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Spray-drying of solutions containing chitosan together with polyuronans and characterisation of the microspheres

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Abstract

Incubation of the rigid and transparent gel obtained upon pouring a chitosan hydrochloride solution into saturated ammonium hydrogen carbonate at 20 °C yielded chitosan carbamate, Chit-$NHCO_2^-NH_4^+$, soluble at alkaline pH values, typically 9.6. Addition of water to the gel isolated by centrifugation promoted the dissolution of the gel. By spray-drying the alkaline solution thus obtained, microspheres of chitosan were obtained. When chitosan carbamate was mixed with alginic acid, polygalacturonic acid, carboxymethyl cellulose, carboxymethyl guaran, acacia gum, 6-oxychitin, xanthan, hyaluronic acid, pectin, k-carrageenan, and guaran, clear solutions were obtained from which chitosan–polyuronan microspheres were easily manufactured by spray-drying. Those made of chitosan–xanthan or chitosan–guaran were unexpectedly found to be soluble in water; similarly, the chitosan–pectin microspheres were almost soluble. The microspheres containing hyaluronic acid or k-carrageenan underwent swelling when contacted with water; the other ones were insoluble. The microspheres were characterized by FTIR, X-ray diffraction spectrometry and scanning electron microscopy. The structural alterations detected were mainly due to interactions between the amino groups and the carboxyl groups.

Keywords: Chitosan solubility; Spray-drying; Chitosan–polyuronan microspheres

1. Introduction

As a drug carrier, chitosan helps overcome certain adverse characteristics of drugs such as insolubility and hydrophobicity, but the semi-crystalline powder does not lend itself to direct compression. While chitosan powders have been evaluated in direct compression tests (Knapczyk, 1993), the formulations so far developed include excipients to facilitate compression (Rege, Shukla, & Block, 1999). A striking example are the commercial chitosan tablets for overweight control, which contain magnesium stearate as a binder, with negative consequences on the efficacy of chitosan.

Chitosan has been spray-dried not very often: chitosan suspensions (Davis et al., 1999; He, Davis, & Illum, 1999; Rege, Garmise, & Block, 2003a,b), chitosan salts (De la Torre, Enobakhare, Torrado, & Torrado, 2003), chitosan gelatin–ethylene oxide (Huang, Yeh, Cheng, & Chiang, 2003; Huang, Yeh, & Chiang 2003), and chitosan ethylcellulose mixture (Shi & Tan, 2002) have been assayed. Spray-drying of chitosan salts solutions provides chitosan microspheres having diameters close to 2–5 μm and improved binding functionality. The chitosan microsphere free-flowing powder is compressible and hence most suitable as a drug carrier (Rege et al., 2003a,b; Sabnis, Rege, & Block, 1997).

The general chemical behaviour of chitosan, however, should be considered in order to avoid certain difficulties stemming from its insolubility at pH higher than 6.5 and its reactivity under the thermal conditions of the sprayer. For example, it seemed easy to spray-dry acetic solutions containing 1–2% chitosan at 168 °C, but the release of a drug from the spray-dried chitosan sharply depended on the acetic acid concentration because of the acetylation reaction

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occurring at that temperature. In fact, the degree of acetylation of chitosan increased during spray-drying and affected its enzymatic degradability (Shi & Tan, 2002).

Chitosan can be spray-dried at neutral pH if a colloidal suspension is prepared with NaOH. Nevertheless this preparation is time-consuming because it is difficult to wash the colloid and to remove excess alkali and salts.

As for alkaline media chitosan has been recently found to be soluble in NH_4HCO_3 solutions, where it assumes the ammonium carbamate form Chit-$NHCO_2^-NH_4^+$, i.e. a transient anionic form that keeps it soluble at pH 9.6, while reversibly masking the polycationic nature of chitosan. These original findings by Muzzarelli, Tosi, Francescangeli and Muzzarelli (2003) have as a valid background some works on glycoprotein synthesis (Cohen-Anisfeld & Lansbury, 1993; Kunz, Hofmeister, & Glaser, 1998; Likhosherstov, Novikova, Derevitskoja, & Kocketkov, 1986; Linek, Alfoldi, & Defaye, 1987; Lubineau, Augé, & Drouillat, 1995; Manger, Rademavcher, & Dwek, 1992; Merchan, Merino, & Tejero, 1997; Muzzarelli, Terbojevich, Muzzarelli, & Francescangeli, 2002; Ortiz Mellet, Jimenez Blanco, Garcia Fernandez, & Fuentes, 1993; Tietgen, Schultz-Kukula, & Hunz, 2000; Vetter & Gallop, 1995; Wang, Zhang, Live, & Danishefsky, 2000). Because ammonium carbamates and NH_4HCO_3 decompose thermally and liberate CO_2, NH_3 and water, this alkaline system is perfectly suitable for producing chitosan microspheres by spray-drying.

It is known that chitosan forms polyelectrolyte complexes with polyanions, such as 6-oxychitin (Muzzarelli, Muzzarelli, Cosani, & Terbojevich, 1999), heparin (Kweon & Lim, 2003), carrageenan (Hugerth, Caram-Lelham, & Sundelof, 1997), pectin (Hoagland & Parris, 1996; Nordby, Kjoniksen, Nystrom, & Roots, 2003), xanthan (Dumitriu, Magny, Montane, Vidal, & Chornet, 1994), acacia gum (Meshali & Gabr, 1993), hyaluronic acid (Laurent, 1998), alginic acid (Kim et al., 1999; Lai, Khalil, & Craig, 2003; Miyazaki, Nakayama, Oda, Takada, & Attwood, 1994), poly(acrylic acid) (De la Torre et al., 2003), carboxymethyl cellulose (Arguelles--Monal & Peniche- Covas, 1988), DNA (Rikimaru, Wakabayashi, Nomizu, & Nishi, 2003) and other macromolecules (Kubota and Kikuchi, 1998). These reactions are very fast and lead in general to the immediate disordered precipitation of the insoluble coacervates upon mixing, particularly when the final pH value is neutral or higher. On their part, the polyanions are normally in sodium salt form and show alkaline hydrolysis that contributes to chitosan insolubilization upon mixing.

As a consequence, chitosan–polyanion complexes have never been manufactured by spray-drying because chitosan itself is insoluble at pH values above 6.3; for instance, it is known that phase separation occurs when hyaluronan is mixed with chitosan particularly for stoichiometric ratios (SR = $-NH_2/-COOH$) close to 1.0.

The complex formation corresponds to charge neutralization at least partially, therefore the degree of complexation by charge neutralization is usually provided. For the system chitosan + hyaluronan the degree of complexation is ca. 1.0 regardless of the stoichiometric ratio value (provided SR $>$ 1); moreover, the degree of complexation is nearly independent of the degree of acetylation of chitosan up to 0.40. For higher degree of acetylation (randomly reacetylated chitosans) the complexation mechanism involves less cooperation. The chitosan–hyaluronan insoluble complexes are stable in acidic and alkaline media: they do not dissolve in NaOH, but dissolve in 0.2 M HCl. The complex is de-stabilized in NaCl brines (Rusu-Balaita, Desbrieres, & Rinaudo, 2003).

The scope of the present work is therefore to produce soluble polyelectrolyte complexes in alkaline solutions suitable for spray-drying, and then manufacture microspheres of chitosan–polyuronan complexes useful for subsequent drug delivery, thus overcoming the present limitations.

A number of anionic polysaccharides deserved immediate consideration, namely alginic acid, polygalacturonic acid, carboxymethyl cellulose, carboxymethyl guaran, acacia gum, 6-oxychitin, xanthan, hyaluronic acid, pectin, k-carrageenan, and guaran, that represent a selection of anionic polyelectrolytes capable of reacting with chitosan and currently studied in the food, pharmaceutical and medical fields (Collins, 1987).

2. Materials and methods

2.1. Materials

Chito-clear FG90, a food grade chitosan manufactured from crustaceans by Primex, Drammen, Norway, distributed by Faravelli Milano, Italy, was used (degree of acetylation 0.10, average MW 128 kDa, viscosity of 1% solution in 1% acetic acid 100 mPa s, ashes 0.3%) was used. Citrus pectin (galacturonic acid 93.5%; methoxy content 9.4%), alginic acid sodium salt from brown seaweeds (200–400 mPa s for 3% solution), polygalacturonic acid ($>$85% pure; ash as carbonate $<$2.5%), carboxymethyl cellulose sodium salt (400–800 mPa s for 4% solution, 99.5% pure), acacia powder from *Acacia senegal* (gum arabic), xanthan from *Xantomonas campestris*, and kappa-carrageenan (a sulfated polysaccharide from red seaweeds, type I), were supplied by Aldrich, Milano, Italy. Guaran, a neutral polysaccharide from *Cyanopsis tetragonolobus*, and carboxymethyl guaran were supplied by Lamberti, Varese, Italy. Cosmetic grade hyaluronic acid was kindly provided by Mavi Sud, Aprilia, Italy. Ultrapure water was obtained with a Millipore MilliQ Academic apparatus. Hen egg white lysozyme was supplied by Calbiochem, Milan, Italy.

2.2. Instrumental methods

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 $75 \times 50\ mm^2$ X–Y stage. In some cases it was necessary to adopt the following procedure: small amounts of the sample, cooled in liquid nitrogen, 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 stretching), 1601 (amine deformation), 1424 (CH deformation), 1383 (C-CH3 amide stretching), 1326, 1159/COC bridge stretching), 1094 (CHOH), 1030 (CO stretching), 894 and 601 cm^{-1}.

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 Kα 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 vs. 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}HNMR, data for FG90 chitosan: 1.98 ($-COCH_3$); 3.17 (t, 2-H); 3.5–4.0; 4.85 (d, H-1); ^{13}CNMR, 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-dryer Buchi-190, Flawil, Switzerland, at a feed rate of 8 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.

2.3. Synthesis of chitosan carbamate ammonium salt

Chitosan hydrochloride salt solution (10 g, containing 1.00 g chitosan and stoichiometric amount of HCl) was poured into a saturated NH_4HCO_3 solution (prepared from 40 ml water and 9.6 g salt; 20 °C; final pH 9.6) and incubated at 20 °C for 5 days with no stirring, to react according to the following equation:

$$\text{Chit-NH}_2 + \text{NH}_4\text{HCO}_3 = \text{Chit-NHCO}_2^-\text{NH}_4^+ + \text{H}_2\text{O}$$

Additions of NH_4HCO_3 were made on the second and fourth day. The rigid and transparent hydrogel was separated by centrifugation at 12,000 rpm, and kept at 4 °C (ca. 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): 581, 843, 1020, 1059, 1155, 1365, 1448, 1681, 1741, 2880, 3363; ^{1}HNMR (75 MHz, D_2O): 1.98 ($-COCH_3$); 3.30 (m 2-H); 3.40 3.9; 4.70 (m 1-H).

2.4. Manufacture of chitosan microspheres

The chitosan carbamate ammonium salt prepared from FG-90 chitosan hydrochloride was poured into a four-fold weight of water and stirred for 30 s with a Silverson emulsifier to obtain a clear solution, that was immediately submitted to spray-drying. At least 5 g of chitosan are necessary for operating the instrument. FTIR (KBr): 580,

Table 1
Operating conditions adopted for spray-drying chitosan carbamate–polyanion mixtures in dilute NH_4HCO_3

Polyanion	Dry weight ratio chitosan/polyanion	NH_2/COOH molar ratio	Total polysaccharide concentration (g/l)	Solubility microsphere	Inlet air (°C)
Alginic acid	1	0.9	4.2	Insoluble	155
Polygalacturonic acid	1	0.9	5.7	Insoluble	155
Carboxymethyl cellulose	1	n.a.	10.2	Insoluble	130
Carboxymethyl guaran	1	n.a.	8.5	Insoluble	135
Acacia gum	0.5	1.6	9.4	Insoluble	150
6-Oxychitin	1.25	1.0	7.5	Insoluble	160
Xanthan	2.5	6.0	3.7[a]	Soluble	155
Hyaluronic acid	1.66	3.9	4.0[a]	Swollen	145
Pectin	1	4.0	9.2	Soluble	145
k-Carrageenan[b]	2	5.0	3.3[a]	Swollen	155
Guaran[c]	1	No	7.7	Soluble	140

[a] These low polysaccharide concentrations were preferred in order to avoid excessive viscosity.
[b] Sulfated polysaccharide. Flow-rate 10 ml/min.
[c] Neutral polysaccharide.

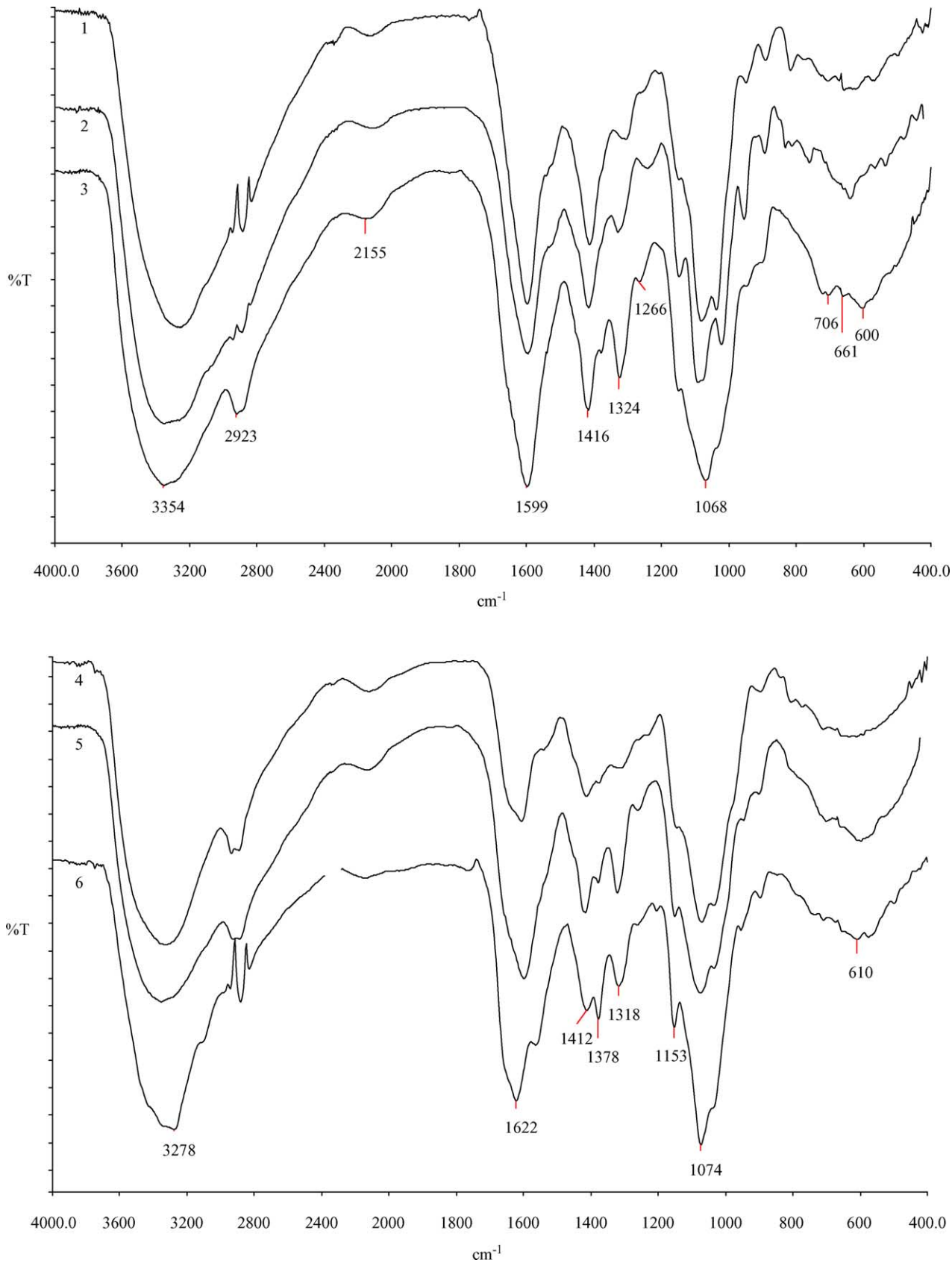

Fig. 1. FTIR spectra of the complexes of chitosan with: 1, alginic acid; 2, polygalacturonic acid; 3, carboxymethyl cellulose; 4, carboxymethyl guaran; 5, acacia gum; 6, 6-oxychitin.

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.

2.5. Manufacture of chitosan–polyanion 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 solution. Each polyanion was dissolved in dilute NH_4HCO_3 solution (same volume as for chitosan). The two solutions were mixed just before spray-drying.

3. Results and discussion

3.1. Alkaline solutions of chitosan carbamate and anionic polysaccharide

When pouring a chitosan acetate solution into a saturated NH_4HCO_3 solution, no precipitation of chitosan occurred. Rather, a self-sustaining and transparent hydrogel formed at once. Upon addition of water, the hydrogel dissolved and the resulting solutions were suitable for spray-drying. 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 microspheres obtained from a system containing HCl were amorphous and their degree of acetylation was coincident with the value of the parent chitosan. 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 (Ogawa & Inukai, 1987). These microspheres 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.

The reactions taking place at the time of spray-drying, namely carbamate decomposition with chitosan restoration, and ammonium hydrogen carbonate decomposition, can be schematised as follows:

$$\text{Chit-NHCO}_2^- \text{NH}_4^+ \xrightarrow{\text{heat}} \text{Chit-NH}_2 + \text{CO}_2 + \text{NH}_3$$

carbamate decomposition

$$\text{NH}_4\text{HCO}_3 \xrightarrow{60-107\ ^\circ\text{C}} \text{CO}_2 + \text{NH}_3 + \text{H}_2\text{O}$$ salt decomposition

3.2. Insoluble microspheres

No phase separation occurred upon mixing a chitosan carbamate solution with a solution of alginic acid, polygalacturonic acid, carboxymethyl cellulose, carboxymethyl guaran, acacia gum and 6-oxychitin. The microspheres manufactured from these mixtures (upper half of Table 1) were insoluble in water, as well as in lysozyme solutions at pH values in the range 4.4–5.3, during an observation period of 1 week.

These microspheres shared very simple features of their FTIR spectra, as shown in Fig. 1. The CO stretching band of free carboxylic acids shifted from 1720 to 1580 cm^{-1} for carboxylates, and in practice these FTIR spectra showed contributions by carboxylate and amino groups. The XRD spectra had in common features indicative of amorphous nature.

At the scanning electron microscope, the microspheres of chitosan alginate were mostly in the diameter range 2–5 μm and exhibited a smooth surface. 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, and indicated the formation of a differently structured material. FTIR (KBr) n 660, 1040, 1082, 1321, 1413, 1597, 2886, 3261 (Fig. 1); X-ray diffraction: 13° 2θ. Data for alginic acid: FTIR (KBr) n 674, 814, 881, 930, 1034, 1243, 1632, 1731, 3488.

In a recent work on polyelectrolyte complex formation between alginate and chitosan at room temperature as a function of pH 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 1750 cm^{-1} band was seen only in complexes where the alginic acid component prevailed (pH 2.0–3.6) (Simsek-Ege et al., 2003). In fact, the carbonyl stretching band of unreacted alginic acid at 1731 cm^{-1} is shifted to 1597 cm^{-1} for carboxylate anion. The formation of complexes was demonstrated even for systems at pH 9.0,

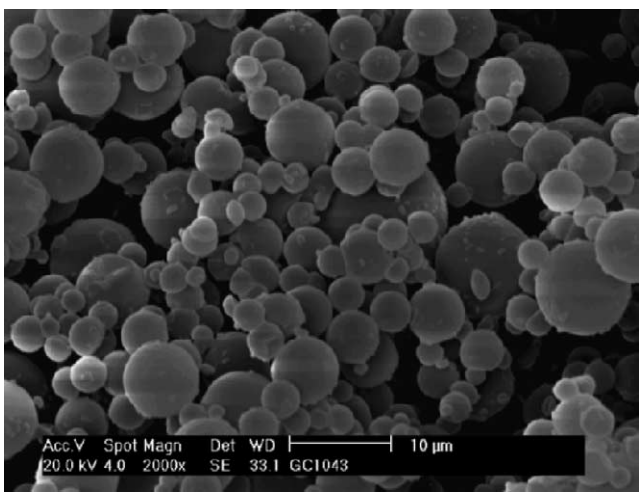

Fig. 2. Microspheres made of chitosan and carboxymethyl guaran complex.

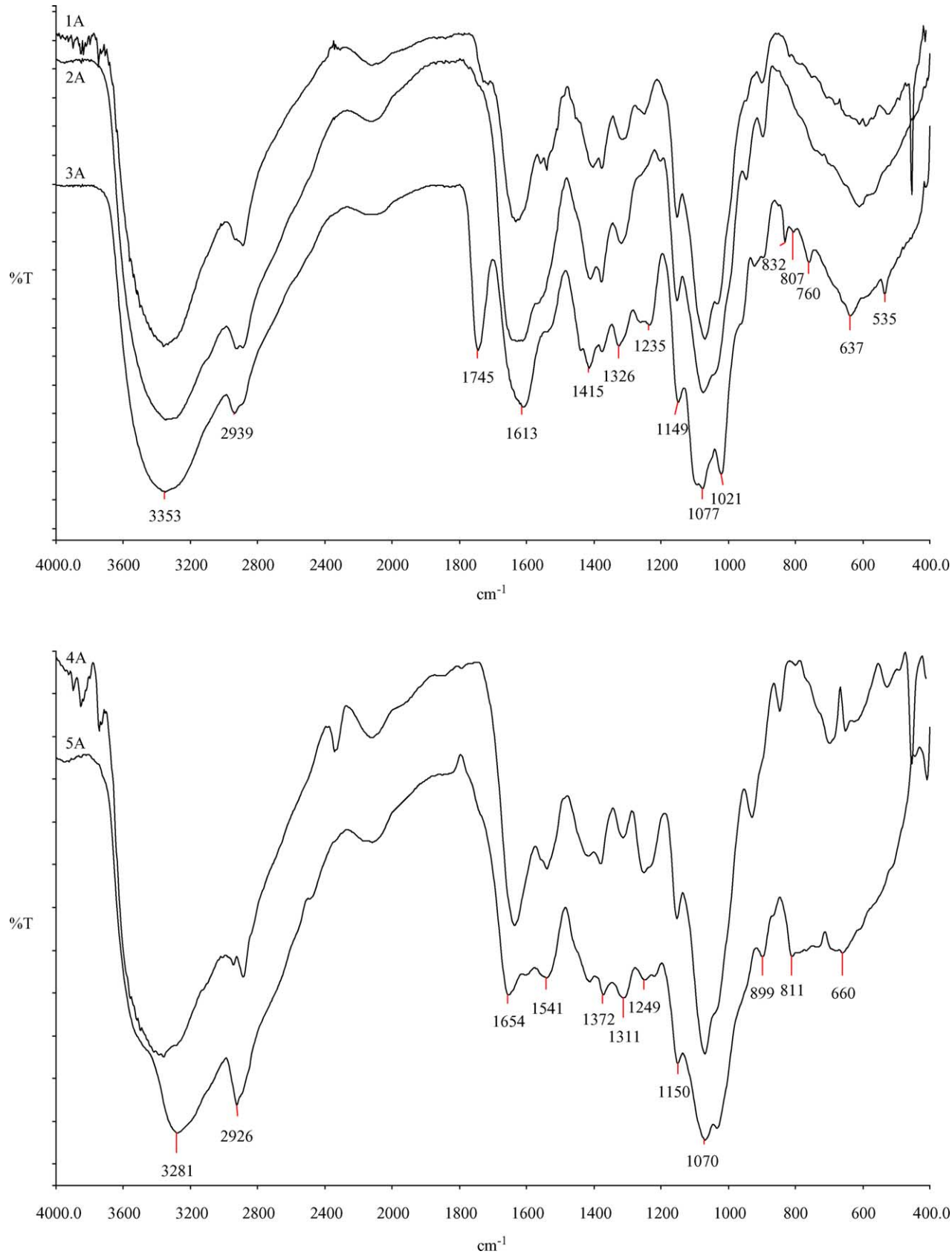

Fig. 3. FTIR spectra of the complexes of chitosan with: 1A, xanthan; 2A, hyaluronic acid; 3A, pectin; 4A k-carrageenan; 5A, guaran.

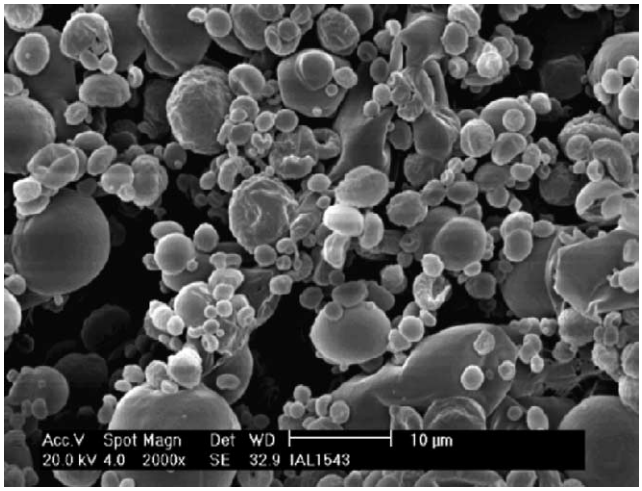

Fig. 4. Microspheres made of chitosan and hyaluronic acid complex.

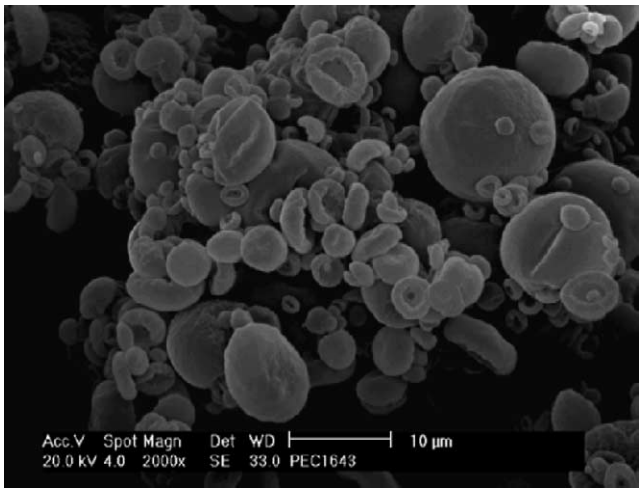

Fig. 5. Microspheres made of chitosan and pectin complex.

for which carboxylate was just a shoulder at 1650–1700 cm^{-1}. Because the spectrum matches those for the complexes never exposed to heat in the cited paper (Simsek-Ege et al., 2003), during spray-drying decarboxylation would not occur at any significant extent while the elimination of NH_4HCO_3 and water suddenly modifies the system. Lactose–chitosan–alginate microspheres were described as ionic complexes.

For chitosan polygalacturonate the X-ray diffraction spectrum was similar to the chitosan alginate spectrum with an additional modest peak at 21 2θ. FTIR (KBr) 642, 894, 954, 1022, 1093, 1148, 1243, 1329, 1415, 1596, 2888, 3355.

The microspheres made of chitosan–carboxymethyl-guaran complex were all spherical with diameters 3–10 μm (Fig. 2), whilst for chitosan carboxymethyl cellulose, the particles were poorly defined, had irregular forms and were agglomerated. The microspheres, made of chitosan–oxy-chitin with diameters 3–12 μm were insoluble in water, acetic acid and NaOH. The X-ray diffraction spectrum exhibited a main peak at 14 2θ, together with a series of four minor peaks at 19, 26, 31.5 and 45.5 2θ.

3.3. Soluble or swelling complexes

For the complexes of chitosan with xanthan, hyaluronan, pectin, k-carrageenan, and guaran (Fig. 3), the FTIR were reminiscent of the chitosan spectrum, mainly because of the high NH_2/COOH molar ratio adopted, and the microspheres either dissolved or swelled in water.

In the case of chitosan–hyaluronan the microspheres were generally smaller than for other complexes but were accompanied by some irregular pellicular fragments (Fig. 4). FTIR (KBr) 611, 1075, 1152, 1319, 1377, 1410, 1613, 2926, 3344.

Notwithstanding the peculiar high apparent density of the chitosan–pectin microspheres, just a minor fraction of the particles exhibited a spherical form (Fig. 5). Chitosan-pectin

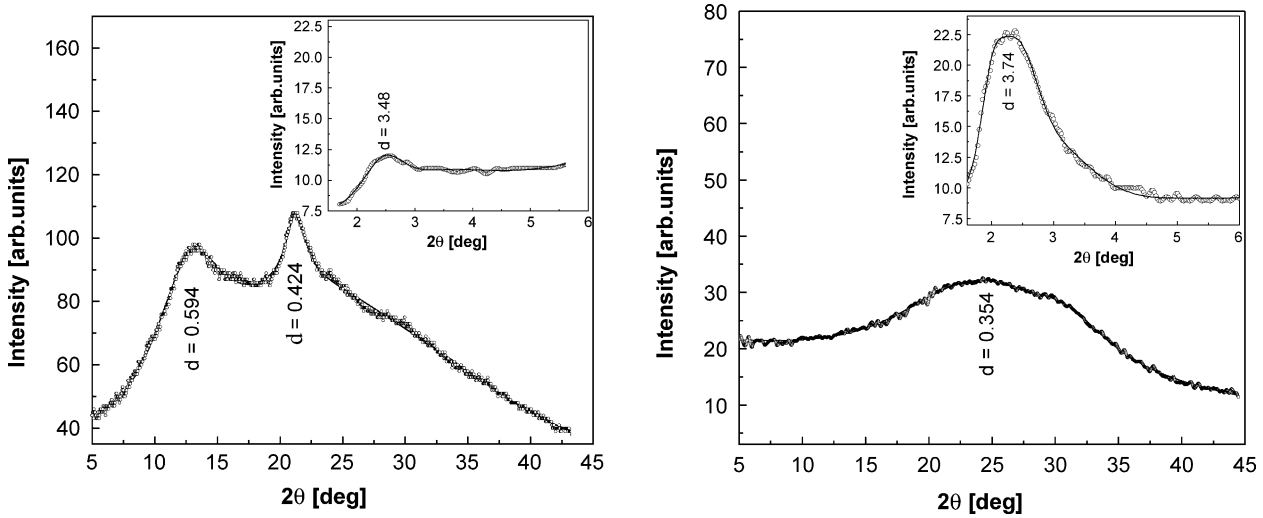

Fig. 6. X-Ray diffraction spectra for pectin (left), and for the chitosan–pectin complex (right). Insets: low angle spectra.

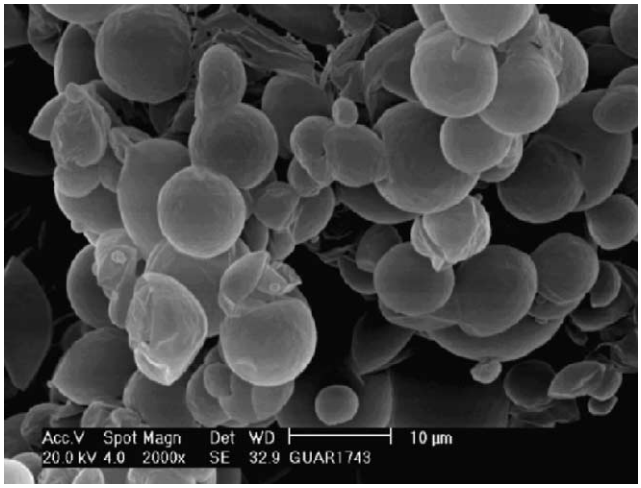

Fig. 7. Microspheres made of chitosan–guaran complex.

FTIR (KBr) 637, 1021, 1078, 1149, 1235, 1327, 1415, 1613, 1745, 2939, 3353. This spectrum included the 1745 band assigned to the methyl ester groups of pectin. The XRD spectra showed that the combination of chitosan with pectin led to a nearly amorphous product, (see Fig. 6).

Also the XRD spectra of microsphere made of chitosan—k-carrageenan and chitosan-guaran indicated that they were amorphous.

The microspheres made of chitosan with guaran, a neutral polysaccharide, were surprisingly soluble in water upon stirring, even after 60-day storage at 30 °C. They were smaller than 10 μm and relatively fragile (Fig. 7). At the XRD they were amorphous, the peaks typical for guaran being quite depressed; however, at small angles, an intense scattering was observed (Fig. 8). For chitosan-guaran FTIR (KBr) 661, 812, 899, 1032, 1069, 1151, 1308, 1374, 1412, 1540, 1656, 2926, 3282.

4. Conclusions

The alkaline solutions of chitosan hydrochloride in NH_4HCO_3 have been found suitable for the preparation of chitosan microspheres by spray-drying. Of course, chitosan hydrochloride is the most convenient salt, because it yields amorphous, highly expanded and underivatized free base chitosan microspheres. The microspheres obtained according to the present method had a better morphology than the corresponding wrinkled and deeply depressed microspheres obtained by He et al. (1999) from acidic solutions.

It should be noted that slightly different characteristics might be obtained depending on the variability of the hydrocolloids (source, time of harvest, isolation process or else).

The chitosan carbamate solution could be mixed with one of a series of anionic polysaccharide solutions without producing precipitation, hydrogel formation or substantial viscosity build-up. Thus, upon mixing, the solutions have been spray-dried with no special precautions, and microspheres of polyelectrolyte complexes have been obtained for the first time.

The instrumental evidence indicates that the final products were actually polyelectrolyte complexes, mainly based on the fact that in general the spectral characteristics of each polysaccharide in pure form were either deeply altered or absent in the spectra of the combinations. Major aspects, among those discussed above, were the alteration of the –COOH band in the FTIR spectra, and the presence of novel peaks in the XRD spectra.

In most cases the microspheres were insoluble. The polysaccharides might be partially cross-linked via amido groups formed by the carboxyl groups of the polyanion and the restored free amino group of chitosan, immediately after the carbamate thermal decomposition. The new links impart

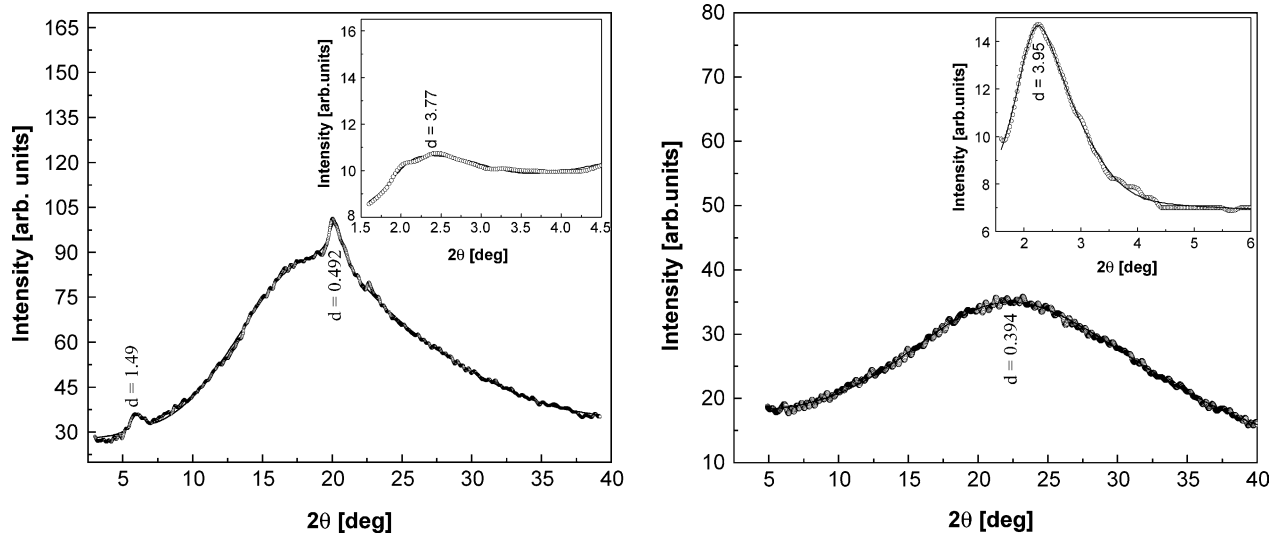

Fig. 8. X-ray diffraction spectra for guaran (left), and for the chitosan–guaran complex (right). Insets: low angle spectra.

new structural order in the complex, of course. The FTIR spectra for all microspheres containing the molar ratio NH_2/COOH close to 1.0 are in fact extremely simple (simpler than the chitosan spectrum) and the few bands in the most significant portion of the spectrum are related to the amino and carbonyl groups.

The susceptibility to enzymatic hydrolysis by lysozyme was poor, mainly because lysozyme, a strongly cationic protein, can be inactivated by anionic polysaccharides (Jollès, 1996).

Most interestingly, the microspheres containing guaran (a neutral polysaccharide, weight ratio 1.0) or xanthan (molar ratio 6.0) were found to be soluble in water. The chitosan–guaran microspheres were amorphous and exhibited low angle diffraction as did the chitosan pectate microspheres (molar ratio 4.0) that were almost soluble. It seems that a minor portion of guaran or xanthan imparts solubility to the respective combinations with chitosan, per se insoluble as a free base.

In spite of the chemical differences (alcohol groups in guaran, carboxyl groups in xanthan, partially esterified carboxyl groups in pectin) these three polysaccharides, once combined with chitosan in the microspheres appear to be able to bring chitosan into solution. This is particularly interesting if one considers that these three polysaccharides of which the solubility in water is well known have important applications in the food and pharmaceutical industries, where they are amply used as food additives, excipients and emulsifiers for the preparation of ice-creams, soups, puddings, cakes, tooth-pastes and spray-dried fixed flavours. The polysaccharide combinations described in the present work might permit a wider use of chitosan in these fields, thanks to the original preparation method and to the unexpected unusual prerogatives of the microspheres.

Acknowledgements

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