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Evaluation of ultrasonic atomization as a new approach to prepare ionically cross-linked chitosan microparticles

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Abstract

Ultrasonic atomization was evaluated as a new approach for the preparation of ionically cross-linked controlled-release chitosan microparticles loaded with theophylline as the model drug, using tripolyphosphate (TPP) as counter-ion. It was possible to nebulize both 2% and 3% (w/v) chitosan solutions as a function of their viscosity, usually not processed by employing the conventional nebulizer. The results of the chitosan molecular characterization using the SEC-MALS analysis revealed that ultrasonic atomization caused a certain depolymerization, probably due to the main chain scission of the 1,4-glycosidic bond; however, Fourier transform-infrared spectroscopy revealed the absence of other chemical modifications. The ultrasonic atomization allowed preparation of TPP cross-linked chitosan microparticles mostly ranging between 50 and 200 μm. As regards manufacturing parameters, the linking time and washing medium were found to affect the properties of the microparticles, while the stirring rate of the TPP solution did not show any influence. The evaluation of the formulation variables revealed that chitosan concentration strongly affected both the feasibility of the ultrasonic atomization and the drug release. All the microparticles showed an encapsulation efficiency of > 50 % and, after an initial burst effect, a controlled release of drug for 48 h. In conclusion, the ultrasonic atomization could be proposed as a robust and innovative single-step procedure with scale-up potential to successfully prepare ionically cross-linked chitosan microparticles.

Introduction

Atomization is a process by which a mass of liquid is converted into small droplets dispersed in a gas (spray). Ultrasonic atomization employs ultrasonic energy to overcome the surface tension forces resulting from an increase in surface area of a liquid during atomization (Morgan 1993). In recent years this technology, widely used in metallurgy industry and in the production of aerosols for pulmonary drug delivery, has been evaluated as a new approach to the preparation of microparticulate drug delivery systems. In particular our group has developed an ultrasonic atomizer that has been successfully employed to prepare microparticles by the spray–congealing technique (Rodriguez el al 1999). Subsequent studies have shown that, selecting a suitable low melting point carrier, the microspheres prepared by ultrasonic atomization can both enhance the dissolution rate of a poorly soluble drug, such as carbamazepine (Passerini et al 2002), and control the release of verapamil hydrochloride (Passerini et al 2003) and theophylline (Albertini et al 2004).

The technique of ultrasonic atomization can be applied to the preparation of polymeric microparticles, too. Until now, only PLA or PLGA microspheres loaded with different peptide and protein have been examined: Bittner & Kissel (1999) and Freitas et al (2004) installed an ultrasonic nozzle in a laboratory spray-dryer, while Felder et al (2003) studied the feasibility of ultrasonic atomization combined with subsequent organic solvent extraction. The results of these works showed that ultrasonic technology has the potential for both aseptic processing and up-scaling.

Further investigation of the potential of ultrasonic atomization employing other polymers could thus be interesting to expand the application of this innovative technology. Hence, this study focused on the manufacturing of natural polymeric (chitosan) microparticles by means of ultrasound.

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Generally, chitosan microparticles are prepared using different techniques (Kas 1997): solvent evaporation or emulsification, simplex or complex coacervation, spray-drying and cross-linking (covalent or ionical). Besides the well-known advantages, solvent evaporation, emulsification and coacervation have the common restriction of the difficulty of scaling-up. Spray-drying is an easy and rapid process quite suitable for scaling-up (Giunchedi et al 2003); however, to obtain controlled-release chitosan microparticles, a hardening step through cross-linking of the polymer is necessary. The covalent cross-linking can be performed during the spray-drying process, adding glutaraldehyde or formaldehyde to the chitosan solution, while ionically cross-linked microparticles require a further hardening step, as reported by Coppi et al (2002). Until now, only beads or big microparticles ($> 500\,\mu$m) have been prepared by ionical cross-linking (also called ionotropic gelation), dropping a chitosan solution by a syringe (Shu & Zhu 2002; Zhang et al 2002) or by spray gun (Ko et al 2002); as counter-ion, tripolyphosphate was employed.

Therefore, in this study ultrasonic atomization, as a new approach for the preparation of ionically cross-linked controlled-release chitosan microparticles, having particle size smaller than beads, was evaluated. The effect of ultrasonic atomization on chitosan properties and the influence of some process variables and formulation parameters on the characteristics of the resulting microparticles were studied.

Materials and Methods

Materials

Chitosan FG90 (degree of deacetylation 97%, viscosity of 1% w/w aqueous solution in 1% v/v acetic acid, 110 mPa s) was purchased from Faravelli, Italy. As model drug, theophylline (batch no. 425785/120702; 90% < 200 μm), supplied by Fluka BioChemika (Buchs, Switzerland) was employed, while tripolyphosphate (TPP), obtained from the same supplier, was used as counter-ion. All other reagents were analytical grade and Milli-RX20 water (Millipore, Molsheim, France) was used throughout.

Preparation of TPP–chitosan microparticles

The ultrasonic atomizer consisted of a sonotrode (Type UIP 250; Hielscher, Berlin, Germany), operating at a frequency of 25 kHz with a full power output of 250 W (amplitude 100%), placed vertically along the axis of the cavity and recessing 2 mm from the minor base of the cone cavity; the sonotrode was also surrounded by a thermostated reservoir containing the solution to be atomized.

Ten millilitres of chitosan solutions at different concentrations (1, 2 and 3% w/v) were prepared by dissolving the polymer in 1% (v/v) acetic acid; different amounts of theophylline were then added and the final solution was stirred to obtain a clear solution and then poured into the reservoir, kept at a temperature of 40°C. The solution was immediately atomized into microdroplets, which fell in a 5, 10 or 15% (w/v) TPP hardening solution (50 mL) at pH 5, magnetically stirred at a fixed rate of 250 rev min^{-1}. After a suitable cross-linking time to allow the ionic interaction between TPP ions and the amino groups of chitosan, the hardened microparticles were washed three times with a suitable solvent to remove non-encapsulated drug and finally freeze-dried for 24 h ($T = -40$°C and $P = 0.3$ mbar). All the experiments were performed in an air-conditioned room at 25°C and two batches for each formulation or set of parameters were produced.

Determination of viscosity

The viscosity determination was performed on 1, 2, 3 and 4% (w/v) chitosan solution with and without the drug. The measurements were carried out on about 5 g of each chitosan solution, which was placed in the small sample adapter of the concentric cylinder viscometer (Visco Star-R; Fungilab S.A., Barcelona, Spain). First the measures were performed at room temperature (25°C) and then at the temperature set for the reservoir surrounding the sonotrode (40°C). After some preliminary tests, the measuring element was selected (spindle number TR9) and the spindle rotating speed was stated at 200 or 100 rev min^{-1} for very viscous solutions, such as 4% (w/v) chitosan solutions.

Size exclusion chromatography – multi-angle laser light scattering (SEC-MALS) analysis

Chitosan molecular characterization was performed by a multi-angle laser light scattering (MALS) photometer on line to a size exclusion chromatography (SEC) system. The SEC-MALS system consisted of an Alliance chromatograph (degasser, pump and autoinjector) from Waters (Milford, MA, USA), a MALS Dawn DSP-F photometer from Wyatt (Santa Barbara, CA, USA) and a 410 differential refractometer from Waters as concentration detector. SEC experimental conditions for chitosan were obtained from the literature (Brugnerotto et al 2001). In detail, the experimental conditions were as follows: 0.3 M acetic acid + 0.2 M sodium acetate pH 4.5 as mobile phase, 35°C temperature, 0.8 mL min^{-1} flow rate and two SynChropak CATSEC (1000 and 100) columns from Micra (USA). The MALS photometer used a vertically polarized He-Ne laser ($\lambda = 632.8$ nm) and simultaneously measured the intensity of the scattered light at 15 angular locations ranging in aqueous solvent from 14.5° to 158.3°. The calibration constant was calculated using toluene as standard, assuming a Rayleigh Factor of 1.406×10^{-5} cm^{-1}. The angular normalization was performed by measuring the scattering intensity of a standard solution of poly(ethylene glycol) with a narrow molar mass distribution ($M = 12.6$ kg mol^{-1}, $D = 1.3$) in the mobile phase, assumed to act as an isotropic scatterer. The SEC-MALS system was described in detail elsewhere (Wyatt 1993; Mendichi & Giacometti Schieroni 2001). The differential refractive

index increment, $dn/dc = 0.190\,mL\,g^{-1}$, for chitosan with respect to the used solvent (acetate buffer pH 4.5) was obtained from literature (Brugnerotto et al 2001). The molecular mass of a 3% (w/v) chitosan solution in 1% (v/v) acetic acid containing the untreated (raw) polymer and of a solution at the same chitosan concentration processed by ultrasonic atomization were determined.

Fourier transform-infrared spectra (FT-IR) analysis

Infrared spectra of raw polymer and of chitosan processed by ultrasonic atomization were obtained by an IR spectrophotometer (Jasco FT-IR 200, Japan) using the KBr disc method. The samples were diluted with KBr and then compressed into a tablet, 10 mm in diameter and 3 mm in thickness, using a manual tablet presser (Perkin Elmer, Norwalk, USA) at $300\,kg\,cm^{-1}$ for 1 min.

Morphological characterization of the microparticles

The morphological characteristics of the final microparticles were observed by scanning electron microscopy (SEM). The samples were sputter-coated with Au/Pd using a vacuum evaporator (Edwards) and examined using a scanning electron microscope (Philips XL30) at 10 KV accelerating voltage.

The shape of microparticles was also observed using an optical microscope (Nikon SNZ-2T) connected through a camera (Panasonic GP KR 222) to an image acquisition system (CV 9000, FKV s.r.l. BG, Italy), to evaluate the influence of several parameters during the optimization of the process.

The size distribution of the microparticles was evaluated by sieve analysis, using a vibrating shaker (Octagon Digital; Endecotts, London, UK) and 5 standard sieves (Scientific Instruments s.r.l., Milano, Italy) of 50, 100, 200, 355 and 400 μm.

Actual drug content of the microparticles

The theophylline content was determined by dissolving about 25 mg of microparticles in 100 mL of pH 1.2 buffer magnetically stirred for 8 h. The solution was then analysed spetrophotometrically at 271.0 nm (UV2 Spectrometer; Unicam, Cambridge, UK). The data reported were the mean of five replicates ± the standard deviation (s.d.). The encapsulation efficiency (EE) was calculated from the following expression: $EE\% = (Wa/Wt) \times 100$, where Wa = actual content and Wt = theoretical content.

Evaluation of the in-vitro theophylline release from microparticles

In-vitro release tests were performed using the USP 24 paddle method (Pharmatest, Steinhein, Germany) rotating at $100\,rev\,min^{-1}$. Microparticles (30–50 mg) were suspended in 500 mL of pH 7.4 phosphate buffer as dissolution medium, thermostatted at a temperature of $37.0 \pm 0.1°C$. The amount of drug dissolved was analysed spetrophotometrically at 271.8 nm (UV2 Spectrometer; Unicam, Cambridge, UK). The results were the mean of five replicates ± s.d.

Statistical analysis

Statistical analysis of the effect of the chitosan concentration and of theophylline–chitosan ratio on the microparticles' actual content was performed using a two-way analysis of variance, while the effect of the TPP amount on the microparticles' actual content and of theophylline–chitosan ratio on the drug release were evaluated using a one-way analysis of variance. In all cases post-hoc comparisons of the means of individual groups were performed by the Student Newman Keuls test using the package SAS system (SAS Institute Inc., New York, NY). $P < 0.01$ denoted significance in all cases.

Results and Discussion

Evaluation of the ultrasonic atomization feasibility

To investigate the possibility of producing chitosan microparticles using the ultrasonic atomizer and to state the maximum concentration of chitosan which it was possible to nebulize, empty chitosan microspheres were first prepared by spraying different chitosan solutions (1, 2, 3 and 4% w/v), setting the reservoir at $T = 25°C$ and the viscosity of those polymeric solutions was also measured at the same temperature.

The results showed that 1% and 2% chitosan solutions were easily processed and the values of viscosity were $\eta = 100$ mPa s and $\eta = 400$ mPa s, respectively, while the 3% chitosan solution was quite difficult to spray ($\eta = 1100$ mPa s) and the 4% chitosan solution was not nebulized because of the too-high viscosity ($\eta = 3600$ mPa s). It is well known that an increase in temperature reduces the viscosity of a fluid; thus, all the chitosan solutions were then nebulized setting the reservoir at 40°C, because it was reported that a temperature $\leq 45°C$ has no influence on chitosan stability (Niederhofer & Müller 2004). The results revealed that both the 2% and 3% chitosan solutions were easily nebulized due to the lower viscosity values ($\eta = 200$ mPa s and $\eta = 600$ mPa s, respectively), while the viscosity of the 4% chitosan solution ($\eta = 1700$ mPa s) was still too high to be atomized. Moreover, as expected, the addition of different amounts of drug to the chitosan solutions did not modify the viscosity values. The atomization of such viscous chitosan solutions (up to 3% w/v), usually not processed employing the spray-drying process, is due to the mechanism involved in the ultrasonic atomization itself, called cavitation, which is more powerful than that of conventional nebulizers like rotary, pressure and two fluids (Killeen 1996).

Therefore, drug-loaded microparticles were prepared using 2% and 3% chitosan solutions.

Evaluation of the effect of ultrasonic atomization on chitosan

Once we had demonstrated the ability of ultrasound to atomize chitosan solutions, the next step was to investigate whether ultrasound could affect the chemical properties of the polymer. In fact, it was reported that ultrasonication could depolymerize biodegradable polymers, as the poly(lactic acid) (Park 1994), while no influence was observed by Bittner & Kissel (1999) on poly(lactide-co-glycolide).

The molecular characterization of raw chitosan solution and of the ultrasound-processed sample is expressed by the values of number (M_n), weight (M_w) and molar (M_z) average molecular mass and of the molecular mass distribution, called polydispersity index ($D = M_w/M_n$ or $D = M_z/M_w$). D is a measure of the polydispersity and it is = 1 for monodisperse polymers, while it is > 1 for etherodisperse systems. The results show that the raw sample solution had got $M_w = 109.3$, $M_n = 47.3$ and $M_z = 231.5$ ($g\,mol^{-1}$) and D values slightly higher than 2 ($M_w/M_n = 2.3$ and $M_z/M_w = 2.1$), indicating that chitosan is etherodisperse. The sonicated chitosan solution displayed lower values of molecular mass with respect to the untreated sample ($M_w = 89.1$, $M_n = 41.8$ and $M_z = 185.7$ ($g\,mol^{-1}$)), indicating that ultrasonic atomization caused a certain degradation, which is probably due to the main chain scission of the 1,4-glycosidic bond. On the other hand, D values of atomized chitosan remained unchanged ($M_w/M_n = 2.3$ and $M_z/M_w = 2.1$), with respect to the raw polymer.

Then the FT-IR analysis was performed to detect other possible chemical modifications after the ultrasonic atomization. The FT-IR spectra of the two chitosan samples are reported in Figure 1. Raw chitosan (Figure 1A) exhibited a broad peak at 3420 cm^{-1} attributed to the OH stretching with intermolecular hydrogen bonding.

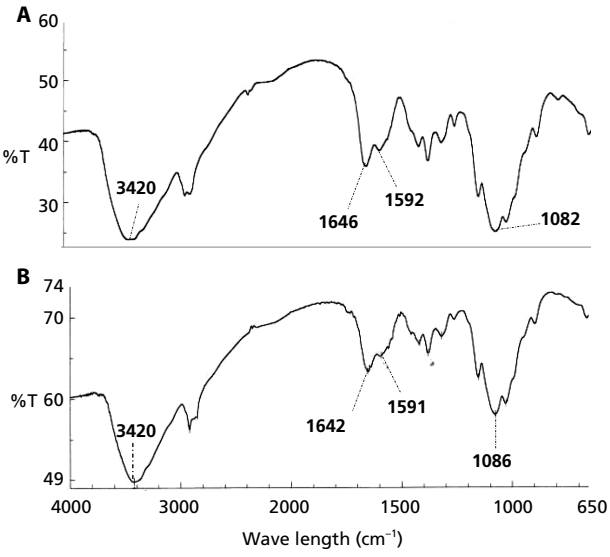


Figure 1 FT-IR spectra of raw polymer (A) and of sonicated chitosan (B).

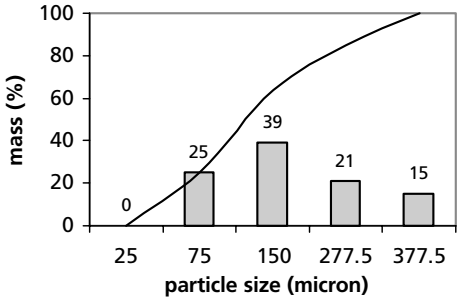
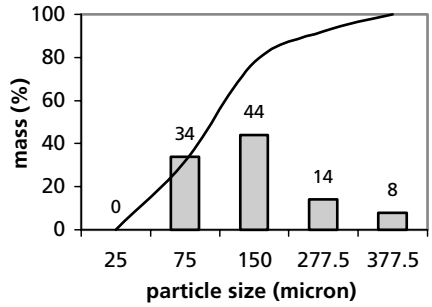
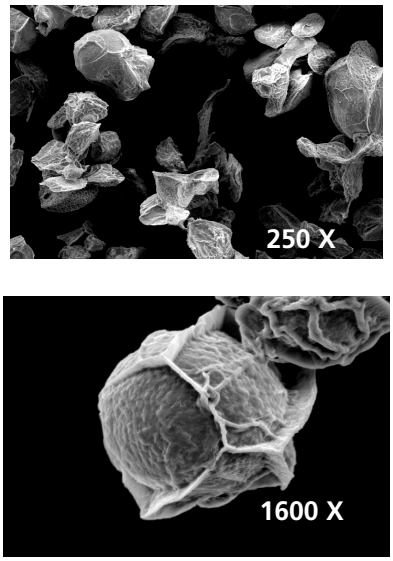
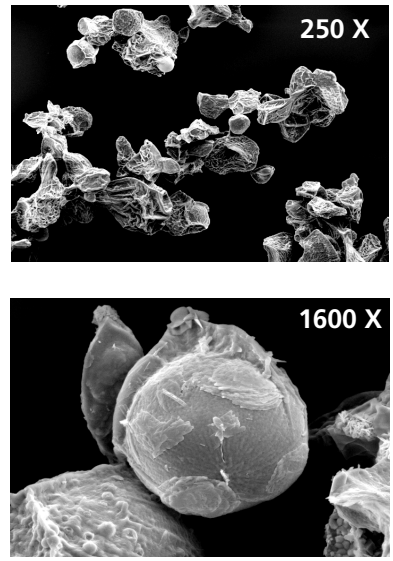
Absorption bands around 1640 cm^{-1} and 1590 cm^{-1} are assigned to CO stretching of the amide (*N*-acetyl-glucosamine) and to the NH_2 bending vibration of the glucosamine functional group, respectively, according to the literature (Nunthanid et al 2004). Finally, the characteristic polysaccharide band, due to the C-O-C stretching, was observed at 1080 cm^{-1} (Möller et al 2004). The absorption peaks of sonicated chitosan (Figure 1B) were comparable with those of raw chitosan. Therefore, the ultrasonic atomization did not appear to cause further chemical modifications, other than a certain depolymerization.

Influence of manufacturing parameters on the microparticle characteristics

Afterwards, to select the best operating parameters to obtain the microparticles, different process variables were considered: polymer solution feed rate, stirring rate of the cross-linking agent solution, linking time, washing medium and drying method; their effect on size and shape of microparticles was also evaluated. A feed rate within 4 and 5 mL min^{-1} was found suitable both for the atomization process and for the yield, whereas when the feed rate became higher, the atomization process was not possible and aggregates and indefinite droplets were formed. Therefore, to process 50 mL of chitosan solution (containing 1 g or 1.5 g of chitosan for 2% and 3% chitosan solution, respectively), about 10–12 min were required. As expected, the stirring rate of the cross-linking agent solution did not affect the size of the microparticles because their dimensions are determined by the size of the droplets formed during ultrasonic atomization. In fact, varying the stirring rate of the TPP solution, the final microparticle size was quite similar. However, stirring rate values higher than 750 rev min^{-1} formed microparticles stuck to each other. Therefore a stirring rate of 250 rev min^{-1} was set throughout. As regards the cross-linking time, the photographs shown in Figure 2 revealed that after 15 min the microparticles were aggregated and only a time longer than 45 min produced quite spherical and not aggregated microparticles. Also the washing medium played an important role during the manufacturing process, affecting the shape of the microparticles as shown in Figure 3. In fact chitosan is insoluble at a pH higher than 6.5 (Kas 1997) and it swelled when washed with de-ionised water, while it remained unmodified when washed with pH 7.4 phosphate buffer. Therefore, all the microparticles were prepared using 45 min of linking time and pH 7.4 as the washing medium. The last considered process parameter was the drying method: vacuum, forced-air oven and freeze-drying were tested; vacuum and forced-air oven produced particles aggregated to a substantial extent, while freeze-dried microparticles were not very aggregated, although a slight sticking effect on the bottom of the tray was still present.

Influence of formulation variables on the microparticle characteristics

The next step was to study the effect of some formulation variables on the microparticle characteristics (size,

Microparticle characteristics	Chitosan solution concentration 2% (w/v)	3% (w/v)
Size(μm)*		
Shape		
EE %	62.7	48.7

* Each value of microparticle size is the average obtained by the analysis of two processed batches.

Figure 2 Effect of different chitosan concentrations on theophylline-loaded microparticle characteristics (the chitosan–theophylline ratio was 1:1 and the TPP concentration was 10% w/v).

shape, encapsulation efficiency and drug release). At first, chitosan microparticles were prepared using different chitosan concentrations, fixing the drug-to-polymer ratio at 1:1, then different drug-to-polymer weight ratios were analysed and finally the effect of different TPP concentrations on the microparticles was studied.

The characteristics of theophylline-loaded microparticles prepared with 2% and 3% chitosan solution are reported in Figure 2. The microparticles' size distribution revealed that in both batches more than 60% of the microparticles were $< 200\,\mu$m; in particular, increasing the chitosan concentration, the mean particle size decreased, as was also observed in our previous study using the ultrasonic device (Albertini et al 2004). Moreover, SEM at low magnification showed that microparticles with varying irregular shapes were obtained in both formulations, indicating that the addition of the drug produced microparticles with a less spherical shape in comparison with unloaded microparticles (shown in Figure 3 with a cross-linking time ≥ 45 min). SEM at higher magnification was then performed to better evaluate their surface characteristics and the results revealed that the surface of both microparticles had some roughness and irregularities. Therefore, it appears that the concentration of chitosan did not affect the shape and the surface of the microparticles, while this parameter has an effect on the encapsulation efficiency (EE%, Figure 2); in fact, the 2% chitosan solution microparticles had an EE%

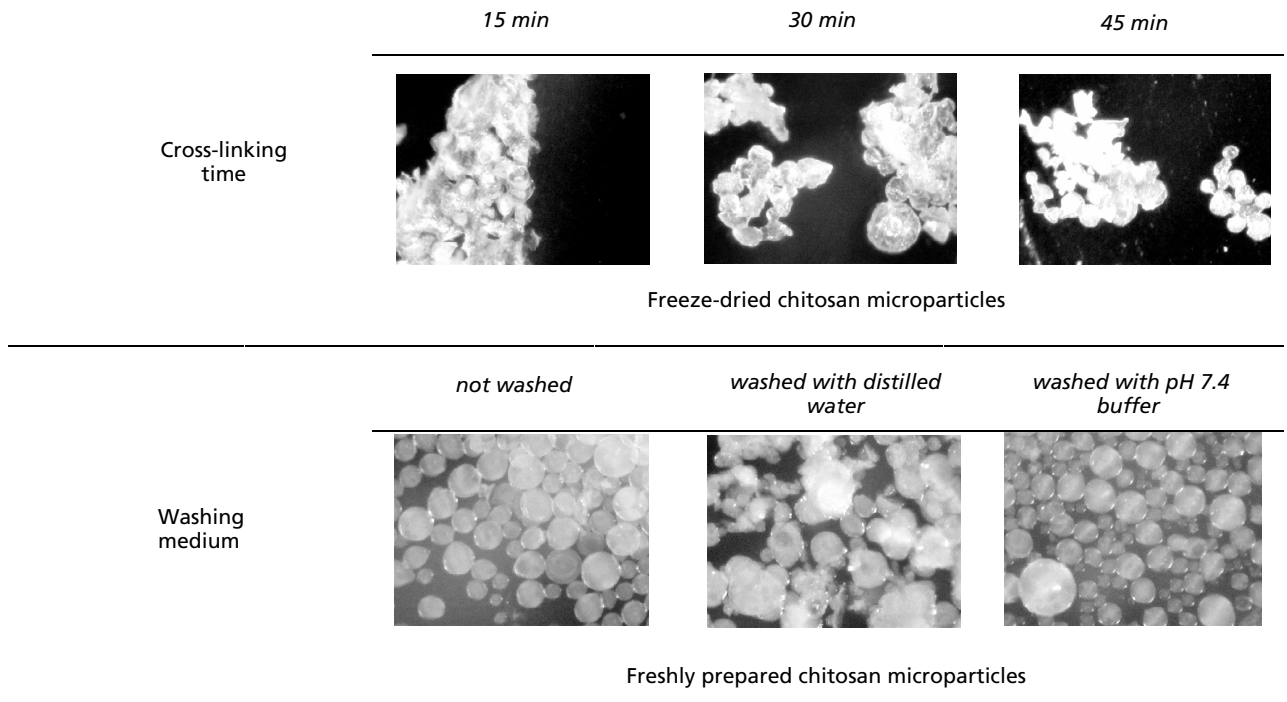


Figure 3 Influence of some process parameters on the shape of unloaded microparticles (magnification 200×).

significantly higher than that of the 3% chitosan solution microparticles ($P < 0.01$). Moreover, a significant difference in encapsulation efficiency was noticeable between microparticles prepared from 2% chitosan solution with a 1:1 drug–polymer ratio and 3% chitosan solution with 2:3 drug–polymer ratio (EE% = 62.7 and 60.1, respectively), which had similar theoretical drug loading. Figure 4 shows the influence of chitosan solution concentration on the theophylline release behaviour from TPP–chitosan microparticles. Both the microparticles with a 1:1 drug-to-polymer ratio exhibited an initial burst effect, which was about 30% for 3% chitosan solution and about 40% for 2%, probably due to the release of the theophylline close to the surface of the microparticles. Unfortunately, initial burst effect is a common phenomenon in chitosan-based delivery systems, in particular with water-soluble drugs (Ko et al 2002; Agnihotri & Aminabhavi 2004). However, after this burst effect, the release of theophylline from microparticles prepared from 3% chitosan solution was significantly lower until 36 h than the release of microparticles with 2% of the polymer ($P < 0.01$), whereas no significant difference ($P = 0.01$) was found at 48 h. Furthermore, microparticles with 2:3 drug-to-polymer weight ratio displayed both a release profile and a burst effect (Table 1) similar to those of microparticles prepared with the same chitosan concentration (theophylline–chitosan, 1:1). It is evident that the drug release is strongly influenced by the polymer amount, even when the drug loading is comparable. These profiles suggest that the release behaviour of the drug is correlated to the viscosity of the starting chitosan solution. It is probable that 3% chitosan solution microparticles comprised a very dense network structure of interpenetrating polymer chains cross-linked to each other by TPP counter-ions, resulting in a lesser permeability, swelling ability and, consequently, a decrease in the diffusion of theophylline from the chitosan matrix. Therefore, the 3% chitosan concentration was the most favourable to control the release of the drug.

Furthermore, the effect of the particle size on the release profiles of microparticles prepared with 3% chitosan solution was investigated. Generally, the microparticles produced using the ultrasonic device ranged between 50 and 400 μm and since the release profiles were not influenced by the microparticles' size (graph not shown), all the release tests were then performed using unsieved microparticles.

The second studied formulation parameter was the drug concentration. Figure 5 reports the dissolution profiles of 2% and 3% chitosan solution microparticles containing different drug-to-polymer weight ratios; the characteristics of these microparticles crosslinked by 10% (w/v) TPP are listed in Table 1. The release of theophylline from microparticles prepared from 2% (w/v) chitosan solution, shown in Figure 5A, revealed that a great amount of the drug (around 40%) was released in the first stage due to the burst effect; then, as expected, the release of drug increased as the drug loading increased. However, the statistical analysis showed that the release profiles of 1:1 theophylline–chitosan ratio and 3:2 theophylline–chitosan ratio were not significantly different, while they were significantly different to that of the 1:2 theophylline–chitosan ratio microparticles ($P < 0.01$).

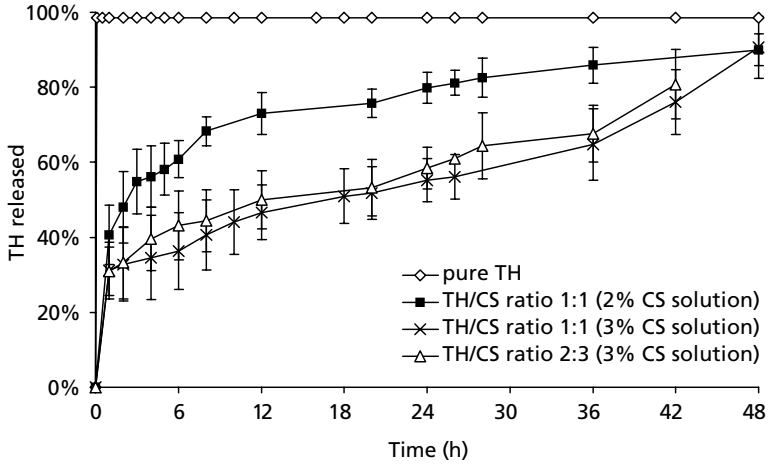


Figure 4 Influence of chitosan solution concentration on the in-vitro theophylline (TH) release profile from TPP–chitosan (CS) microparticles.

Table 1 Effect of the chitosan concentration and of theophylline–chitosan ratio on the characteristics of microparticles (the TPP concentration was 10% w/v)

Chitosan solution (%)	Theophylline–chitosan ratio	Theoretical content (%)	Actual content (%)	EE (%)	Burst effect (% in 1 h)
2	1:2	1.89	0.83 ± 0.06	43.9	36.9
	1:1	3.70	2.32 ± 0.09	62.7	40.5
	3:2	5.45	2.95 ± 0.10	54.1	44.6
3	1:3	1.85	0.86 ± 0.03	46.5	22.1
	2:3	3.63	2.18 ± 0.08	60.1	30.8
	1:1	5.36	2.61 ± 0.06	48.7	31.0

Table 1 shows that increasing the theophylline theoretical content resulted in a significant increase in the actual content ($P < 0.01$); however, it is evident that the EE% of microparticles with a 3:2 theophylline–chitosan ratio was significantly lower than that of microparticles with a 1:1 drug-to-polymer weight ratio (54.1 and 62.7, respectively), suggesting that in the first case the amount of drug was too high with respect to the polymer to be encapsulated. Therefore, this data indicates that microparticles prepared with 2% chitosan solution and with a 1:1 chitosan–theophylline ratio displayed the best performance. Figure 5B shows the release profiles of microparticles prepared from 3% chitosan solution; it was noticed that, increasing the drug amount, the release profiles showed the same trend shown in the previous graph. Anyway, it was found that microparticles prepared from 3% chitosan solution displayed the lower burst effect ($\leq 30\%$, depending on the drug loading) and the better controlled release.

The last parameter studied was the concentration of cross-linking agent (Table 2). An increase in the TPP concentration (from 5% to 15%) led to a significant decrease in drug loading efficiency ($P < 0.01$) (e.g., 5% TPP microparticles had an EE of 44%, while 15% TPP microparticles had an EE of 29.5%). Probably, when a concentration of 15% TPP is used, the extent of cross-linking of the chitosan–TPP matrix is too high to determine the lowering of the drug content.

Finally, as regards the effect of different TPP concentrations on the theophylline release behaviour from the chitosan microparticles, the results of the dissolution tests (not shown) provided evidence that this parameter did not significantly affect the release profiles of theophylline from the microparticles; thus, even the cross-linking agent at the lowest concentration is sufficient to control the drug release from these systems.

Conclusions

The results of this preliminary study showed the feasibility of the ultrasonic nebulization technique using high viscosity polymer solutions. Determination of the chitosan molecular mass showed that, after ultrasonic atomization, the polydispersity index (D) did not change and only a certain depolymerization of the longer macromolecular chains, at least at the highest concentration, was observed. FT-IR did not reveal any chemical modifications.

TPP cross-linked chitosan microparticles had prevalent dimensions between 50 and 200 μm, depending on the viscosity of the starting chitosan solution. Moreover, they displayed varying spherical shape and controlled release patterns (48 h), which could be modulated by varying the chitosan concentration. In conclusion, ultrasonic atomization could be proposed as a robust and an innovative single-step procedure with scale-up potential to successfully prepare ionically cross-linked chitosan microparticles, which could be orally administered by introducing them into enteric-coated capsules.

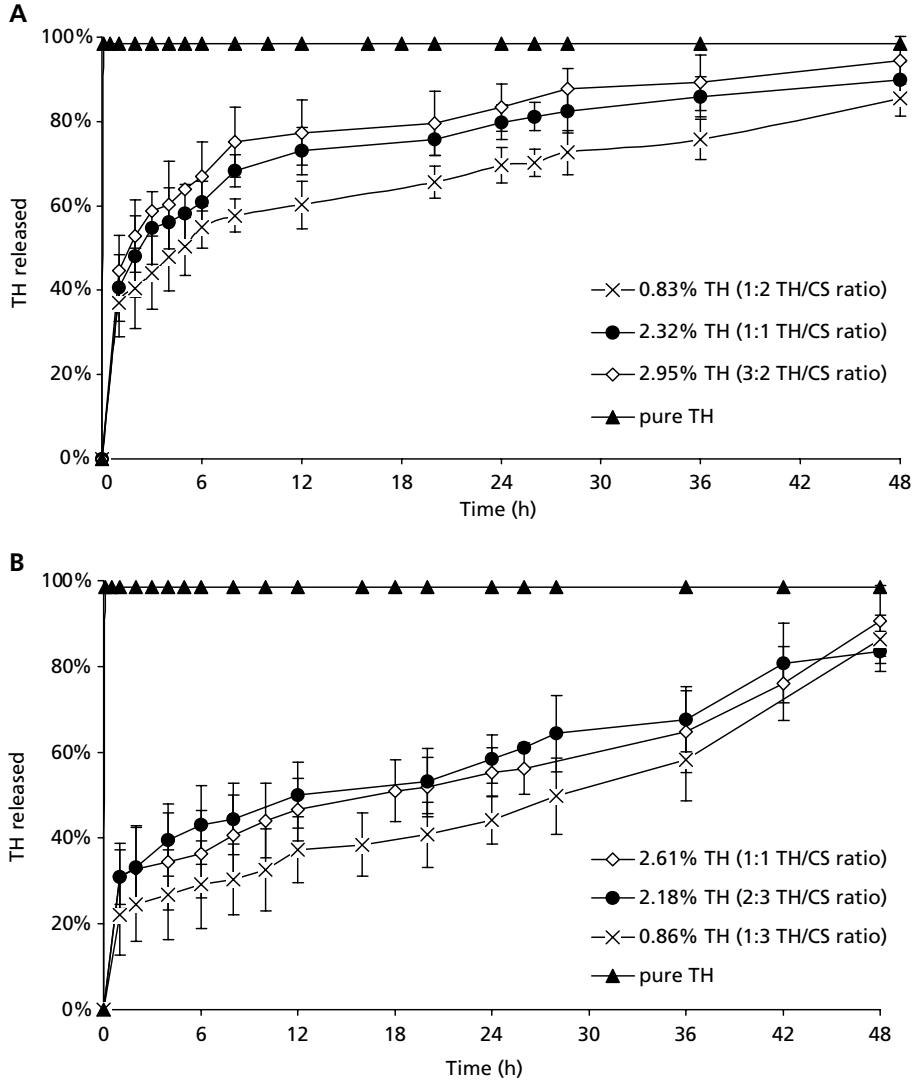


Figure 5 Influence of drug (theophylline, TH) loading on the in-vitro dissolution profiles of chitosan (CS) microparticles cross-linked by 10% of TPP, prepared from 2% chitosan solution (A) and 3% chitosan solution (B)

Table 2 Effect of the TPP amount on the characteristics of chitosan microparticles (theophylline–chitosan ratio 1:1)

Chitosan solution (%)	TPP (%)	Theoretical content (%)	Actual content (%)	EE (%)	Burst effect (% in 1 h)
3	5	9.68	4.23 ± 0.09	43.7	25.0
	10	5.36	2.61 ± 0.06	48.7	30.8
	15	3.70	1.09 ± 0.06	29.5	24.3

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