LECITHIN-CHITOSAN SELF-ORGANIZING NANOPARTICLES AS DRUG CARRIERS FOR LIPOPHILIC DRUGS

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Introduction

Nanotechnologies provide innovations in various fields of medicine, namely diagnostics, imaging and drug delivery (Barratt 2000; Freitas 2005; Sonvico 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. Even if some liposomal drugs have been approved or are under evaluation for clinical application, in particular for cancer treatment (Torchilin, 2005), in most of 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.
Recently, a great deal of attention has been directed to colloidal preparations obtained with polysaccharides and lipids. These materials are considered biocompatible, biodegradable and safe. In a previous work, nanoparticles prepared by direct injection of soybean lecithin alcoholic solution into water solutions containing different concentrations of chitosan were studied (Cagnani, et al., 2004). The aim of this study was to investigate the physico-chemical characteristics of chitosan/lecithin nanoparticles prepared using chitosan with various molecular weights (MW) and to optimize progesterone loading in these vectors in view of transmucosal drug delivery.

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 1% v/v). Lecithin (Lipoid S45) was obtained from Lipoid AG (Ludwigshafen, Germany). The isopropylmyristate used for nanoparticle preparation was furnished by ACEF s.p.a. (Fiorenzuola d'Arda, Italy), with purity superior to 95%. The progesterone was provided by FLUKA (Buchs, Switzerland).

Nanoparticle preparation
Nanoparticle suspensions were obtained by injecting 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). Chitosan solutions were prepared by diluting with distilled water appropriate volumes of 1% w/v chitosan solution in HCl 0.275 N, in order to obtain after injection lecithin/chitosan weight ratio 20:1. Nanoparticles were prepared using chitosan batches having different viscosity ranging from 8 to 715 cP. Batches of colloidal suspensions, obtained by injection of the lecithin alcoholic solution in water without the polysaccharide, were produced for comparison.

Nanoparticle characterization
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.
Colloidal suspensions 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 minutes. In the case of Phase Analysis Light Scattering (ZetaPALS, Brookhaven Instruments Corp., Holtsville, USA) 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.
Progesterone (PG) was entrapped within nanoparticles consisting of a lecithin/chitosan weight ratio of 20:1 dissolving PG in the lecithin ethanol solution (2-20 mg / 100 ml colloidal suspension). The nanoparticle suspension was centrifuged at 5,000 rpm (~2,200 x g) for 10 minute 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 (l=241 nm, acetonitrile/water 62:38 v/v, Novapack® C18, 150 x 3.9 mm, Waters, Vimodrone, Italy). The suspension was further ultra-centrifuged at 43,000 rpm for 90 minutes in order to separate nanoparticles (~120,000 x 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 minutes before PG assay by HPLC. For each batch the encapsulation efficiency (amount of progesterone encapsulated/total amount of progesterone x 100) was determined.
In order to optimize progesterone incapsulation, a batch of nanoparticles having lecithin/chitosan 20:1 w/w ratio was produced by adding to the ethanolic solution progesterone (final concentrations 20 mg/ 100 ml of colloidal suspension) and isopropyl myristate (IPM, final concentration 5 mM). The amount of progesterone loaded in nanoparticles was not determined directly on nanoparticles after ultracentrifugation, because when IPM is present particles tend to cream, due to decreased density and could not be recovered. Progesterone encapsulated was then determined indirectly as the difference between the total amount of progesterone in the colloidal suspension and the sum of the progesterone in solution and precipitated.

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). The injection of alcoholic lecithin solution into aqueous chitosan solution led to the formation of nanoparticles instead of liposomes. Indeed, the colloidal particles produced in same conditions by injection of the lecithin alcoholic solution in water were characterized by small size (72.6 ± 1.5 nm) and high negative surface charge (-52.6 ± 0.7 mV). 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 Figure 1. While the charge of particles produced remained positive and almost constant for all samples (around 40 mV), a steady increase of the size of the particles versus chitosan viscosity was found. In these systems, the effect of molecular weight of polysaccharide on nanoparticle size suggested that the polymer was present on their surface; in fact, longer hydrophilic polymer chains extend farther from the surface, influencing the hydrodynamic radius.

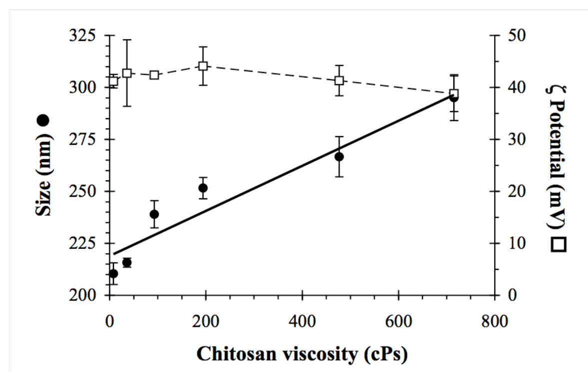

Figure 1 – Lecithin/chitosan nanoparticle size and surface charge versus chitosan viscosity

In the AFM scan of nanoparticle sample, the presence of spherical structures of various sizes ranging from 200 to 500 nm was recorded. The comparison of these pictures 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 nanoparticles exhibited well-defined and projecting round shapes (Fig. 2).

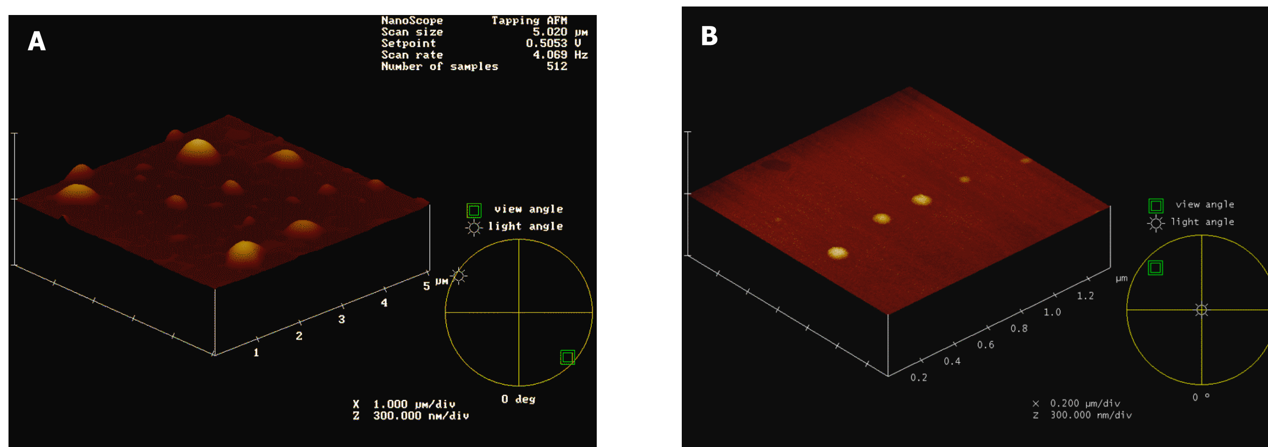

Figure 2 – Atomic Force Microscopy images of lecithin/chitosan nanoparticles (A) and of lecithin vescicles (B).

Preliminary experiments on encapsulation of metoclopramide HCl in lecithin/chitosan nanoparticles showed efficiencies below 1% (Cagnani, et al., 2004). We studied the encapsulation of progesterone, as model lipophilic drug. The encapsulation efficiency and the drug loading for lecithin/chitosan nanoparticles produced using increasing amounts of drug and by adding isopropyl miristate are presented in Table 1. Up to 10 mg of drug in 100 ml of suspension, the efficiency of encapsulation was maximized (around 60%). For higher quantities of drug, a higher variability in results was found along with the progressive increase of the quantity of progesterone precipitated during nanoparticle production. This inconvenience was counteracted by the use in the preparation of a small amount of isopropyl myristate that

Total Progesterone (mg/100 ml)	Encapsulation Efficiency (%)	Precipitated drug (%)
2	57.2 ± 0.1	9.0 ± 1.5
5	55.1 ± 2.2	7.2 ± 4.0
10	62.4 ± 5.3	7.0 ± 1.6
15	44.9 ± 16.4	33.5 ± 26.9
20	18.2 ± 1.1	70.9 ± 2.2
20 + IPM	82.9 ± 3.2	2.0 ± 0.2

Table 1 - Encapsulation efficiency for progesterone loaded lecithin/chitosan nanoparticles (mean ± SD, n=3)

Conclusions

The nanoparticles obtained can be considered as a self-organized structure, resulting from the electrostatic interaction of the polycation chitosan and lecithin, due to the presence of negatively charged components in the lipid mixture. Progesterone was encapsulated with a good efficiency, while, on the contrary, poor drug loadings were obtained using water-soluble drugs. An optimization of the encapsulation efficiency of progesterone could be obtained by adding a lipophilic excipient, as isopropyl myristate.

Acknowledgments

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