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Formation of self-organized nanoparticles by lecithin/chitosan ionic interaction

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Abstract

In this work the production of auto-assembled nanoparticles obtained by the mixing of chitosan and lecithin is presented. The size and surface charge of the nanoparticles were studied as function of the weight ratio between components, the viscosity of the polysaccharide and the pH of the colloidal suspension.

In order to elucidate the structure of nanoparticles, micro-FT-IR and elastic neutron scattering experiments have been performed. Results evidenced a strong electrostatic interaction between components and a structure that is neither that of homogeneous spheres nor of coated unilamellar vesicles. Preliminary encapsulation experiments with progesterone, as model lipophilic drug, showed good encapsulation efficiencies.

Keywords: Chitosan; Lecithin; Self-organizing structures; Nanoparticles

1. Introduction

Nanotechnologies provide innovations in various fields of medicine, namely therapy, diagnostics, imaging and drug delivery (Barratt, 2000; Freitas, 2005; Sonvico et al., 2005). In drug delivery, colloidal carriers have been proposed for effective administration of drugs having problems, such as toxicity, low bioavailability or poor water solubility. For these purposes, different colloidal drug delivery systems have been fabricated such as liposomes, micelles, nanoemulsions and nanoparticles. Polymeric or lipidic nanosystems in several cases improved drug bioavailability, modified pharmacokinetics or protected the encapsulated drug from enzymatic attack. Several liposomal drugs have been approved or are under evaluation for clinical application, in particular for cancer treatment (Torchilin, 2005). However, in many cases the application of colloidal drug carriers has been hindered by specific drawbacks, such as the limited stability in solution, the use of organic solvents, the difficulties in scaling up.

A great deal of attention has been directed to colloidal preparations obtained with from polysaccharides and lipids. These materials are considered biocompatible, biodegradable and safe. Chitosan has been used for the production of nanoparticles by ionotropic gelation with tripolyphosphate (Agnihotri et al., 2004; Janes et al., 2001). Additionally, this polysaccharide, has been investigated for stabilization of microemulsions, in which lecithin was one of the emulsifying agents (Alonso et al., 1997; Calvo et al., 1997). Lecithin is a lipid mixture of phospholipids that has been frequently used for liposome and micelle formation (Batzri and Korn, 1973; Betageri et al., 1993). Interestingly, the coating of these lipid-based nanostructures with chitosan has been found to increase their stability (Henriksen et al., 1994; Henriksen et al., 1997) and to provide them with mucoadhesive properties (Takeuchi et al., 2003). Recently, films and gels based on the interaction between negatively charged phospholipids and chitosan has been also proposed for the delivery of poorly soluble anticancer drugs (Grant et al., 2005; Ho et al., 2005).

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In this work, nanoparticles prepared by direct injection of soybean lecithin alcoholic solution into chitosan water solution are studied. The nanoparticles were obtained from the supramolecular self-organizing interaction of negative lipid material in presence of the positively charged polysaccharide. The aim of this study was to investigate the influence of several processing parameters on the physico-chemical and structural properties of the nanoparticles. The ability of these nanostructures to encapsulate a model lipophylic drug was also investigated. Progesterone was chosen as model drug due to its poor water solubility and low oral bioavailability.

2. Materials and methods

Seven different batches of chitosan (Chitoclear FG), provided by Primex (Haugesund, Norway), were used without further purification (specifications: deacetylation degree 92–99%; viscosity 8–715 cP at 1% (w/v) in acetic acid). Lecithin (Lipoid S45) was obtained from Lipoid AG (Ludwigshafen, Germany). Progesterone USP was kindly provided by IBSA Pharmaceutics (Lugano, Switzerland). Ethanol, acetic acid, acetonitrile, deuterated water and phosphotungstic acid were of analytical grade.

2.1. Nanoparticle preparation

Nanoparticle suspensions were obtained by injecting through a glass pipette (internal diameter 0.75 mm, injection rate 40 ml/min) 16 ml of a lecithin ethanol solution (25 mg/ml) into 184 ml of a chitosan solution mechanically stirred (Ultraturrax TP 18/10–10N, IKA Werke, Staufen, Germany). The chitosan solutions were prepared by diluting with distilled water appropriate volumes of 1% (w/v) chitosan solution in HCl 0.275N, in order to obtain after injection lecithin/chitosan ratios ranging from 5:1 to 80:1 (w/w). Chitosan used for these experiments had a viscosity of 93 cP, as determined by the supplier on a 1% solution (w/v) in acetic acid 1%, and a deacetylation degree of 95%. Batches of colloidal suspensions of lecithin, obtained by injection of the alcoholic solution in water without the polysaccharide, were produced for comparison.

Nanoparticles with a lecithin/chitosan weight ratio of 20:1 were prepared as described above, using chitosan batches having different viscosity ranging from 8 to 715 cP; these chitosan lots had a deacetylation degree higher than 92%.

2.2. Nanoparticle morphology

Nanoparticles were analysed with transmission electron microscopy (EM 208 S, Philips, Eindhoven, The Netherlands). The colloidal suspension was diluted tenfold with distilled water, an aliquot of 15 μl was then deposited on a Formvar coated grid (300 mesh, AGAR Scientific, Stansted, United Kingdom) and stained with a phosphotungstic acid solution 2% (w/v) for 20 s. The excess of reagents was removed by means of filter paper.

Atomic force microscopy (AFM) was used to study the surface morphology of the colloids produced. Nanoparticle suspension was tenfold diluted with distilled water and a drop was deposited on a mica thin layer fixed on a metallic magnetic support. The drop was dried overnight. The AFM images were collected in air with a Nanoscope III (Digital Instruments, Santa Barbara, USA) operating in tapping mode.

2.3. Nanoparticle size and surface charge

Colloidal suspensions produced were characterized for size using dynamic light scattering (ZetaPALS, Brookhaven Instruments Corp., Holtsville, USA). Having verified that size measurements did not change diluting with water or water/ethanol 92:8, samples were diluted with distilled and 0.45 μm filtered water in order to obtain a photon count of 50–150 kcts avoiding multiple scattering. Measurements were performed at 25 °C, collecting scattered light at 90° for 12 min. In the case of phase analysis light scattering samples were measured directly without dilution, allowing the instrument to automatically optimize signal intensity of the sample. Measurements were performed at 25 °C, collecting scattered light at 15° and repeated 10 times for each sample. The instrument software, applying Smoluchowski approximation, calculated the zeta potential of samples.

2.4. Micro-FT-IR analysis of nanoparticles

The micro-FT-IR spectra were collected at room temperature in the wave-number range 4000–650 cm^{-1} with a Jasco MFT-2000 apparatus (Jasco Inc., Tokyo, Japan), supported by Jasco FT software. For the spectra collection a drop of the nanoparticle suspension was placed on an aluminium foil, dried and analyzed in reflectance mode. The spectra of the single components, i.e. chitosan and lecithin, were obtained in transmittance mode by placing a small amount of the dry material under investigation on a KBr disc. In both cases the background was previously measured at a microscopic aperture size matching the sample size and set to the shape of a square with sides ranging from 50 to 100 μm. The number of scans was adjusted automatically as a function of sample concentration.

2.5. Elastic neutron scattering of nanoparticles

Elastic neutron scattering experiments on nanoparticles having 20:1 lecithin/chitosan weight ratio have been performed at the Institut Laue Langevin (Grenoble, France) on lyophilized nanoparticles suspension and on partially re-hydrated samples. Samples of the colloidal suspension of lecithin were also studied for comparison. Colloidal suspensions were lyophilized for 48 h (Modulyo, Edwards, Crawley, United Kingdom) and assayed for residual water content by Karl–Fisher titration (Titromatic 1S, Crison, Allela, Spain). Water content was $3.1 \pm 0.4\%$ for lyophilized suspensions containing lecithin/chitosan nanoparticles and $3.8 \pm 0.6\%$ for those containing lecithin. Freeze dried samples were then re-hydrated at room temperature, in a D_2O saturated atmosphere for 48 h approximately. The hydration level (weight D_2O/weight dry sample) was then 0.33 for lecithin/chitosan nanoparticles and 0.42 for lecithin samples. The incoherent elastic neutron scattering scans have been performed using the thermal backscattering spectrom-

eter IN13, that use thermal neutrons selected by a CaF_2 monochromator ($\lambda = 2.23$ Å, $E = 16.45$ meV). In the elastic scattering configuration adopted, the neutrons scattered by the sample are then energy-analyzed in backscattering geometry by a set of CaF_2 analyzers kept at the same temperature of the monochromator. The momentum transfer (Q) range covered by the analyzer configuration was 0.3–4.5 $Å^{-1}$ (see http://www.ill.fr/YellowBook/IN13/). The elastic scattering intensities were measured as a function of temperature from 20 to 340 K. They were corrected for empty cell contribution and normalized to the lowest temperature (20 K) data, where only a very small vibrational contribution is expected for the mean square atomic fluctuation. The mean square atomic fluctuations $\langle u^2 \rangle$ were calculated from the Q-dependence of elastic scattering intensity $S(Q, \omega = 0)$ in the Q-region ($Q \leq 2$ $Å^{-1}$) where the Gaussian approximation is valid:

$$S(Q, \omega = 0) \propto e^{-Q^2 \langle u^2 \rangle}$$

where ω is the energy transfer between neutrons and matter.

2.6. Nanoparticle density

Density measurements were performed using an Anton Paar high precision density meter (DMA 5000, Anton Paar GmbH, Austria). The instrument was calibrated using both air and degassed distilled water at 20, 40 and 60 °C. The particles density (d_p) was calculated with the following equation:

$$\frac{1}{d_p} = \frac{1}{C_w}\left(\frac{1}{d_{sp}} - \frac{1 - C_w}{d_{sv}}\right)$$

having determined the solvent density (d_{sv}), the suspension density (d_{sp}) and the concentration (w/w) of the nanoparticles (C_w).

2.7. Progesterone-loaded nanoparticles

Progesterone (PG) was entrapped within nanoparticles consisting of a lecithin/chitosan weight ratio of 20:1 using the technique described above and dissolving PG in the lecithin ethanol solution. The nanoparticle suspension was centrifuged at 5000 rpm (~2200 × g) for 10 min in order to separate the PG crystals that could have been formed in the preparation process. The sediment was dissolved in ethanol and the amount of progesterone assayed by HPLC for PG content ($\lambda = 241$ nm, acetonitrile/water 62:38 (v/v), Novapack® C18, 150 mm × 3.9 mm, Waters, Vimodrone, Italy). The suspension was further ultracentrifuged at 43,000 rpm for 90 min in order to separate nanoparticles (~120,000 × g). The supernatant was recovered and assayed for the dissolved PG. The sediment containing progesterone-loaded nanoparticles was freeze-dried and then, accurately weighted. The loaded nanoparticles were then dispersed in ethanol and sonicated 20 min before PG assay by HPLC. For each batch the encapsulation efficiency (amount of progesterone encapsulated/total amount of progesterone × 100) and drug loading (weight of progesterone encapsulated/weight of dry nanoparticles × 100) were determined. The formation yield of nanoparticles was measured as percentage ratio between recovered nanoparticles and total weight of solid components employed in their manufacturing.

3. Results and discussion

The injection of alcoholic solutions of appropriate combinations of phospholipids in water is a well-established method for liposome preparation (Batzri and Korn, 1973). In the present study, the injection of an alcoholic lecithin solution into an aqueous chitosan solution led to the formation of nanoparticles rather than liposomes. More specifically, these nanoparticles could be obtained by combining a lecithin alcoholic solution 25 mg/ml with chitosan aqueous solutions in the range from 0.025 to 0.4 mg/ml.

Indeed, the colloidal particles produced in the same conditions by injection of the lecithin alcoholic solution in water was characterized by small size (72.6 ± 1.5 nm) and high negative surface charge (−52.6 ± 0.7 mV). Thus, we investigated the physico-chemical characteristics of the nanostructures obtained by mixing lecithin and chitosan solutions. Preliminary experiments showed that the speed and type of mixing had a small effect on particle size distribution of these colloidal systems (data not shown). On the contrary, the size and surface charge of the nanoparticles obtained were dependent on the content of chitosan in the aqueous solution. Fig. 1 illustrates the variation of the size and zeta potential of the nanoparticles depending on the ratio of lecithin and chitosan in the preparation. The graph shows three different zones delimiting evident modifications of the obtained particle characteristics in dependence on the two components' weight ratio. Mixing lecithin/chitosan in ratios from 5:1 to 20:1, the obtained colloidal particles had positive surface charge (about 40 mV); the size was lower than 280 nm and size distribution was narrow, having a polydispersity index below 0.2. When lecithin and chitosan were mixed in the weight ratio interval from 30:1 to 50:1, the formation of aggregates a few minutes after the production of the colloidal suspension was observed. This aggregation was very evident especially in preparations having 30:1 and 40:1 component ratios. A quick sedimentation of particles larger than 3 μm was observed. We

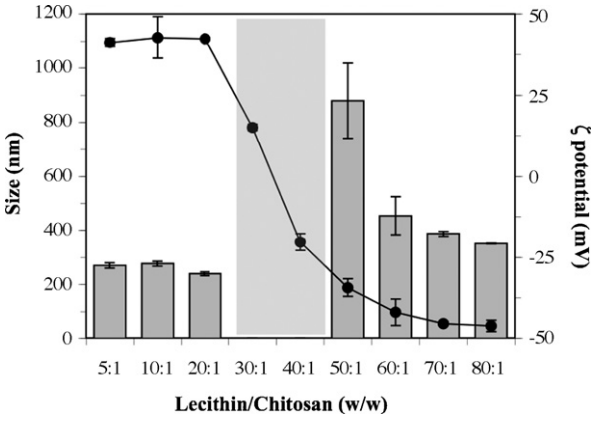

Fig. 1. Nanoparticle size (bars) and surface charge (circles) for various lecithin–chitosan (w/w) ratios (mean ± S.D., $n = 3$). The gray zone corresponds to weight ratios for which aggregates were obtained instead of colloidal suspensions.

considered that the aggregation probably occurred because of a lack of electrostatic stabilization of the nanostructures. In fact, a progressive inversion of surface charge of particles from positive to negative was measured in correspondence on the composition values of these two formulations. For the component weight ratio 50:1, the aggregation was less relevant, but particles had a wide size distribution and a mean volume diameter around 1 μm. Further reducing the chitosan content, as in the case of lecithin/chitosan weight ratios from 60:1 to 80:1, colloidal particles with negative surface charge (about −42 mV), size around 400 nm and higher polydispersion index (≥0.231) were produced.

The behavior of size and stability of these colloidal particulate systems was determined by the net charge on the particle surface. When the zeta potential was largely positive or negative, the size of particles was sub-micron and the colloidal suspension was stable. On the contrary, in the composition region where the particle charge was close to neutrality, the colloidal suspension settled due the increased in size of aggregated particles. From these results, the system under study can be described as a particulate stabilized structure obtained by electrostatic interaction between the negative lecithin and the positive chitosan. When the polysaccharide is enough, the particle surface is positive; on the contrary, small amounts of chitosan are not sufficient to completely neutralize the negative charges present in the lipid structure. Interestingly, the production of nanoparticles starting from pure phosphatidylcoline, the zwitterionic main component of soybean lecithin, was not feasible; this confirmed that the presence of a net negative charge in the lipid mixture was requested for obtaining the assembling with chitosan by electrostatic interaction. It was reported that lecithin contains lipids, such as phosphatidic acid, giving rise to a net negative charge to the colloidal particles (Alonso et al., 1997).

Because of their size slightly above 200 nm and of their positive surface charge, nanoparticles produced with a lecithin/chitosan ratio of 20:1 were selected for the characterization and development of the colloidal system. The analysis of this system by transmission electron microscopy showed homogeneous spherical objects with a size between 200 and 300 nm characterized by a compact core surrounded by a transparent

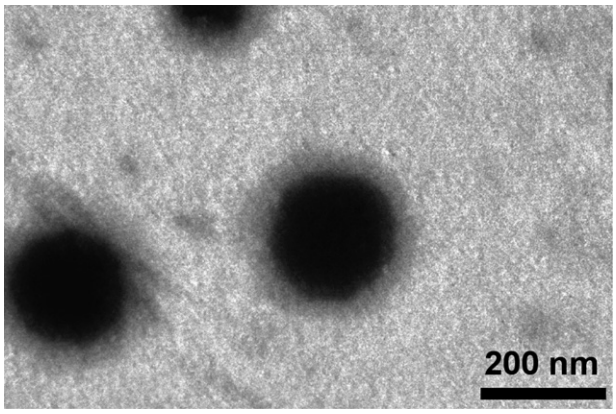

Fig. 2. Transmission electron micrographs of lecithin/chitosan nanoparticles (weight ratio 20:1).

corona (Fig. 2). Further investigation of their morphology using atomic force microscopy confirmed their size and shape (Fig. 3). In the AFM scan of the nanoparticle sample, the presence of spherical structures of various sizes ranging from 200 to 500 nm was recorded. In addition, almost no aggregation of the sample seemed to have occurred during the drying of the suspension. The comparison of these results with those obtained by the samples produced with lecithin alone, evidenced dimensional and morphological differences. In fact, in the case of lecithin samples AFM pictures showed smaller and almost flat structures, as typically described for vescicles during AFM tapping mode determination (Ruozi et al., 2005); on the contrary, the lecithin/chitosan nanosystems exhibited well defined and projecting round shapes (Fig. 3).

We performed a study of size and surface charge of particles obtained by using chitosan batches having different molecular weights and, accordingly, different viscosity. These results are presented in Fig. 4. While the charge of particles produced remained almost constant for all samples, a quasi-linear increase of the size of the particles versus chitosan viscosity was found. In these systems, the influence of the molecular weight of the polysaccharide on nanoparticles size suggested that the polymer was present on their surface, because longer hydrophilic polymer

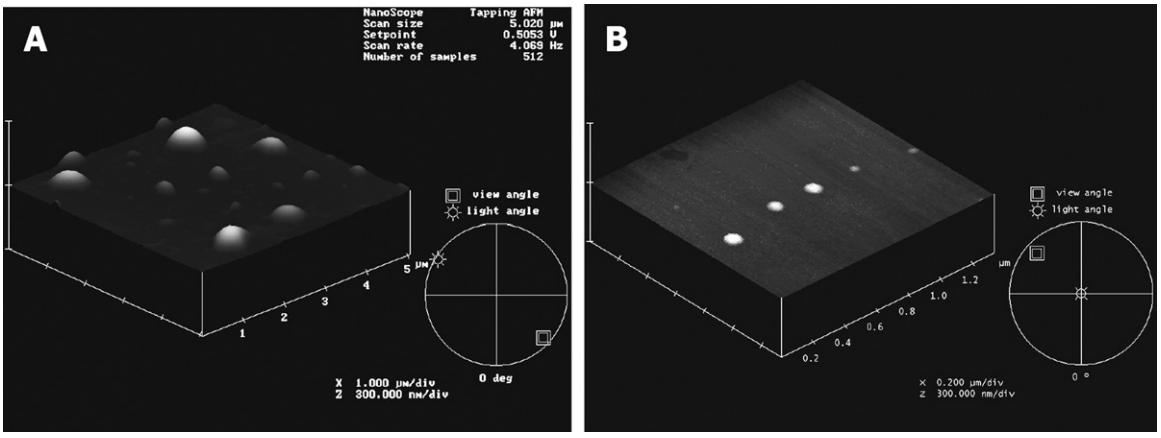

Fig. 3. Atomic force microscopy images of lecithin/chitosan nanoparticles (weight ratio 20:1) (A) and of lecithin vesicles (B).

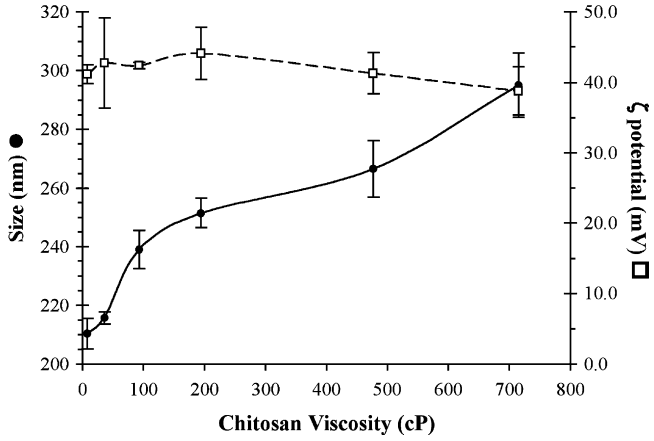

Fig. 4. Size and surface charge of lecithin/chitosan nanoparticles (weight ratio 20:1) as function of the viscosity of the chitosan (mean ± S.D., $n = 3$).

chains are able to extend farther from the surface, influencing the hydrodynamic radius.

We also studied the dependence of particles characteristics from the pH value of the suspension. The results are displayed in Fig. 5. Increasing the suspension pH from 2.5 to 5.0, the surface charge of the particles slightly decreased. Further increasing the value of pH above 5.0 the zeta potential dropped rapidly. In parallel, an increase of particles size of the sample until aggregation at pH values over 5.0 was evidenced. These results confirmed that the stabilization of particles was of electrostatic nature and that the polysaccharide component defined the particle surface properties. In fact, the chitosan pK_a value around 6.5 explained why at pH values near 6.0 the surface charge of the colloidal system approached neutrality.

In order to disclose the structure of these lipid/polysaccharide nanoparticles, the interactions between the polysaccharide and the lipid have been studied by micro-FT-IR and quasi-elastic neutron scattering. The micro-FT-IR spectra of nanoparticles and of the components lecithin and chitosan alone are reproduced in Fig. 6. The infrared spectrum of lyophilized nanoparticles shows the typical absorption bands of lecithin, the main component of the nanosystem. In fact, in the nanoparticle spectrum the characteristic band of chitosan at 1590 cm^{-1},

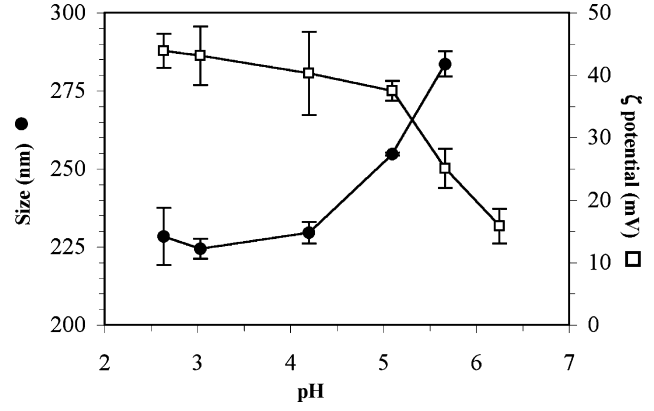

Fig. 5. Size and surface charge of lecithin/chitosan nanoparticles 20:1 as function of the pH of the suspension (mean ± S.D., $n = 3$).

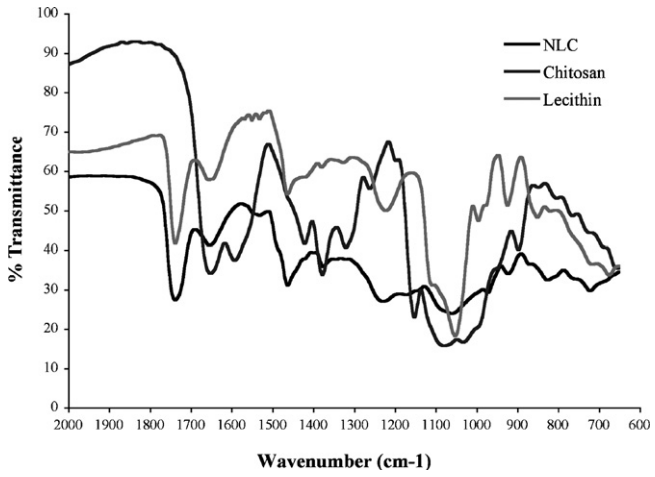

Fig. 6. Micro-FT-IR spectra of lecithin/chitosan nanoparticles and of the two components separately.

corresponding to the NH_2 scissoring vibration of primary amino group, was not present. This event was probably due to the formation in acidic medium of chitosan ionized form and to the ionic interaction of the ammonia groups of chitosan with the phosphate groups of lecithin. On the contrary, the typical absorption peak of the chitosan amino group was still present at 1660 cm^{-1}. Furthermore, in the nanoparticle spectrum the intensity of the absorption band at 1738 cm^{-1}, due to the stretching of the carbonyl groups of the fatty acids, decreased with respect to lecithin alone and the absorption band of the phosphate group shifted from 1236 cm^{-1} in the lecithin to 1217 cm^{-1} in the nanoparticles sample. This was indicative to the fact that ionic interactions between phosphate groups of lecithin and amino groups of chitosan have taken place.

The hints about the two component interactions have been confirmed by elastic neutron scattering. Briefly, it was possible to determine the mean square fluctuations of hydrogen atoms of lecithin, which is the main component of the nanoparticles, with or without chitosan over a broad range of temperatures (20–340 K). A full interpretation of the data set has been published elsewhere (Sonvico et al., in press). The neutron scattering signal was dominated by the hydrogens of the lipid component (lecithin), being the contribution due to chitosan two orders of magnitude smaller. The signal from hydration water was also negligible because of the low scattering cross-section of deuterium with respect to hydrogen: it was about 2% of the total intensity for the sample at the highest hydration. The same analysis has been performed on a lecithin sample. The calculated mean square fluctuations, $\langle u^2 \rangle$, are reported in Fig. 7. The temperature dependence of $\langle u^2 \rangle$ was qualitatively similar to that observed in most biopolymers: it showed a rapid increase above ~220 K that corresponded to a kinetic transition similar to the glass-transition observed in amorphous systems. Below the transition $\langle u^2 \rangle$ behaved according to a quasi-harmonic dynamics almost hydration-independent. The strong increase of $\langle u^2 \rangle$ above the transition was generally attributed to the onset of fast conformational transitions of the lipid-chain groups (Deriu and Natali, 2002). This behavior was qualitatively

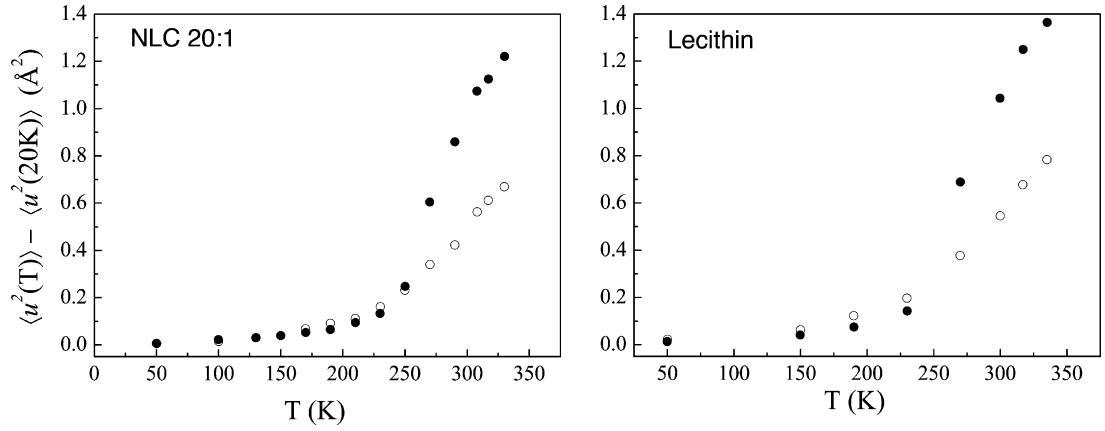

Fig. 7. Mean square fluctuations $\langle u^2\rangle$ of hydrogen atoms determined for a wide range of temperatures for dry (●) and D_2O re-hydrated samples (◯) of lecithin/chitosan nanoparticles (NLC 20:1, left) and lecithin alone (right). Mean square fluctuations $\langle u^2\rangle$ values are subtracted with the value registered at 20 K.

Table 1
Encapsulation efficiency, drug loading and amount of drug precipitated for progesterone-loaded lecithin/chitosan nanoparticles (mean ± S.D., $n = 3$)

Total progesterone (mg/100 ml)	Encapsulation efficiency (%)	Drug loading (%)	Precipitated drug (mg/100 ml)
2	57.2 ± 0.1	0.7 ± 0.1	0.18 ± 0.03
5	55.1 ± 2.2	1.5 ± 0.2	0.36 ± 0.20
10	62.4 ± 5.3	3.3 ± 0.5	0.70 ± 0.16
13	43.2 ± 15.9	3.0 ± 0.2	4.12 ± 0.37
15	44.9 ± 16.4	3.6 ± 1.0	5.03 ± 4.03
20	18.2 ± 1.1	2.1 ± 0.2	14.18 ± 0.44

similar for both samples. However, it was of interest to compare the behavior of the nanoparticle systems with that of the pure lecithin component. The lecithin sample showed mean square fluctuations slightly larger than those of the nanoparticles either for dry and re-hydrated samples. The effect of the chitosan was therefore to reduce the mean square fluctuation $\langle u^2\rangle$ with respect of that of the pure lipid. This result was not unexpected. It is known that a saccharide coating can significantly reduce the mean square fluctuations in polipeptides leading to a quasi-harmonic behavior almost up to room temperature (Cordone et al., 1999). In our nanoparticles we observed a similar effect: the presence of a chitosan layer stabilized the nanostructure reducing the thermally induced atomic mean square fluctuations of the lipid component. The strong interaction occurring between the two materials was also demonstrated by the difference showed between the NLC and lecithin alone when the colloidal particulate density was calculated. In fact, a density of 0.873 g/cm^3 was determined for samples containing lecithin alone, while 1.175 g/cm^3 was the density value found for lecithin/chitosan nanoparticles. These results suggested that the self-organization of the two components led to a more packed and denser structure and not to the simple coating of lecithin vescicles. Furthermore, small angle neutron scattering data collected for NCL suspensions, still under interpretation in order to disclose further information about the particle structure, had in any case excluded simple structures as homogeneous spheres or polysaccharide coated unilamellar liposomes.

Preliminary experiments the encapsulation of hydrophilic drugs showed that encapsulation of metoclopramide HCl in lecithin/chitosan nanoparticles was nearly impossible, with efficiencies below 1% (Cagnani et al., 2004). We studied also the encapsulation of progesterone, as model lipophilic drug. The encapsulation efficiency and the drug loading determined for lecithin/chitosan nanoparticles produced using increasing amounts of drug are presented in Table 1. Up to 10 mg of drug in 100 ml of suspension, the efficiency of encapsulation was maximized (around 60%) and the drug loading increased reaching a value around 3.5%. For higher quantities of drug a higher variability in results was found along with the progressive increase of the quantity of progesterone precipitated during nanoparticles production. The total amount of PG precipitated, PG dissolved and PG encapsulated in nanoparticles was 99.3 ± 5.8% of the amount of drug used for nanoparticle preparation. The overall formation yield for progesterone-loaded nanoparticles was 59 ± 11%.

4. Conclusions

In this work a novel nanoparticulate carrier, constituted of lecithin and chitosan and produced without preliminary vesicle formation, was presented. The nanoparticle surface properties were characterized by a charge moving from positive to negative in dependence on the decrease of the cationic polysaccaride content. When the surface charge was close to the neutrality the nanoparticles aggregated. The nanoparticles obtained can be considered as a self-organized structure, result of the electrostatic interaction of the polycation chitosan and lecithin, due to the presence of negatively charged components in the lipid mixture. With regard to the structure of the system, neutron scattering experiments evidenced a strong interaction between

components and preliminary structure elucidation excluded homogeneous particles, as well as, simply coated unilamellar vesicles. Concerning drug encapsulation, progesterone was encapsulated with a good efficiency, while, on the contrary, poor drug loadings were obtained using water-soluble drugs.

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References

Agnihotri, S.A., Mallikarjuna, N.N., Aminabhavi, T.M., 2004. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. J. Control. Rel. 100, 5–28.

Alonso, M.J., Calvo, P., Remunan, C., Vila-Jato, J.L., 1997. Stabilization of colloidal systems by the formation of ionic lipid-polysaccharide complexes. EU Patent EP0771566(A1).

Barratt, G.M., 2000. Therapeutic applications of colloidal drug carriers. Pharm. Sci. Technol. Today 3, 163–171.

Batzri, S., Korn, E.D., 1973. Single bilayer liposomes prepared without sonication. Biochim. Biophys. Acta 298, 1015–1019.

Betageri, G.V., Jenkins, S.A., Parsons, D.L., 1993. Liposome drug delivery systems. Technomic Publishing Company, Lancaster PA, USA.

Cagnani, A., Sonvico, F., Colombo, P., Bettini, R., Alonso, M.J., Colombo, G., Rossi, A., 2004. Chimerical agglomerates of lecithin/chitosan nanoparticles for powder nasal delivery. AAPS J. 6, 1190.

Calvo, P., Remunan-Lopez, C., Vila-Jato, J.L., Alonso, M.J., 1997. Novel hydrophilic chitosan-polyethylene oxide nanoparticles as protein carrier. J. Appl. Polym. Sci. 63, 125–132.

Cordone, L., Ferrand, M., Vitrano, E., Zaccai, G., 1999. Harmonic behavior of trehalose-coated carbon-monoxy-myoglobin at high temperature. Biophys. J. 76, 1043–1047.

Deriu, A., Natali, F., 2002. Recent neutron investigation on biomolecular dynamics: from model systems towards complex macromolecular machines. J. Neutron Res. 10, 115–122.

Freitas Jr., R.A., 2005. What is nanomedicine? Nanomedicine 51, 325–341.

Grant, J., Blicker, M., Piquette-Miller, M., Allen, C., 2005. Hybrid films from blends of chitosan and egg phosphatidylcholine for localized delivery of paclitaxel. J. Pharm. Sci. 94, 1512–1527.

Henriksen, I., Smistad, G., Karlsen, J., 1994. Interactions between liposomes and chitosan. Int. J. Pharm. 101, 227–236.

Henriksen, I., Vagen, S.R., Sande, S.A., Smistad, G., Karlsen, J., 1997. Interactions between liposomes and chitosan. II. Effect of selected parameters on aggregation and leakage. Int. J. Pharm. 146, 193–204.

Ho, E.A., Vassileva, V., Allen, C., Piquette-Miller, M., 2005. In vitro and in vivo characterization of a novel biocompatible polymer-lipid implant system for the sustained delivery of paclitaxel. J. Control. Rel. 104, 181–191.

Janes, K.A., Calvo, P., Alonso, M.J., 2001. Polysaccharide colloidal particles as delivery systems for macromolecules. Adv. Drug Deliv. Rev. 47, 83–97.

Ruozi, B., Tosi, G., Forni, F., Fresta, M., Vandelli, M.A., 2005. Atomic force microscopy and photon correlation spectroscopy: two techniques for rapid characterization of liposomes. Eur. J. Pharm. Sci. 25, 81–89.

Sonvico, F., Di Bari, M.T., Bove, L., Deriu, A., Cavatorta, F., Albanese, G., in press. Mean square hydrogen fluctuations in chitosan/lecithin nanoparticles from elastic neutron scattering experiments. Physica B.

Sonvico, F., Dubernet, C., Colombo, P., Couvreur, P., 2005. Metallic colloid nanotechnology, applications in diagnosis and therapeutics. Curr. Pharm. Des. 11, 2095–2105.

Takeuchi, H., Matsui, Y., Yamamoto, H., Kawashima, Y., 2003. Mucoadhesive properties of carbopol or chitosan-coated liposomes and their effectiveness in the oral administration of calcitonin to rats. J. Control. Rel. 86, 235–242.

Torchilin, V.P., 2005. Recent advances with liposomes as pharmaceutical carriers. Nat. Rev. Drug Discov. 4, 145–160.