which was found to be a (partly protonated) pure chloride salt with a degree of deacetylation of 90% based on XPS data (reported degree of deacetylation of 75−90%). Sodium alginate (medium viscosity; 3500 cps for 2% at 25 °C) derived from *Macrocystis pyrifera* (kelp) was supplied by Sigma. The M/G ratio was determined to be 1.6 from ^{1}H NMR studies following the method outlined by Grasdalen et al.[13]

Calcium chloride, sodium citrate (tribasic), potassium chloride, and sodium chloride were from Sigma, sodium dihydrogen phosphate dihydrate was from Aldrich, sodium hydroxide was from Selby Biolab, barium chloride was from Merck, dipotassium hydrogen phosphate, hydrochloric acid (37%), hydrogen peroxide (30%), and *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES) buffer were all from Ajax Finechem, lactic acid was from WWR International, Ltd., anhydrous citric acid was purchased from Rowe Scientific, and sulfuric acid (98%) was from LabScan. Milli-Q water was used throughout.

Methods. Single-component films of either alginate or chitosan were produced by casting a solution of the biopolymer into either a glass petri dish or into polystyrene tissue culture plates, and subsequently heating at 40 °C either in a regular or vacuum oven until the solvent had evaporated. Sodium alginate was dissolved in Milli-Q water, and chitosan (Sigma) was dissolved in either hydrochloric acid (1% w/v) or lactic acid (1% w/v). Chitosan (Nova MatriX) was dissolved in a phosphate buffer (0.05 M, initial pH of 2.94 was used to dissolve chitosan, which was subsequently adjusted to pH = 4.8)

The alginate film was immersed in either $CaCl_2$ or $BaCl_2$ solution (0.1 M) for 20 min at room temperature. The film was washed thrice using Milli-Q water, ensuring that excess salt was washed away. The chitosan film that had been prepared from lactic acid solution was immersed in a $CaCl_2$ solution (0.1 M) and washed in a similar manner.

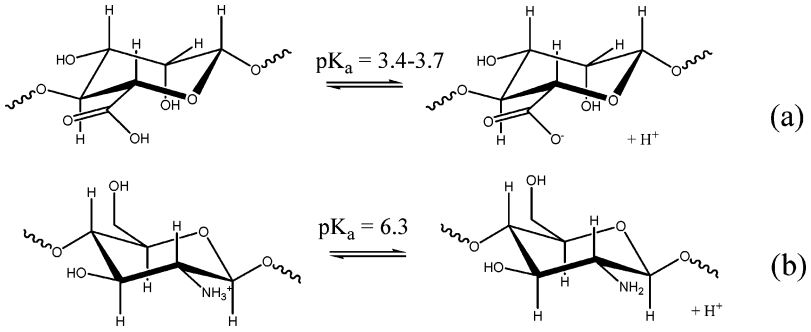
An alginic acid hydrogel was prepared from an alginate solution (1.5% alginate in Milli-Q water) by the addition of excess hydrochloric acid solution (1% HCl). The gel was washed with 1% HCl solution three times and with water three times. The alginate gel was isolated by centrifugation and dried in a vacuum oven at 40 °C.

Three different approaches were taken to produce alginate−chitosan PECs:

(1) Chitosan (NovoMatrix) solution (1% w/v, pH 2.94, 0.05 M phosphoric acid buffer), which was carefully titrated up to pH 4.8 using 0.1 M NaOH, was added dropwise to an alginate solution (1% w/v, pH 4.51, 0.05 M phosphoric acid buffer) under vigorous stirring. The formed precipitate was isolated and washed with water before drying in a vacuum oven at 40 °C.

(2) A chitosan (Sigma) solution (1.5% w/v in 1% lactic acid) was added to a sodium alginate solution (1.5% w/v in Milli-Q water) under vigorous stirring and subsequently left on a magnetic stirrer for an additional 30 min before standing for 2 h to minimize any air bubbles before casting. The resulting solution was cast into films. These were immersed into a $CaCl_2$ solution (0.1 M) for 20 min at room temperature.

Scheme 2. Illustration of the States of Protonation of the Biopolymers (a) Alginate and (b) Chitosan



The resulting film was washed thrice using Milli-Q water, ensuring that excess $CaCl_2$ was washed away.

(3) An LbL assembly was produced as follows: For the XPS and contact-angle studies, glass slides were used as the substrate. For external reflection FTIR characterization, silicon wafers were used. These substrates were cleaned using acidic piranha solution (3:1 conc H_2SO_4 and concn H_2O_2) at 90 °C for 4.5 h. The slides were then rinsed with Milli-Q water four times and dried in a vacuum desiccator overnight. The slides were subsequently immersed in a chitosan (Nova MatriX) solution (1 mg/mL, pH 3.02, 0.05 M citric acid buffer, 0.5 M NaCl) and an alginate solution (1 mg/mL in 0. 5M NaCl) alternately for 20 min each followed by thorough rinsing with Milli-Q water between each layer until a total of 10 layers had been assembled.

Characterization. Contact-angle measurements of the LbL assemblies were obtained using a sessile drop method[14] using the setup described previously.[15] Advancing contact angles (θ_A) were determined by placing 5, 10, 15, and 20 μL drops of Milli-Q water. The tip of the needle was removed, and an image was recorded. Duplicate measurements were obtained on different areas of each sample. The image of the drop was recorded by a digital camera interfaced to a computer. Contact angles were calculated using NIH IMAGE software. Using the equation $2h/\Delta = \tan\theta/2$, contact angles (θ) were calculated (Δ is the base diameter of the drop, and h is the height of the drop).[14]

XPS survey and high-resolution spectra were collected from an Axis Ultra XPS spectrometer (Kratos Analytical, UK) at an analyzer pass energy of 160 and 20 eV, respectively. The spectrometer had a monochromatic Al Kα X-ray source operating at 15 kV, 10 mA (150 W) for all data acquisitions. The overall information depth was 3λ (where λ is the electron free path), which, for Al Kα, is $\lambda = 3.5$ nm, resulting in a total depth of ~10 nm. Binding energies were charge-corrected to 285.0 eV for aliphatic carbon. High-resolution spectra were resolved into individual Gaussian−Lorentzian peaks using a least-squares fitting program (PeakFIT, Jandell Scientific Software). Component energies, number of peaks, and peak widths (full width at half-maximum (fwhm) of 1.0 and 1.2 for all Cs and Ns, respectively) were fixed initially, and refinement was performed for peak heights only. In a final optimization cycle, component energies and peak widths were also refined, and these changed by less than 1.0%. Peak fit results were imported into a graphic software package (Origin, OriginLab Corp.) for displaying the modeled data.

Attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectra were acquired using a Nicolet Nexus 870 FTIR spectrometer equipped with a Smart Endurance diamond ATR accessory (64 scans, 4 cm^{-1} resolution, wavenumber range 4000−550 cm^{-1}). Spectral manipulations were performed using the spectral analysis software GRAMS/32 (Galactic Industries Corp., Salem, NH). External reflection FTIR was recorded on a Specac grazing angle accessory using an s-polarized beam at an angle of incidence of 40° and a mercury cadmium telluride (MCT/A) detector. Spectra were acquired for 512 scans with a resolution of 8 cm^{-1} in the range of 6000−650 cm^{-1}. A piranha-treated silicon wafer was used as the background.

A selection of films was investigated for solution stability by immersion of the films in an "ionic solution", which contained the inorganic ions Ca^{2+}, Na^+, K^+, and Cl^- at concentrations of $[Ca^{2+}] =$ 2 mM, $[Na^+] = 112$ mM, $[K^+] = 4$ mM and $[Cl^-] = 120$ mM. The solution was buffered to pH 7 using HEPES buffer (0.05 M). The choice of ions and their concentrations were based on their presence in body fluids such as blood plasma and synovial fluid.[16] Visual monitoring of the films in this solution was performed over a 2 h period.

Results and Discussion

Characterization Techniques. FTIR reveals information about the molecular structure of chemical compounds and is useful for the characterization of biopolymers. The carbonyl vibrations of a carboxylate and a carboxylic acid group occur at very different wavenumbers, as does the N−H vibrations of amines and protonated amines. Specific vibrations for these groups all appear in the 1400−1750 cm^{-1} spectral window. It is therefore a very useful technique for discriminating the different states of protonation of both alginate and chitosan. It is, however, difficult to quantify the amount of individual components using FTIR when the vibrational bands overlap, as is the case for the biopolymers under consideration in this study. XPS is a complementary technique yielding information about the atomic composition of a material's surface, thus it can quantify the amount of monoatomic ionic species, which are silent in FTIR, (e.g., Na^+, Ca^{2+}, and Cl^-). In addition, XPS is capable of quantifying the extent of protonation of amine groups through examination of the N 1s narrow scan; it is, however, difficult to obtain the degree of deprotonation of carboxylic acid groups. The electrostatic interaction between alginate and either simple divalent cations or positively charged biopolymers (both chitosan and polylysine) in self-assembled complexes has been studied by either chemical characterization (FTIR[17−20] and XPS[21]) or by physical characterization (scanning electron microscopy,[22] mechanical testing[23] and swelling studies[12,20,24]). Detection of the specific interactions has been proposed in the literature using FTIR; however, disagreement in band assignments for the PECs is evident.[18,19] Very few studies have explored the exact nature of the interaction between the carboxylate groups (alginate) with protonated amine groups (chitosan or polylysine) in PECs. In this study, the combination of XPS and FTIR has allowed a more in-depth exploration of various interactions between alginate, chitosan, and other species present.

FTIR and XPS Spectra of Alginate. The solubility of alginate is related to the pH of the solution. In acidic solution at a pH below 3.6, an alginic acid hydrogel forms as the carboxylate groups are protonated (the pK_a of the carboxylic acid group being ~3.5 (Scheme 2a)[25]), whereas, at neutral pH, alginate is water soluble. Sodium alginate (as received) displayed two vibrations in the infrared spectrum due to the carboxylate group; an antisymmetric stretch at 1596 cm^{-1} and a symmetric stretch at 1412 cm^{-1}. A film obtained by casting from sodium

Table 1. FTIR Bands of Sodium Alginate and Alginic Acid with Assignments

sodium alginate vibration (cm^{-1})	alginic acid vibration (cm^{-1})	assignment
3700−3000 (broad)	3700−3000 (broad)	OH stretch
3000−2850	3000−2850	CH stretch
	1722	C=O stretch of COOH
	1635	water
1596		antisymmetric CO_2^- stretch
1412		symmetric CO_2^- stretch
	1385, 1347	O−H deformation and C−Ostretch modes
1297	1237	skeletal vibration
1081−1027	1081−1027	antisymmetric stretch C−O−C

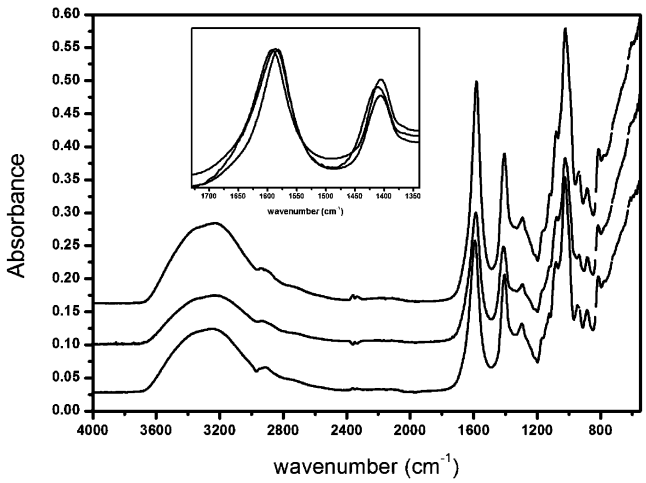


Figure 2. FTIR spectra of Na−alginate film (bottom) and films obtained after subsequent cross-linking in $CaCl_2$ (middle) and $BaCl_2$ (top) solutions. Inset illustrates the small changes in band position of the C=O vibrational modes.

alginate solution yielded a spectrum where no significant shifts (i.e., less than 5 cm^{-1}) in these bands were observed. The band positions are in agreement with those previously reported.[17] For the full assignment of infrared bands of alginate, refer to Table 1. The XPS spectrum of sodium alginate confirmed the presence of the anticipated peaks for sodium, carbon, and oxygen, with an O/Na ratio of 6, as expected.

The XPS spectrum of an alginic acid hydrogel, obtained by the precipitation of alginate using hydrochloric acid, revealed no sodium but only carbon and oxygen (and a small chlorine contamination). This indicates complete protonation of the carboxylate groups. The carbonyl vibration of this carboxylic acid moiety occurred at 1722 cm^{-1}. In addition, a band observed at 1635 cm^{-1} can be assigned to water associated with the biopolymer. For full assignment of the infrared bands of alginic acid, please refer to Table 1.

Interaction of Ionic Species with Alginate. The alginate film produced by casting the sodium salt of alginate from aqueous solution was subsequently immersed in a solution of either $CaCl_2$ or $BaCl_2$. The XPS spectra of the cross-linked alginate films showed no evidence of residual sodium, thus all sodium ions had been exchanged for calcium or barium ions. It was observed that the antisymmetric carbonyl vibration (at 1596 cm^{-1}) was most sensitive to the presence of these cross-linking agents with the Ca^{2+} cross-linked film displaying this vibration at 1586 cm^{-1} and the vibration for the Ba^{2+} film occurring at 1582 cm^{-1}. Thus, this band shifts to lower energy as the strength of the ionic interaction increases (Na^+ to Ca^{2+} to Ba^{2+};[26] Figure 2). The stability of the alginate film was influenced by cross-linking with calcium ions. In our ionic solution, an alginate film was observed to be stable up to 5 min, at which point it began to dissolve, whereas the Ca^{2+} cross-linked film was stable beyond 2 h. This indicates that the calcium ions in the film are physically cross-linking adjacent chains of alginate, in agreement with the generally accepted "egg-box" model.[9]

In a parallel study, we used low viscosity alginate (Sigma, 250 cps for 2% at 25 °C) with an M/G ratio of 1.5 and found similar changes in the infrared bands. It is, however, possible that alginate polymers with different M/G ratios will display different positions in their infrared bands upon binding to divalent ions. Sartori et al.[27] studied alginate polymers with M/G ratios in the range of 0.43−1.08 and found that the symmetric stretch at 1413 cm^{-1} shifts to 1431 cm^{-1} upon calcium exchange in the alginate matrix, which indicates that the M/G ratio may influence the spectral changes. Tam et al.[21] on the other hand, studied alginate with a reported M/G ratio of 0.67 or less and observed a shift to lower energy from 1604 to 1598 cm^{-1} for the antisymmetric and a shift from 1412 to 1409 cm^{-1} for the symmetric stretching vibration when comparing their sodium alginate and calcium alginate preparations. These changes are similar to our findings but are in contrast to the study by Sartori. It is therefore still unclear whether the M/G ratio or perhaps more subtle differences in the alginate structure (such as the length of G blocks) cause the differences in the FTIR spectral features, and this warrants a more detailed study.

In traditional coordination compounds incorporating carboxylate ligand(s), the separation (Δ) between the antisymmetric and symmetric stretching vibrations either increases or decreases compared to that observed for the carboxylic acid salt.[28] The value for Δ generally increases for complexes in which the ligand coordinates to the metal center in a unidentate manner and decreases when carboxylate acts as a chelating or bridging ligand. In the current study, $\Delta = 184$ cm^{-1} for the sodium salt and 175 and 176 cm^{-1} for the calcium and barium cross-linked films, respectively. These differences in the values for Δ are, however, not thought to be significantly different.

FTIR and XPS Spectra of Chitosan. Chitosan with a degree of deacetylation of 85% displayed two strong vibrations at 1645 and 1584 cm^{-1}. These have previously been assigned to amide I and amide II vibrations.[29,30] However, since only 15% or less of the nitrogen atoms occur as amides (confirmed by XPS, see below), the remaining atoms being amines, these assignments are unlikely to be accurate. Amine deformation vibrations usually produce strong to very strong bands in the 1638−1575 cm^{-1} region.[31] We therefore propose that the band at 1583 cm^{-1} is the N−H bending vibration overlapping the amide II vibration and that the 1645 cm^{-1} band is the amide I vibration. To further verify this, curve fitting to the spectrum in the 1760−1500 cm^{-1} region was performed, revealing three bands at 1648, 1594, and 1573 cm^{-1} (Figure 3). These positions correspond to the amide I, amide II, and N−H bending vibrations, respectively. In addition, C−N stretching vibrations occur in the 1190−920 cm^{-1} region and overlap the vibrations from the carbohydrate ring. N−H stretching also occurs in the 3315−3215 cm^{-1} region overlapping the OH stretch from the carbohydrate ring. The full assignment is provided in Table 2. An XPS survey scan of the as-received (Sigma) chitosan powder displayed carbon, oxygen, and nitrogen peaks, in agreement with the molecular structure. The N 1s narrow scan (Figure 4A) displayed a peak at 400 eV, which required two peaks for the curve fit: one peak at 399.4 eV and one at 400.5 eV, corresponding to amine and amide, respectively. The atomic ratios of these peaks were 88 and 12% (Chi; Table 3), respectively, agreeing well with a degree of deacetylation of 85% quoted by the supplier.

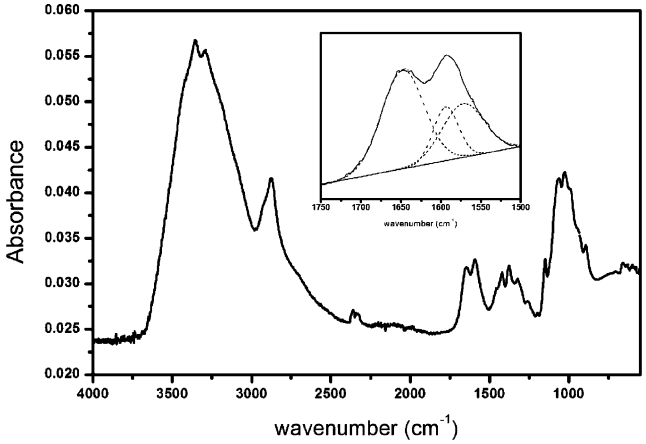


Figure 3. FTIR spectrum of chitosan powder (Sigma). Inset shows the curve fit to the 1760−1500 cm^{-1} region.

Table 2. FTIR Bands of Chitosan with Assignments

chitosan vibration (cm^{-1})	assignment
3290	O−H and N−H stretch
2864	C−H stretch
1645	amide I
1584	N−H bending from amine and amide II
1414	−CH_2 bending
1375	CH_3 symmetrical deformation
1150	antisymmetric stretch C−O−C and C−N stretch
1026	skeletal vibration of C−O stretching

FTIR and XPS Spectra of Chitosan Films. The solubility of chitosan is pH dependent and requires the primary amines to be protonated to dissolve in aqueous solutions; a pH of 6.3 ensures sufficient numbers of amine groups are protonated (pK_a of 6.3, Scheme 2b[32]) to allow dissolution. When producing a chitosan film by casting from an acidic solution, the conjugated base will also be incorporated into the structure. Depending on the chemical structure of the acid used, the counterion may be FTIR silent (i.e., chloride ion) or may display vibrational modes in the spectral region of interest (i.e., lactate ion).

The XPS spectrum of a chitosan film cast from a hydrochloric acid solution of chitosan displayed no changes in the C 1s spectrum in comparison to the as-received chitosan powder (data not shown), but large changes were seen in the N 1s narrow scan (Figure 4B). The N 1s peak required three peaks for the curve fit: at 399.4 eV (amine), at 400.5 eV (amide), and at 401.4 eV (protonated amine). In this film, less than half of the amine groups were protonated (Table 3, 48 atom % amine; 38 atom % protonated amine). The atomic percent of chloride in this film was equal to that of the atomic percent of protonated amine. On the basis of this data we can describe the chitosan structure as possessing just under half of the amine groups protonated with chloride ions as the counterion. From the FTIR spectrum (Chi-HCl film; Table 3), it was found that major bands in the spectral region of interest occurred at 1628 and 1524 cm^{-1}.

The spectrum of a chitosan film prepared from dissolving chitosan in lactic acid is shown in Figure 5. Residual lactic acid is evident in this film (carbonyl vibration of the carboxylic acid at 1715 cm^{-1}) as a consequence of the low volatility of lactic acid compared to hydrochloric acid. The analysis of the chitosan spectrum is only possible after subtraction of the lactic acid spectrum from the chitosan film spectrum to give the spectrum shown in Figure 5 where no band can be observed at 1715 cm^{-1}.

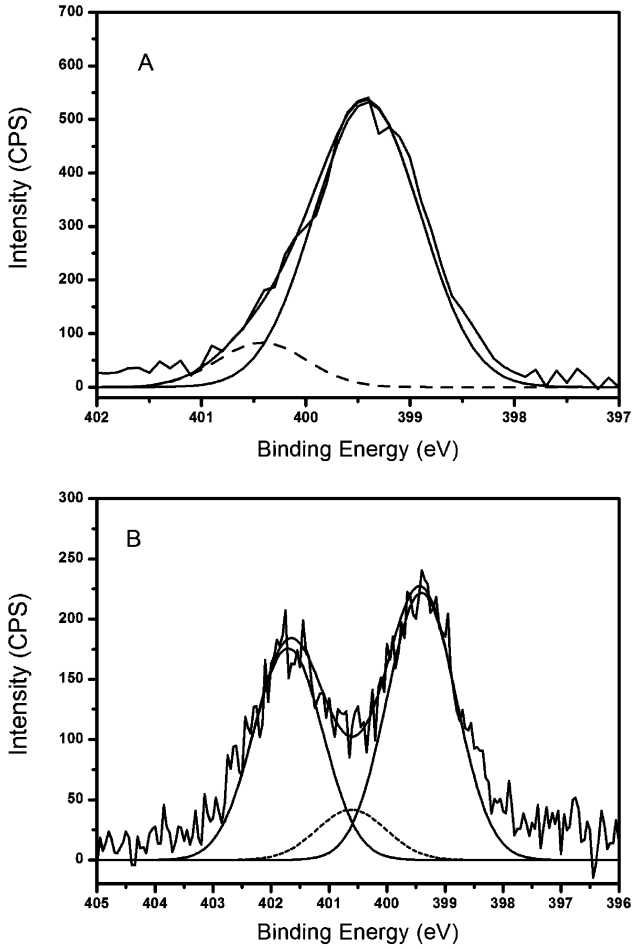


Figure 4. XPS N 1s narrow scans with the curve fit of (A) chitosan powder (Sigma) and (B) chitosan film cast from HCl solution.

Table 3. XPS and FTIR Data for Chitosan (Chi) and Alginate−Chitosan (Alg−Chi) Mixtures[a]

sample	N1[b]	N2[c]	N3[d]	IR bands (cm^{-1})
Chi[e]	88	12		1645, 1584, and 1414
Chi-HCl film	48	14	38	1628 and 1524
Chi-LA film	28	15	57	1633, 1529, and 1453[f]
Chi-LA($CaCl_2$) film	27	11	62	*g*
Chi-buffer film	24	16	60	1632, 1527, and 1455
Chi-LA/Alg film	77	13	10	*g*
Chi-LA/Alg($CaCl_2$) film	43	9	48	1585 and 1411
Chi-buffer/Alg precipitate	30	15	55	1606 and 1416
Chi/Alg LbL assembly	36	9	55	1614, 1533(sh), and 1401

[a] The atomic percent of various nitrogen species was determined through curve fitting to the N 1s peak in the XPS spectra. The IR bands were in the 1400−1750 cm^{-1} spectral region only. [b] 399.4 ± 0.4 eV. [c] 400.5 ± 0.4 eV. [d] 401.5 ± 0.4 eV. [e] Chitosan flakes supplied from Sigma. [f] After subtraction of lactic acid and lactate bands. [g] Subtractions not performed.

Subtracting the spectrum of sodium lactate removes residual lactate bands (e.g., ∼1580 and ∼850 cm^{-1} bands), resulting in the spectrum in Figure 5 (bottom) where only bands associated with chitosan remain. Bands can now be observed at 1633 and 1529 cm^{-1} (Chi-LA film; Table 3), in close agreement with the bands observed for the hydrochloric acid film described above. The XPS spectrum of this chitosan film displayed an N 1s peak, which again required three peaks for the curve fit with a large amount of amines being protonated (57%, Chi-LA film; Table 3).

A chitosan film was prepared from a phosphate-buffered solution. The choice of the phosphate buffer was based on the

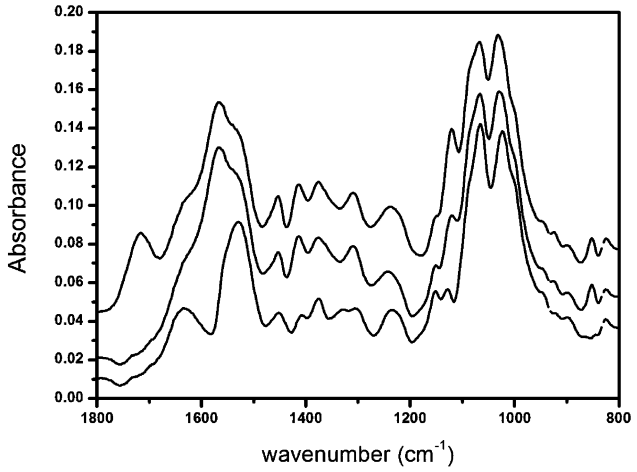


Figure 5. (Top) FTIR spectrum of chitosan film cast from lactic acid (Chi-LA); (middle) the spectrum after subtraction of the LA spectrum; (bottom) the spectrum after an additional subtraction of sodium lactate.

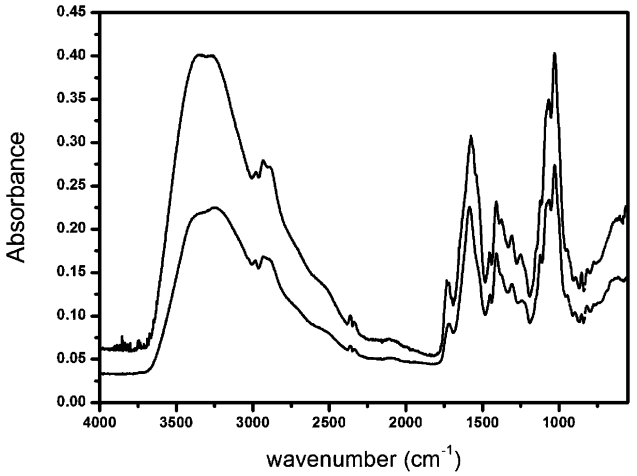


Figure 6. FTIR spectra of an alginate−chitosan mixture (bottom) and the addition spectrum of Na−alginate and chitosan cast from lactic acid (top).

absence of P−O or O−H vibrational modes in the spectral region under examination. The FTIR spectrum of the precipitate displayed vibrational bands at 1632 and 1527 cm^{-1} (Chi-buffer film; Table 3), again in good agreement with the films described above. The XPS spectrum of this chitosan precipitate displayed an N 1s peak, which required three peaks for the curve fit, and, as for the lactic acid film, a large amount of amines were protonated (60%, Chi-buffer film; Table 3).

From the XPS data of partly protonated chitosan present in the films described above, it was found that the extent of protonation of amine groups ranges from 44 to 71%; however, the FTIR spectra are similar, thus, FTIR is not a technique that can be used to assess the extent of protonation of chitosan. The FTIR spectra all showed two main bands: one at ∼1628−1633 cm^{-1} and one at ∼1524−1529 cm^{-1}. Considering that protonated amines display an antisymmetric deformation in the 1625−1560 cm^{-1} range and a symmetric deformation in the 1550−1505 cm^{-1} range,[31] and that amide and amine moieties are also present in the film, the two observed bands must represent an envelope of (at least) five bands in close proximity. It is, however, not viable to curve fit that many parameters to the data. The two bands can therefore be assigned as follows: the band at ∼1628−1633 cm^{-1} contains the amide I and antisymmetric $-NH_3^+$ deformation, while the band at ∼1524−1529 cm^{-1} contains the amide II, N−H bending vibration as well as the symmetric $-NH_3^+$ deformation.

Chitosan Lactic Acid Films Immersed in $CaCl_2$ Solution. When a chitosan film produced from a lactic acid solution was immersed in a $CaCl_2$ solution and subsequently dried, the resulting film displayed an uptake of Ca^{2+} ions (atom % of 3.2) and chloride ions (atom % of 2.1). The N 1s peak in the XPS spectrum required three peaks for the curve fit, with protonated amine amounting to 62% of all nitrogen species (Chi-LA($CaCl_2$) film; Table 3) leading to 2.3 atom % protonated amine in the film. First, the atomic percent of protonated amine increased slightly upon calcium and chloride ion addition. Second, in order to maintain an assembly that is charge neutral overall, a concurrent increase in the lactate content from excess lactic acid in the film must have occurred, and this is in agreement with the decrease in the 1715 cm^{-1} vibrational band (data not shown). This decrease can also, in part, be due to the removal of some lactic acid during the immersion of the film in $CaCl_2$ solution. It is worth noting that the atomic percent of protonated amine and chloride ion is the same within experimental error. The stability of the chitosan film was influenced by the addition of calcium and chloride ions. In our ionic solution, the chitosan film was observed to be stable for up to 60 min, whereas the film containing Ca^{2+} and Cl^- ions disintegrated immediately. This suggests that the calcium ions in the film do not interact with amine groups to cross-link individual strands. It is likely that the calcium ions are associated with the lactate, as this is a good ligand for calcium ions. It is possible that the lactate ions present in the film before treating with $CaCl_2$ solution help to stabilize the film and therefore exhibit some cross-linking ability. However, after calcium uptake, this effect is nullified. The observed stabilities can, with this in mind, be understood in terms of the relative solubility of chitosan lactic acid and chitosan hydrochloric acid salts in neutral solution.

Alginate−Chitosan Mixed Films. Two component systems can be produced from mixtures of alginate and chitosan solutions. When chitosan was dissolved in hydrochloric acid and mixed with alginate in water, one reaction that takes place is the protonation of alginate. This was evident from a carbonyl vibration at 1722 cm^{-1} arising in the FTIR spectrum of the resulting film. This occurs simply as a consequence of a high local concentration of acid in the sodium alginate solutions and could not be prevented, even with vigorous stirring. Thus, this is not a viable method for controlled production of PECs.

When chitosan is dissolved in lactic acid and mixed with an aqueous alginate solution, the resulting 1:1 film displays an FTIR spectrum that is very similar to the addition spectrum of the two components (Figure 6) as a consequence of all the components coprecipitating. Since this spectrum contains contributions from lactic acid and/or lactate (bands at 1720, 1458, 1242, and 1121 cm^{-1}), a detailed spectral analysis is not possible. This two component film was analyzed by XPS. It was found that the film contained residual Na^+ (from the alginate solution). From the N 1s narrow scan it was found that only 10% of the nitrogen species were protonated amines (Chi-LA/Alg film; Table 3). A comparison of the amount of sodium (3.29 atom %) and protonated amine (0.27 atom %) in the film revealed that the carboxylate groups arising from alginate observed in the FTIR spectrum would mostly be associated with sodium ions rather than with protonated amines from chitosan. This film was stable in our ionic solution for only 20 min, after which it disintegrated, thus we can conclude that a weak PEC had formed.

After incubation of this film in a $CaCl_2$ solution, an uptake of Cl^- ions was evident from the XPS survey scan; however, no Na^+ or Ca^{2+} ions were observed. The N 1s narrow scans of

the two-component film after $CaCl_2$ immersion was significantly different from that prior to immersion (Chi-LA/Alg($CaCl_2$) film; Table 3). Before immersion, only 10% of all nitrogen species were protonated amines compared to 48% after immersion in $CaCl_2$. The chloride content (2.9 atom %) was found from the survey scan to be similar to the protonated amine content (3.1 atom %). This suggests that, although large amounts of amines are protonated, there are adjacent chloride ions that may be counterbalancing the charge, and so only a small portion of these protonated amine groups are available to interact with the carboxylate groups of alginate. The FTIR spectrum shows the absence of a band at ~1720 cm^{-1}, indicating that all alginate is in the deprotonated state. The bands at 1458, 1242, and 1121 cm^{-1} are also absent, indicating the absence of lactate in this film. A broad band at 1584 cm^{-1} with a prominent shoulder at ~1530 cm^{-1} as well as a band at 1411 cm^{-1} are observed in the FTIR spectrum of the film (Chi-LA/Alg($CaCl_2$) film; Table 3). The two main bands are assigned to the carbonyl vibrations of the carboxylate groups of alginate. The broadness of these bands arises from overlapping bands from the partly protonated chitosan moieties and the prominent shoulder corresponding to the 1529 cm^{-1} band seen in the chitosan film cast from lactic acid (Figure 5). The stability of this film was slightly higher than that observed before immersion in $CaCl_2$ solution; it maintained its integrity for 1 h in our ionic solution. This suggests that a weak PEC formed, and this, combined with the spectroscopic data, leads us to conclude that only a slight change in the degree of interaction between the protonated amine group of chitosan and the carboxylate group has occurred after immersion in $CaCl_2$ solution.

Alginate−Chitosan PEC Precipitate. For the preparation of an alginate−chitosan precipitate, both biopolymers were in a phosphoric acid buffer at pH 4.8. The precipitate formed upon mixing the two solutions displayed an FTIR spectrum with broad bands at 1606 and 1416 cm^{-1}, which is similar to the bands observed in the alginate−chitosan film described above. Since the precipitate was formed in a phosphate buffer, coprecipitation of sodium phosphate species was evident from the XPS survey scan. The XPS narrow scan of this alginate−chitosan precipitate displayed an N 1s peak, which required three peaks for the curve fit, with a large amount of amines being protonated (55%, Chi-buffer/Alg precipitate; Table 3). The stability of this precipitate was very high, and it maintained its integrity well beyond 2 h in our ionic solution. This suggests the presence of a strong PEC. The presence of the ionic forms of both polyelectrolytes (controlled by using a buffer system) promotes the formation of a strongly associated complex.

LbL Assembly. A number of studies relating to alginate−chitosan PECs involve the formation of controlled structures through the addition of each component to a substrate as a subsequent layer and, as such, can be identified as the LbL approach, which has been used in capsule and thin-film fabrications.[33,34] In the current study, an LbL assembly was produced on a glass slide to explore the interaction between alginate and chitosan in a controlled model system. Successful assembly of 10 layers (beginning with a chitosan layer) onto a glass slide was verified by contact-angle measurements and XPS (Figures 7 and 8). After deposition of the first chitosan layer, the contact angle increased compared to the value for a native glass surface. The subsequent addition of the first alginate layer did not show a significant change in contact angle; however, in all subsequent layers, it was observed that the contact angle of the chitosan layer was significantly higher than that of the alginate layer, illustrating that alginate is more hydrophilic than

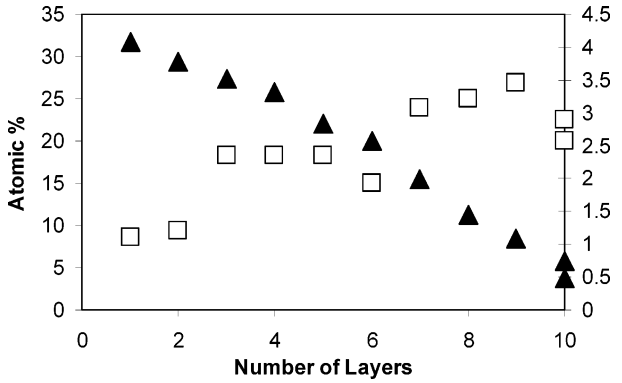


Figure 7. Atomic percent from XPS survey scans of Si (left *y*-axis) and N (right *y*-axis) as a function of the number of layers in the alginate−chitosan LbL assembly.

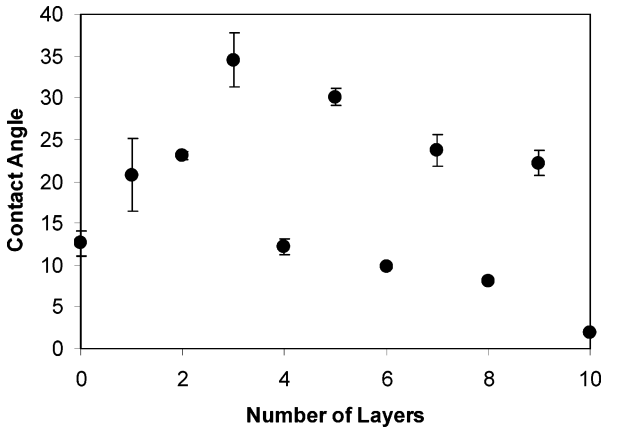


Figure 8. Advancing water contact angle as a function of the number of layers in the alginate−chitosan LbL assembly.

chitosan. From the XPS survey scans, it could be seen that the Si 2s peak decreases in intensity with increasing number of layers deposited, while the N 1s peak increases concomitantly. The fact that the silicon peak in the XPS spectrum decreases to ~5 atom % after the deposition of 10 layers suggests that the thickness of the 10 layers is less than 50 Å, in agreement with previous literature.[33,34]

When chitosan is the outermost layer in an LbL assembly, the amine groups extending into solution during fabrication will become deprotonated (neutral) upon washing and drying in air. However, when alginate is the outermost layer, the amine groups of the underlying chitosan layer will be protonated to a larger degree due to interaction with the deprotonated carboxylate groups of alginate. It is therefore expected that a higher amount of protonated amines will be present when alginate is the outmost layer, and thus the LbL assembly was fabricated such that the final layer was alginate. The N 1s narrow scan of each of the layers needed three peaks for the curve fit. The atomic percent of the peak corresponding to the protonated amine (at 401.4 eV) ranged from 45−60%, with the higher values occurring when alginate was the outmost layer. The final assembly displayed an atomic percent of protonated amine of 55% (Chi/Alg LbL assembly; Table 3). It is important to emphasize that, in an LbL assembly, ionic interactions between the carboxylate groups of alginate and the protonated amines of chitosan are a requirement for a stable assembly, thus this system serves as a positive control in characterizing PEC formation.

External reflection FTIR was performed on the LbL assembly. Since alginate is the outermost layer, it is expected that some protonated alginate will be present, as the carboxylate groups

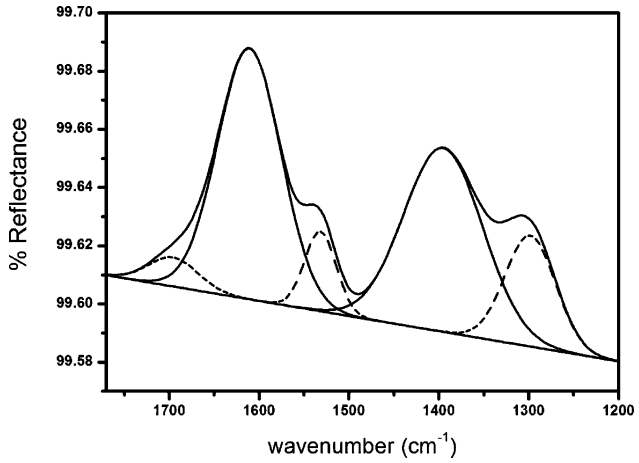


Figure 9. External reflection FTIR spectrum (after applying smoothing) of LbL assembly in the 1770−1200 cm^{-1} spectral region with a curve fit.

become protonated (neutral) upon washing and drying in air. The 1200−1800 cm^{-1} region of the spectrum is displayed in Figure 9 together with a curve fit to the spectrum. This curve fit is intended only to guide the reader and does not represent all the bands that are the origin of the overall spectrum. The two main bands observed at 1610 and 1394 cm^{-1} are the C═O antisymmetric and symmetric stretch of the carboxylate group. Considering an estimated error of ±10 cm^{-1} in band position when using the external reflection technique, these are not considered significant changes compared to the band positions of the sodium salt or to those of the alginate−chitosan film and precipitate described above.

The remaining bands in the FTIR spectrum can be assigned as follows: The band at 1297 cm^{-1} is the skeletal vibration of alginate, and the shoulder at ∼1530 cm^{-1} corresponds to one of the main bands observed for the partially protonated chitosan (arising from amide II, N−H bending, and symmetric $-NH_3^+$ deformation). In addition, a broad shoulder is seen on the high wavenumber side of the 1610 cm^{-1} band, corresponding to the other main band observed for the partially protonated chitosan (arising from amide I and antisymmetric $-NH_3^+$ deformation) as well as small amounts of alginic acid.

Use of FTIR to Identify PEC Formation in Alginate−Chitosan Mixtures. Our studies show that the FTIR spectra of all the alginate−chitosan mixtures prepared are very similar, despite the varying degree of interaction between the functional groups in the two polyelectrolytes. This renders FTIR as limited in its ability to recognize PEC formation.

A number of studies have attempted to identify the formation of PECs between alginate and chitosan on the basis of FTIR spectra. Simsek-Ege et al.[18] proposed that the band observed at 1420 cm^{-1} in 1:1 alginate−chitosan mixtures formed at pH values between 2 and 9 was due to the interaction of $-NH_3^+$ (from chitosan) with $-COO^-$ (from alginate). They came to this conclusion by comparing the FTIR spectra of their Chi/Alg mixtures with those of pure alginic acid (pH = 2) and neutral chitosan (pH = 9). Two other groups subsequently adopted the same analysis for PEC formation.[35,36] From our work it is clear that the band at ∼1420 cm^{-1} is due to the symmetric stretching vibration of the $-COO^-$ group from alginate and that this band is present in all films containing the deprotonated form of alginate, whether its counterion is Na^+, Ca^{2+}, Ba^{2+}, or $-NH_3^+$. Therefore its presence does not in itself signify PEC formation. Since the study by Simsek-Ege et al. does not report the concentration of the counterions Na^+ and Cl^- (use of HCl and NaOH for pH adjustment), the degree of PEC formation cannot be assessed.

In a separate study of alginate−chitosan mixtures by Wang et al.,[19] the band at 1620 cm^{-1} was assigned to the carboxylate group of alginate associated with chitosan, and a band at 1530 cm^{-1} was assigned to the amino group of chitosan associated with alginate. The band at 1620 cm^{-1} can also be seen in their spectrum of sodium alginate, and we assign this to the antisymmetric stretch of the $-COO^-$ group of alginate, although it is observed at a somewhat higher value than in our spectra. The band at 1530 cm^{-1} is not present in either of their pure materials, assuming that the chitosan spectrum that they display is of the neutral form. We have assigned this band to the $-NH_3^+$ group of chitosan. As above, the presence of these bands does not in itself signify PEC formation.

Implications for Materials Development. The detailed spectroscopic investigation of the nature of the interaction between alginate and chitosan when brought together in a range of structural assemblies has illustrated the difficulty in quantifying the strength of this interaction. There is no doubt that electrostatic attraction is sufficient to induce an interaction between the two biopolymers through LbL deposition, forming a stable construct. However, the mode of assembly preparation has substantial influence on the subsequent stability of the films, as assessed through solution studies reflecting the relative strength of the PEC associations. Indeed, in most systems investigated, the formation of a PEC is only through weak ionic associations. With the widespread use of PEC assemblies in drug delivery, cell microencapsulation, and tissue engineering initiatives, the reliance solely on the ionic association between oppositely charged polysaccharides is cautioned. A strongly associated matrix can only be achieved through careful selection of solution environments and, in many systems, can only be developed through modification of the association through the introduction of either covalent bonds or conjugating species.

Conclusion

This study has challenged the current literature interpretation of FTIR spectra of alginate−chitosan PECs. We have demonstrated that no significant change in band position of the two carbonyl vibrations from alginate occurs upon interaction with different ionic species. However, protonation of the carboxylate group causes a new band to appear at 1710 cm^{-1}, as anticipated. Also, we find that partial protonation of the amine group of chitosan causes the appearance of one new band (∼1530 cm^{-1}) due to one of the $-NH_3^+$ vibrational modes (the other mode overlaps the amide I band). Importantly, the position of the two main bands in the spectral region of interest in partly protonated chitosan films is not dependent on the extent of protonation. To assess this, XPS N 1s narrow scans must be obtained. In our chitosan−alginate film, precipitate, and LbL assembly, the bands observed in the FTIR correspond to the species $-COO^-$ and $-NH_3^+$, but their position is not different from each of the single components. Thus, FTIR cannot be used directly to identify the presence of PECs. However, in combination with XPS, using both survey scans and narrow N 1s scans, a more complete description of the structure can be obtained.

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