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Note

Alkaline chitosan solutions

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Abstract

Rigid and transparent hydrogels were obtained upon pouring chitosan salt solutions into saturated ammonium hydrogen carbonate. Incubation at 20 °C for 5 days yielded chitosan carbamate ammonium salt, Chit-$NHCO_2^-NH_4^+$ a chemical species that either by hydrolysis or by thermal treatment decomposed to restore chitosan in free amine form. Chitosans of different degrees of acetylation, molecular sizes and origins (squid and crustaceans) were used as hydrochloride, acetate, glycolate, citrate and lactate salts. Their hydrogels obtained in ammonium hydrogen carbonate yielded chitosan solutions at pH values as high as 9.6, from which microspheres of regenerated chitosans were obtained upon spray-drying. These materials had a modest degree of crystallinity depending on the partial acylation that took place at the sprayer temperature (168 °C). Citrate could cross-link chitosan and impart insolubility to the microspheres. Chloride on the contrary permitted to prepare microspheres of chitosan in free amine form. By the NH_4HCO_3 treatment, the cationicity of chitosan could be reversibly masked in view of mixing chitosan with alginate in equimolar ratio without coacervation. The clear and poorly viscous solutions of mixed chitosan carbamate and alginate were spray-dried at 115 °C to manufacture chitosan–alginate microspheres having prevailing diameter approx 2 micron.
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Keywords: Chitosan solubility; Alkaline chitosan solution; Microspheres; Regenerated chitosan; Chitosan–alginate complexes; Chitosan carbamate ammonium salt

Once dissolved as a salt, chitosan can be precipitated with the aid of aqueous NaOH or ammonia solutions.[1,2] Carbonic acid has been recently found to dissolve chitosan as a consequence of the formation of chitosan bicarbonate salt, provided that chitosan is used as a re-precipitated hydrogel rather than a powder. Chitosan bicarbonate has been assayed for cellulose coating in view of the easy removal of the anion.[3,4]

Ammonium hydrogen carbonate, a volatile salts commonly used in baking powder formulations, has been used in connection with glycosylamine preparation by several authors[5–14] who reported that excess NH_4HCO_3 transforms the hydroxy group at the anomeric carbon of substituted *N,N′*-diacetylchitobioses into $-NH_2$, yielding *N,N′*-diacetylchitobiosylamines, while keeping the β anomeric configuration. Several reviews[15–22] underline the importance of this reaction for the preparation of glycoproteins; other reactions involving glycosylamines are the FMOC derivatization,[23] the reaction with bifunctional bridges between carbohydrates and peptides[24] and the production of neoglycoproteins.[25]

D-Glucose, D-galactose, lactose, cellobiose, melibiose and maltose have been treated at 42 °C for 36 h with aqueous ammonia in the presence of NH_4HCO_3 to afford the corresponding glycosylamines.[12,26] The formation of glucosylcarbamate with 100% yield was observed when glucose solutions were saturated with NH_4HCO_3. The disaccharide glycosylamines obtained after 5 days were mainly in the form of N-glycosylcarbamates. A 5-day treatment with NH_4HCO_3 at 20 °C led to a molar ratio 1.5 to 1 of $-NH_2$ to $-NHCOO^-NH_4^+$ for a number of disaccharides.[9,18,26]

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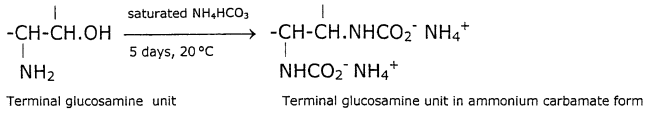


Scheme 1.

Moreover, the formation of diglucosylamine at temperatures higher than 50 °C was reported.[7] The literature data however do not provide any information on the behaviour of chitosan and D-glucosamine in the presence of saturated NH_4HCO_3.

The scope of the present work is to report the dissolution of chitosan salts into NH_4HCO_3 solutions at pH values as high as 9.6, and to prepare chitosan carbamate ammonium salt solutions for spray-drying (Scheme 1). These solutions would expectedly be suitable for polyelectrolyte complex formation in microsphere form, based on the following rationale: the chitosan carbamate polyanion would be compatible with other polyanions and no precipitation would occur but, as soon as sprayed, it would release carbon dioxide thus regaining its polycationic nature.

In this context, the extent of carbamate formation and the concentration of the accompanying NH_4HCO_3 are scarcely relevant for the production of chitosan-polyanion microspheres, because the role of the carbamate species is simply: (i) to impart solubility at pH 9.6; and (ii) to mask transiently the cationicity of chitosan. Possible thermal reaction (amide bond formation) would involve the free amino groups of chitosan, regardless of how many were in free or carbamate form, because the carbamate group would decompose thermally.

1. Results

When pouring a chitosan acetate solution into a saturated NH_4HCO_3 solution, we noticed that the expected precipitation of chitosan did not occur. Rather, a self-sustaining and transparent hydrogel formed at once. Table 1 collects the observations made on the freeze-dried powders obtained from commercial chitosans after incubation in saturated NH_4HCO_3 solution. After 21 h, one (FG90) out of three crustacean chitosans yielded a hydrogel amenable to an instant soluble freeze-dried powder, while the other two (CSVF 1430 and Sirc) required a longer incubation period to yield a soluble material. On the other hand, the low molecular weight squid chitosan (M151) required just 6 h.

Further on, each chitosan hydrogel was kept in its mother liquor at 20 °C for different lengths of time (2–10 days) in order to get instrumental evidence of the progress of the chemical reaction. At the end of the desired incubation period, the rigid transparent hydrogels obtained from chitosan acetate and NH_4HCO_3 isolated by centrifugation dissolved in water or dilute NaOH more or less promptly depending on the chitosan used and the length of the incubation time; the same was observed for the corresponding freeze-dried powders. The freeze-dried samples were promptly water soluble even several weeks after isolation.

This behaviour of the five chitosans tested in alkaline solution was peculiar and indicative of alterations brought about by NH_4HCO_3 when the incubation time was at least 21 h; 2 h were not enough to modify appreciably the chitosan behaviour in alkaline solution. The M151 squid chitosan was more prone than crustacean chitosan, in agreement with its reported enhanced reactivity.[27] Alkalimetric measurements indicated that equimolar NH_4HCO_3 and NaOH buffer solutions were particularly suitable for dissolution of the hydrogel. In all these systems (water, dilute NaOH, buffers) dissolution took place in 30 s with stirring.

The freeze-dried products were submitted to ^1H NMR, FTIR and X-ray diffraction analysis. The infrared spectra in Fig. 1, for incubation times of 2, 3 and 5 days, are reminiscent of the parent FG90 chitosan spectrum, but reveal certain spectral alterations that become more evident with incubation time: on the second day, the main features are preserved while a new band appears at 1497 cm^{-1}; on the third day, bands at 1487 and 1364 cm^{-1} are more marked and the novel bands at 835 and 706 cm^{-1} appear (all due to carbamate/bicarbonate); on the fifth day, the 1741 (carboxylate) band appears, whilst the ones at 2880 (CH stretching) and 1593 cm^{-1} (amine deformation) disappear.

The supernatant isolated by centrifugation after 10-day incubation in NH_4HCO_3 was treated with NaOH and methanol (5 vol) to precipitate residual soluble chitosan, for which hydrogel permeation chromatography showed a very low average degree of polymerisation, approx 20. FTIR spectra showed quite evident alterations compared to the spectra of the parent chitosan. The OH, NH, CH stretching region at 3400–2900 cm^{-1}, the band at 1076 and the amide band centred at 1560 cm^{-1} were depressed or hardly detectable. A number of novel bands appeared in the spectra, namely those centred at 2546, 1355, 1031, 834 and 706 cm^{-1}, the latter two most prominent and sharp (otherwise absent in chitosan) were assigned to carbonate. While reproducible, these spectra depended on the quantity of NaOH added and crystallisation of various forms of carbonate; the quantity of chitosan in these supernatants was very small compared to the hydrogels obtained.

The ^1H NMR spectra for the same products from the supernatants as well as for the centrifuged hydrogels at 5 days showed two sharp signals at δ 4.70 H-1 and 3.30 H-2, significantly different from the parent chitosan

Table 1
Observations made on the freeze-dried chitosan transparent and homogeneous hydrogels obtained by pouring a chitosan acetate solution into saturated ammonium hydrogen carbonate and incubating for various periods of time at 20 °C

No.	Chitosan	Time (h) in NH_4HCO_3	Solubility in water	Solubility after adding 1 M NaOH
1	FG90	2	partially soluble	soluble
2	FG90	21	fully soluble	remains soluble
3	M151	6	fully soluble	remains soluble
4	M151	21	fully soluble	remains soluble
5	M642	2	partially soluble	soluble
6	CSVF1430	21	partially soluble	soluble
7	Sirc	21	partially soluble	soluble

Last column refers to solubility in dilute NaOH.

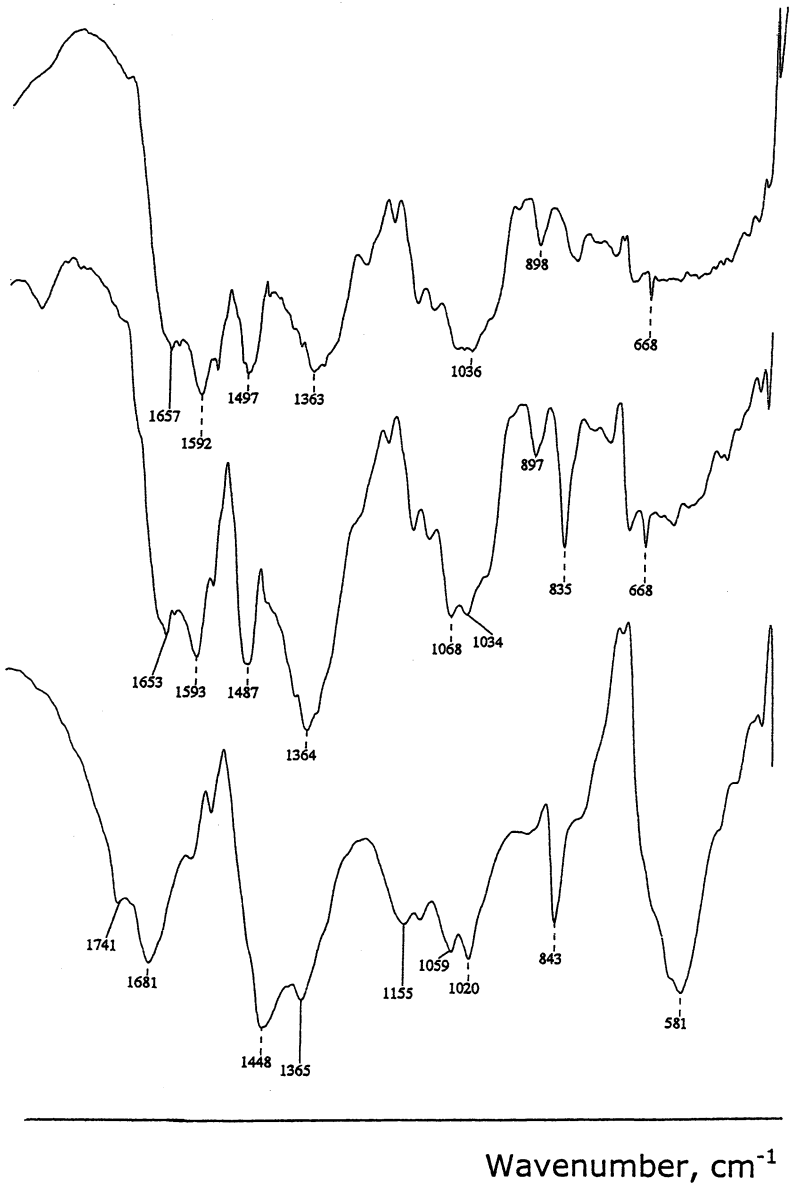


Fig. 1. FTIR spectra for the freeze-dried hydrogels isolated from saturated ammonium hydrogen carbonate after 2-day (upper curve), 3-day (middle curve) and 5-day (lower curve) incubation. Alterations indicative of carbamate formation are in the range 1741–1448 cm^{-1}. See text for band assignments.

signals, due to the carbamate function in agreement with the literature,[9] and small signals at 1.85 and 1.97 for the acetyl groups. Based on the NMR spectra features, evidence was obtained that chitosan incubated in saturated NH_4HCO_3 for 5 days or longer was fully in the carbamate form.

The consequences of the incubation in NH_4HCO_3 for chitosan were also clearly detectable by means of X-ray diffraction spectrometry carried out on the freeze-dried hydrogels. While the positions of the peaks were very close to the usual values for crustacean chitosans (interplanar spacings 1.05 and 0.45 nm),[14] the intensity of the peak at approx 20° 2θ value was surprisingly much lower than the one at approx 8° 2θ . For the squid chitosan, peaks were located at angular positions corresponding to interplanar spacings 1.10, 0.79, 0.49 and 0.39 nm, in good agreement with the general trend and specific values provided by other authors;[28] again, the intensity of the peaks decreased with increasing 2θ values (see Fig. 2).

Based on the instrumental evidence, the NH_4HCO_3 treatment led to important swelling of the polysaccharide, altered and weakened the hydrogen bond network in the chitosan, depressed crystallinity and generated the $-NHCO_2^-$ carbamate function. Essentially, in agreement with the cited literature, the amino functions at the terminal C-1 (glycosylamine function) and at the C-2 position became ammonium carbamate functions, Chit-$NHCO_2^-$, that masked the cationicity of chitosan and imparted solubility to the polysaccharide, according to

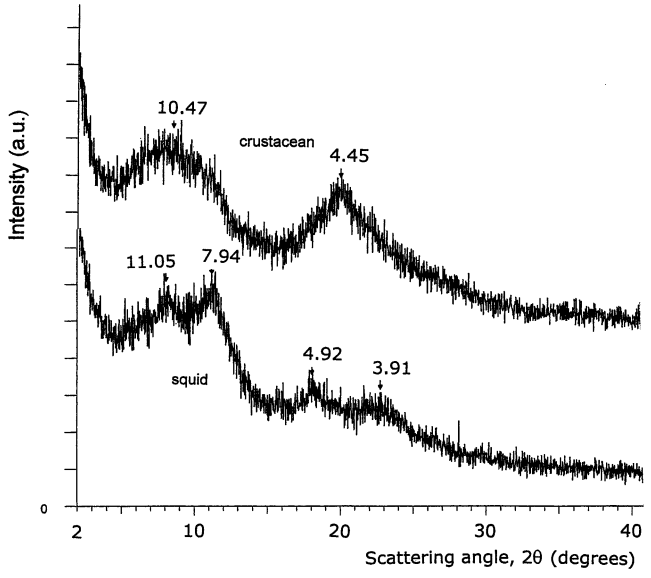


Fig. 2. X-Ray diffraction patterns for the freeze-dried gels obtained from the FG90 crustacean chitosan (upper curve) and the M151 squid chitosan (lower curve) after incubation in saturated ammonium hydrogen carbonate for 21 h. A remarkable feature is the low intensity of the peaks at high scattering angles. Values are in Å.

the equilibrium:

$$\text{Chit-NH}_2 + HCO_3^- NH_4^+ = \text{Chit-NHCO}_2^- NH_4^+ + H_2O$$

Moreover, the glycosylcarbamate groups may be stabilised by the acetamido group at C-2 in chitosan chains ending with a GlcNAc unit.[23] Excess carbonate accompanied the polysaccharides in hydrogel form, as already noted by other authors.[18] The behaviour described here was common to all chitosans tested, regardless of origin, molecular weight, degree of acetylation and manufacture.

1.1. Alkaline solutions of chitosan carbamate

The solutions prepared from the hydrogels upon dilution with water were suitable for spray-drying, because the excess NH_4HCO_3 decomposed thermally between 60 and 107 °C; on the other hand, the carbamate function released carbon dioxide under the effect of the temperature at which the spray-drier was operated, thus regenerating chitosan, as demonstrated by infrared spectrometry. The general characteristics of the white chitosan microspheres depended on the nature of the acid used to dissolve chitosan. Five acids were used, namely HCl, acetic, glycolic, citric and lactic (Table 2).

The degree of acylation of the microspheres (0.35–0.40) was much higher than the original degree of acetylation of the parent chitosan (0.03) when acetate, glycolate and lactate were present. These microspheres exhibited X-ray diffraction spectra where four peaks indicated a modest degree of crystallinity (Fig. 3).

The interplanar distance values indicated by peaks, namely 1.10, 0.43, 0.28 and 0.21 nm for microspheres obtained from samples containing citric acid and glycolic acid, mean that the typical values for chitosan are preserved but, in addition, a structural alteration took place.

On the contrary, the microspheres obtained from a system containing HCl were amorphous and their degree of acetylation was coincident with the value of the parent chitosan (Fig. 4). No chloride was present in these microspheres in agreement with the fact that they were insoluble in water besides being amorphous: it is known that chitosan hydrochloride is crystalline.[29] These microspheres showed high chelation ability for copper sulfate; they dissolved promptly in acetic acid where absence of effervescence was indicative of absence of carbamate or bicarbonate groups. Therefore, their preparation led to amorphous chitosan free base, a convenient chemical form for further use.

Microspheres obtained from a citrate containing system were noticeably insoluble in acetic acid, and the $CuSO_4$ test was negative (Table 2); the complexing and cross-linking ability of citric acid for chitosan has been reported.[30] In practice, these microspheres were partially cross-linked. Amide bond formation in chitosan salts of

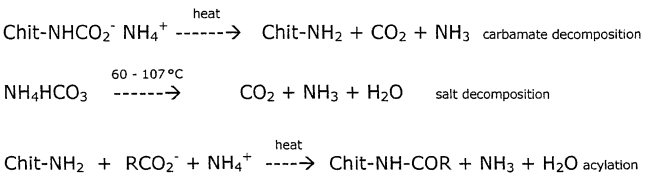
Table 2
Data for microspheres obtained by spray-drying chitosan carbamate solutions generated from various chitosan salts

Chitosan salt	Degree of acylation, alkalimetry	Crystallinity	Solubility 1% acetic acid [a]	$CuSO_4$ chelation test
Chloride	0.03	amorphous [c]	soluble	blue
Acetate	0.40 [b]	crystalline	soluble	pale blue
Glycolate	0.35	crystalline [c]	soluble	pale blue
Citrate	n.d.	crystalline [c]	insoluble	white
Lactate	0.40	crystalline	soluble	pale blue

n.d., not determined due to insolubility.
[a] All samples insoluble in water and 0.1 M NaOH.
[b] By FTIR according to Ref. 44.
[c] See Fig. 3.

hydroxyacids has been described at certain temperatures as low as 80 °C for 3 h.[31,32]

The reactions taking place at the time of spray-drying can be schematised as in Scheme 2.



Scheme 2.

1.2. Alkaline solutions of chitosan carbamate and alginate

Mixing of plain chitosan with alginate normally leads to instant precipitation of a coacervate, that prevents spray-drying due to clogging of the nozzle. In the present instance, no phase separation occurred upon mixing a chitosan carbamate solution with an equimolar solution both containing excess NH_4HCO_3, at pH 9.6. The resulting clear chitosan carbamate+alginate solution was submitted to spray-drying and white microspheres were manufactured.

At the scanning electron microscope, the microspheres were mostly in the diameter range 2–5 micron and exhibited a smooth surface (Fig. 4). These microspheres were insoluble in water, acetic acid and NaOH: in the latter they aggregated. The X-ray diffraction spectrum of this material was significantly different from the spectra of chitosan and alginic acid, as shown in Fig. 5 (compare also with Fig. 2), and indicated the formation of a differently structured material. As for the FTIR spectrum of the chitosan–alginate microspheres in Fig. 6, it can be noticed that its main features are the two bands at 1597 and 1413 cm^{-1}; in this spectral region, no signal appeared for the carboxyl groups of alginic acid. In a recent work on polyelectrolyte complex formation between alginate and chitosan at room temperature as a function of pH[33], the band at 1413 cm^{-1} was attributed to the $-NH_3^+$ groups of chitosan interacting with the $-CO_2^-$ groups of alginate, while the

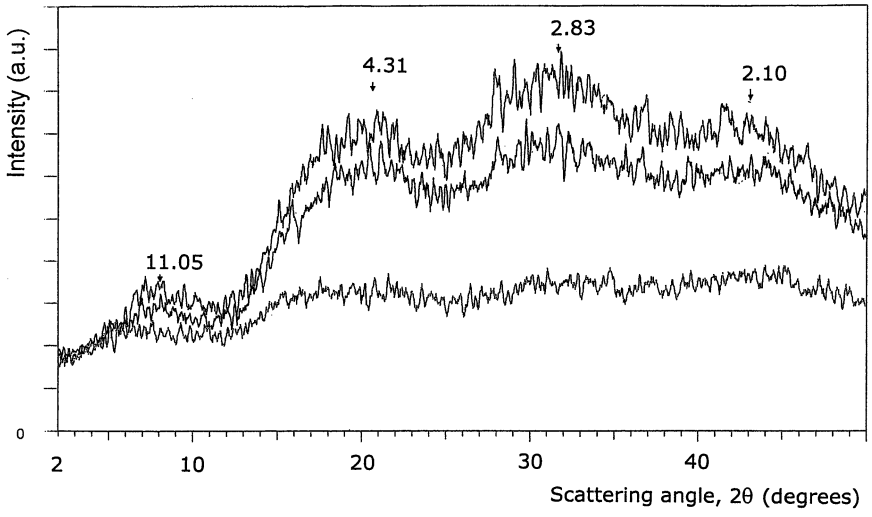


Fig. 3. X-Ray diffraction patterns for microspheres obtained from FG90 chitosan citrate (upper curve), glycolate (middle curve) and hydrochloride (lower curve), after incubation in saturated ammonium hydrogen carbonate. for 5 days. Values are in Å.

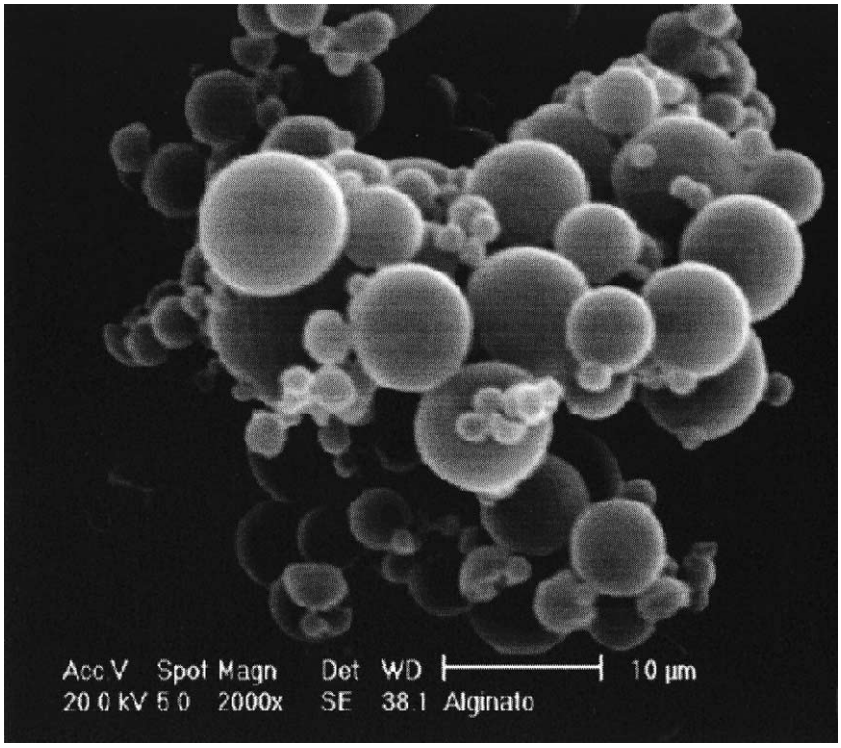


Fig. 4. Scanning electron microscopy of microspheres obtained from a clear alkaline solution of chitosan carbamate ammonium salt and ammonium alginate.

1750 band was seen only in complexes where the alginic acid component prevailed (pH 2.0–3.6). The formation of complexes was demonstrated even for systems at pH 9.0, for which carboxylate was just a shoulder at 1650–1700 cm^{-1}. Because the spectrum in Fig. 6 matches those for the complexes never exposed to heat in the cited paper,[33] we suggest that during spray-drying decarboxylation would not occur at any significant extent while the elimination of NH_4HCO_3 and water suddenly modifies the system. Even though alginic acid is susceptible to thermal and HCl-promoted decarboxylation,[34,35] alginate-poly-L-lysine. HBr and alginate-poly(vinyl)amine,[36,37] as well as bovine serum albumin-alginate[38] and lactose–chitosan–alginate microspheres were prepared;[39] the latter were described as ionic complexes. On the other hand, the thermal stability of chitosan during spray-drying is amply recognized.[40–43]

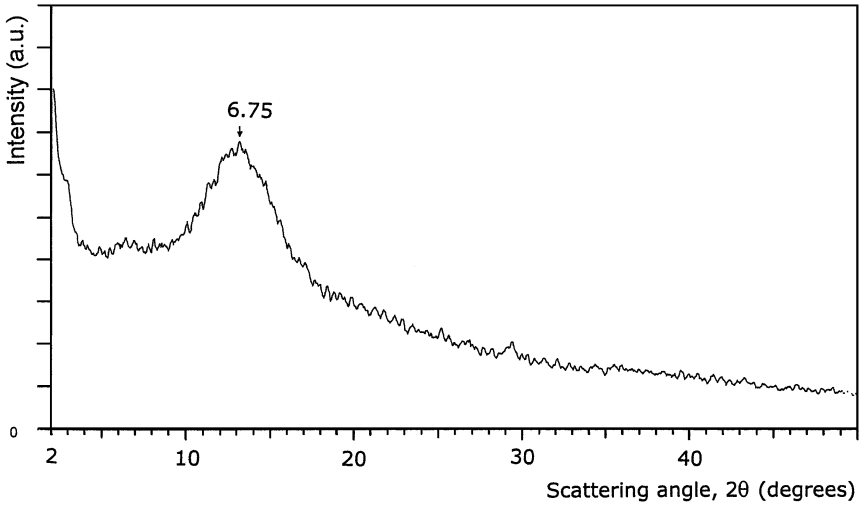


Fig. 5. X-Ray diffraction pattern for microspheres obtained from a clear alkaline solution of chitosan carbamate ammonium salt and ammonium alginate. Values are in Å.

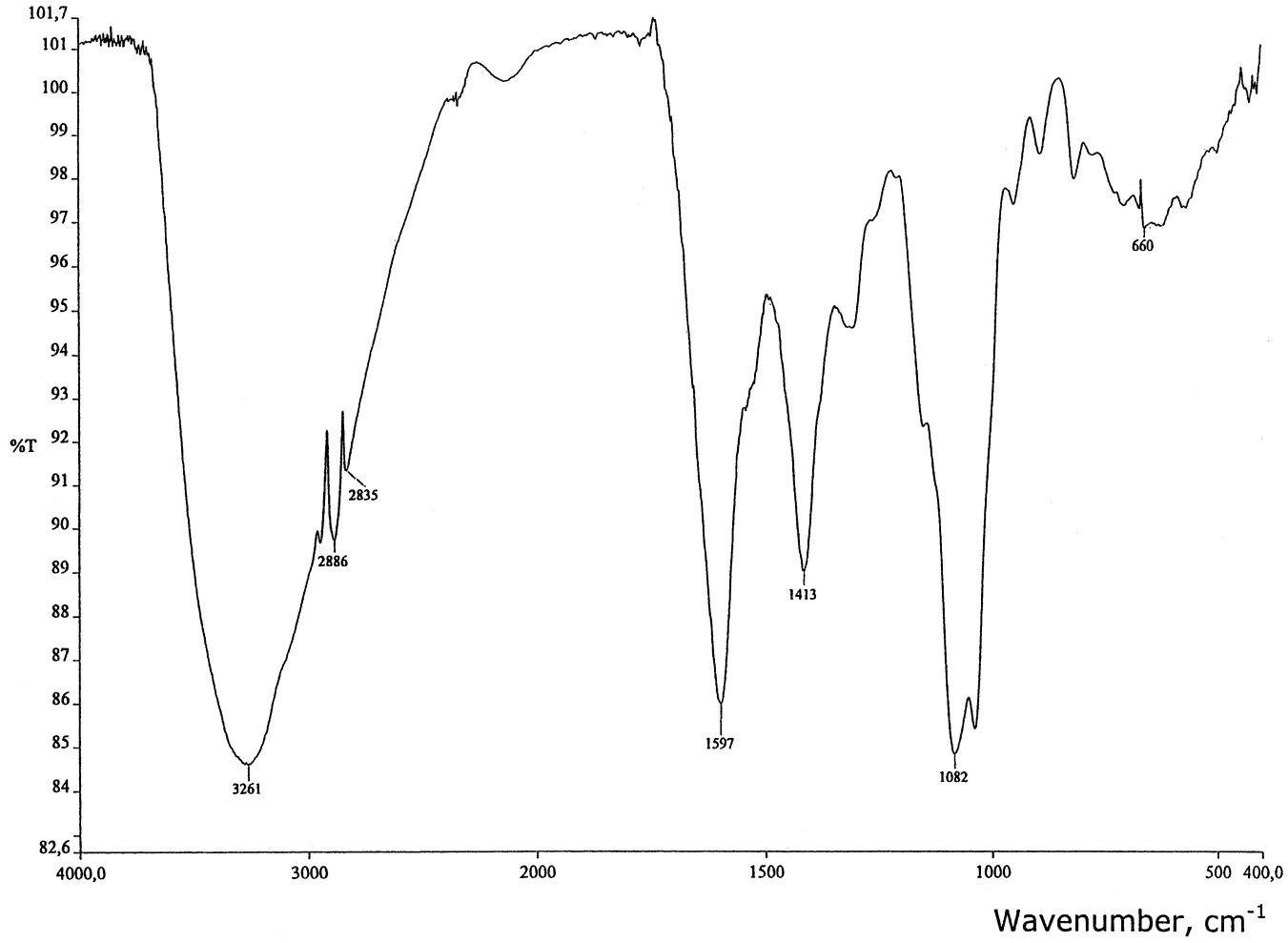


Fig. 6. FTIR Spectrum for microspheres obtained from a clear alkaline solution of chitosan carbamate ammonium salt and ammonium alginate.

In conclusion, the present work offers the only reliable process for spray-drying chitosan solutions where concomitant acylation or depolymerisation are avoided. This work removes the difficulties inherent to the insolubility of chitosan in alkaline media by providing a simple medium, namely ammonium hydrogen carbonate solutions, where highly deacetylated and high molecular weight chitosans are soluble. Moreover, it discloses the possibility of mixing chitosan with polyanions without producing coacervate precipitation: thus, for the first time chitosan–alginate microspheres are manufactured by spray drying and characterised.

2. Experimental

In order to assess the general validity of the process, the following commercial chitosan sample showing widely different characteristics were selected: Chito-clear FG90, a food grade chitosan manufactured by Primex, Drammen, Norway, distributed by Faravelli from crustaceans (degree of acetylation 0.03, average M_W 100 kDa, viscosity of 1% soln in 1% acetic acid 110 mPa s, ashes 0.3%). Vanson CSVF1430 manufactured by Vanson, Redmond WA, USA from crustaceans (degree of acetylation 0.14, average M_W 450 kDa, viscosity of 1% soln in 1% acetic acid 543 mPa, ashes 0.3%); Mahtani Chitosan M642, manufactured by Mahtani Chitosan Ltd., Veraval, India, from squid (degree of acetylation 0.20, average M_W 55 kDa, viscosity of 1% soln in 1% acetic acid 60 mPa); Mahtani Chitosan M151, manufactured by Mahtani Chitosan Ltd., Veraval, India, from squid, (degree of acetylation 0.20, average M_W 6 kDa, viscosity of 1% soln in 1% acetic acid 12 mPa); Sirc Chitosan, supplied by Sirc, Milano, Italy; from crustaceans (degree of acetylation 0.20, average M_W 120 kDa, viscosity of 1% soln in 1% acetic acid 100 mPa s, ashes 0.2%). Chemicals and alginic acid were supplied by

Aldrich, Milano, Italy. Ultrapure water was obtained with a Millipore MilliQ Academic apparatus.

A Perkin–Elmer Spectrum GX FT-IR spectrometer equipped with a Perkin–Elmer Multiscope system infrared microscope (MCT-SL detector) was used to record Attenuated Total Reflectance, ATR, spectra. The microscope was equipped with a movable 75 × 50 mm X–Y stage. In some cases, it was necessary to adopt the following procedure: small amounts of the sample, cooled in liquid N_2, were ground with KBr and the spectra were obtained by using a Spectra Tech. Diffuse Reflectance (DRIFT) accessory. In both cases, the spectral resolution was 4 cm^{-1}. The absorption spectra were the results of 16 scans. Treatments of the data were achieved with a Perkin–Elmer Spectrum and with a Grams/32 Galactic Corp. software package. Data for FG 90 chitosan: typical bands at 3446 (NH and OH stretching), 2873 (CH stretching), 1663 (amide CO strecthing), 1601 (amine deformation), 1424 (CH deformation), 1383 (C–CH3 amide stretching), 1326, 1159 (COC bridge stretching), 1094 (*CH*OH), 1030 (CO stretching), 894 and 601 cm^{-1}. The degree of acetylation was determined according to a published method.[44]

X-Ray diffraction measurements on powder samples were performed with the Bruker AXS General Area Detector Diffraction System (GADDS) equipped with a two-dimensional (2D) gas-filled sealed multiwire detector (scattering-angle resolution of 0.02°). Monochromatized Cu–K_α radiation ($\lambda = 0.154$ nm) was used. The powder samples were placed in 0.8-diameter Lindemann glass capillaries. The sample-detector distance was 10 cm. The intensity versus scattering-angle spectra were obtained after radial average of the measured 2D isotropic diffraction patterns. Data for FG90 chitosan: 8.22, 19.30 ° 2θ.

NMR spectra were recorded with a Bruker CXP-300 (75 MHz) spectrometer (polymer concentration 100 mg/0.6 mL D_2O, 25 °C, deuterated acetic acid). ^{1}H NMR data for FG90 chitosan: 1.98 (–COC*H*$_3$); 3.17 (t, 2-H); 3.5–4.0; 4.85 (d, H-1); ^{13}C NMR data for FG90 chitosan hydrochloride: 23.2 (CH_3), 57.1 (C-2); 60.8 (C-6); 75.1 (C-3,5); 81.1 (C-4); 104.7 (C-1); 173.8 (C=O).

Microspheres were prepared with a spray-drier Buchi-190, Flawil, Switzerland, at a feed rate of 5 mL/min; the air inlet temperature was 168 °C, outlet 100 °C unless otherwise indicated and air flow 600 L/h. Microspheres were gold-coated for examination with a Philips SEM 505 scanning electron microscope.

The molecular size determinations were made by hydrogel permeation chromatography with a Beckman Systemgold 116 pump equipped with a Systemgold 406 Analog Interface, a Shodex RI-SE-61 detector, a TSK-GEL G-oligo PW colums operated at the flow rate of 0.7 mL/min. Chitosan samples (10 μL, 5 g/L) were eluted with a mixture of acetic acid (0.5 M) and sodium acetate (0.2 M) at 25 °C.

2.1. General procedure for the synthesis of chitosan carbamate ammonium salt

Chitosan hydrochloride salt soln (10 g, containing 1.00 g chitosan and a stoichiometric amount of HCl) was poured into a saturated NH_4HCO_3 soln (prepared from 40 mL water and 9.6 g salt; 20 °C; final pH 9.6) and kept at 20 °C for 5 days with no stirring. Additions of NH_4HCO_3 were made on the 2nd and 4th day. The rigid and transparent hydrogel was separated by centrifugation at 12,000 rpm, and kept at 4 °C (approx 10 g); no syneresis and no microbial growth occurred over 30-day storage at 4 °C. For the analysis, it was freeze-dried. Yield 0.93 g. FTIR (KBr): *ν* 581, 843, 1020, 1059, 1155, 1365, 1448, 1681, 1741, 2880, 3363; ^{1}H NMR (75 MHz, D_2O): δ 1.98 (–$COCH_3$); 3.30 (m, 2 H); 3.40–3.9; 4.70 (m, 1 H).

Alkalimetric measurements indicated that 113.5 mL of 1.0 M NaOH facilitated the dissolution of 0.5 g (chitosan dry weight approx 3 mmol) in the form of chitosan hydrogel in the presence of 9.5 g NH_4HCO_3 and 50 mL water. The numbers of moles of NH_4HCO_3 (9.5:79 = 0.12) and NaOH (0.113) were practically the same, and the final pH was 9.6. Therefore, the solution was at its maximum buffering capacity, by analogy with the sodium carbonate/bicarbonate buffer solutions.

2.2. General procedure for the manufacture of chitosan microspheres

The chitosan carbamate ammonium salt prepared from FG-90 chitosan hydrochloride was poured into a fourfold weight of water and stirred for 30 s with a Silverson emulsifier to obtain a clear soln, that was immediately submitted to spray-drying. At least 5 g of chitosan are necessary for operating the instrument. FTIR (KBr): *ν* 580, 1075, 1153, 1321, 1378, 1401, 1594, 1645 (shoulder), 2883, 3288; ^{1}H NMR (75 MHz, D_2O): 1.98 (–$COCH_3$); 3.17 (t, 2 H); 3.5–4.0; 4.85 (d, H 1); Degree of acetylation: 0.03. X-Ray diffraction amorphous. The expanded form of these microspheres was revealed by their bulk density (0.025 g/cm^3) that was much smaller compared to microspheres from systems containing acids other than HCl.

2.3. General procedure for the manufacture of chitosan–alginate microspheres

The chitosan carbamate ammonium salt prepared from chitosan hydrochloride was poured into a fourfold weight of water, and stirred for 30 s with a Silverson emulsifier to obtain a clear soln. Alginic acid (same weight as chitosan) was dissolved in dilute NH_4HCO_3 soln (same vol as for chitosan). The two solutions were mixed just before spray-drying at 115 °C. FTIR (KBr) *ν* 660, 1040, 1082, 1321, 1413, 1597, 2886, 3261 (Fig. 6);

X-ray diffraction: 13 ° 2θ. Data for alginic acid: FTIR (KBr) ν 674, 814, 881, 930, 1034, 1243, 1632, 1731, 3488.

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