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PLGA-based nanoparticles: Effect of chitosan in the aggregate stabilization. A dielectric relaxation spectroscopy study

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ABSTRACT

Chitosan-modified polylactic-co-glycolic acid (PLGA) nanoparticles with average diameter of 200 nm in PBS buffer solution have been investigated by means of dielectric relaxation spectroscopy measurements in the frequency range (1 MHz–2 GHz) where interfacial polarizations occur. PLGA-based nanoparticles offer remarkable advantages in different biotechnological fields, such as their biocompatibility, easiness of administration and rather complete biodegradation. However, despite the use of these drug delivery systems is increasing, little is known about the basic process involved in the formation of complexes and in the subsequent release kinetics. In the present work, we have characterized the colloidal behavior of PLGA-based nanoparticles in the presence of oppositely charged chitosan polyelectrolyte by means of dynamic light scattering, electrophoretic mobility and radiowave dielectric relaxation measurements. In particular, we have emphasized how the presence of a coating layer at the nanoparticle surface could exert a marked slowing-down in the drug release. The consequence of this finding is briefly discussed at the light of some biological implications.

1. Introduction

Charged colloidal nanoparticles in the presence of oppositely charged polyelectrolytes form different hierarchical supramolecular structures which represent an interesting and not yet completely investigated system [1–4]. In particular, the resulting mesoscopic aggregates are influenced by the presence of two correlated effects, i.e., the charge inversion and the re-entrant condensation, both of them governed by electrostatic long-range repulsive and short-range attractive interactions [5–7]. These concomitant effects give rise to the formation of stable aggregates whose size and overall charge can be tuned, in an appropriate interval of values, by environmental parameters such as the polyelectrolyte concentration, bulk electrical conductivity and, to a some extent, the temperature. This rather complex phenomenology has been extensively investigated because, from a fundamental point of view, these hybrid assemblies, built up by a particle core surrounded by a polyelectrolyte shell render these materials an intermediate state between hard spheres and soft-star polymer systems [8–10].

This interest is even more justified because the resulting aggregates have emerged as promising entities in different fields of biotechnology and, above all, in drug delivery, where they provide numerous advantages such as high payload of active substances and a high chemical versatility [11–15].

Among the different charged colloidal particles showing these peculiar aggregation phenomena governed by electrostatic interactions, polymeric nanoparticles based on biodegradable polymers such as poly(lactide-co-glycolid) (PLGA) polymers are the most widely used [16,17]. PLGA-based nanoparticles (10–1000 nm) or microparticles (1–100 μm) have been employed in the past for the controlled release of drugs because of different advantages, such as (i) good bio-degradation and biocompatibility; (ii) easy administration; (iii) commercial availability [18].

However, while PGLA microspheres have been extensively investigated, for example as particulate vaccine delivery systems [19–21], less attention has been addressed to PLGA nanoparticles [22], despite the fact that the reduced size has been proved to be particularly successful in delivery drugs to lung tumors [23].

In this work, we have investigated the colloidal behavior of PLGA nanoparticles, 200 nm in diameter, in the presence of increasing concentration of chitosan (CS) by means of dielectric relaxation spectroscopy measurements. The presence of chitosan, a cationic linear nontoxic, biodegradable and biocompatible polyelectrolyte [24], contributes to the reduction of the particle surface charge through strong electrical interactions with the negative charge of the PLGA nanoparticle surface, resulting in an enhanced stability of the resulting complexes. This feature can be conveniently exploited

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in the design of systems of potential interest for drug encapsulation and subsequent controlled release, as we will show as an example in last section of the paper.

A colloidal charged particle aqueous suspension generally exhibits a rather intricate dielectric spectrum, but, in the frequency range roughly from 1 MHz to 1 GHz, the dielectric response is relatively simpler, being mainly influenced only by the surface properties of the dispersed phase. Consequently, this technique allows us to follow the changes in the electrical properties of the particle surfaces as a consequence of the polyelectrolyte-induced interactions. In particular, we have measured the complex dielectric constant $\epsilon^*(\omega)$ of PLGA particle aqueous solutions in the presence the different amount of chitosan and have observed the influence of different coating on dielectric behavior, from the initial formulation, with negatively charged particles, to the final formulation, when the particles have reversed their charge. The dielectric spectra have been described in the framework of the standard electrokinetic model [25,26].

Recently, significant efforts have been directed towards the development of systems that allow drug delivery to specific cells over an extended period of time, possibly without systemic side effects. Polymeric systems based on PLGA nanoparticles, belonging to the class of bulk eroding forms, where the drug release is controlled by the imbibition of water, appear to be a good candidate as controlled drug delivery carrier [16]. Moreover, the release profile, depending markedly on average particle size, can be conveniently adjusted by checking the size of the polymeric aggregates.

In order to evaluate the performance of chitosan-coated PLGA nanoparticles in retaining a drug for long period of time, in comparison with the bare nanoparticles, we have investigated the release of dexamethasone (DXM), a synthetic steroidal anti-inflammatory drug largely used as a potent immune suppressive therapeutic agent for several inflammatory diseases. The release of DXM (in PBS buffer solution) as a function of time has been monitored by the measurement of the time evolution of the dielectric response of the particle aqueous suspensions and results have been compared with the ones obtained from more conventional techniques. The good agreement we have found suggests that dielectric measurements can be usefully considered as a candidate for the measurement of the *effective* drug release from a polymeric nanoparticle, avoiding to analyze the aqueous solution, where the creation of sink conditions for water insoluble compounds could limit the accuracy of the measurement.

2. Experimental

2.1. Materials

Poly-D,L-lactide-co-glycolide 50:50, Mw 40,000–75,000 Da, Dexamethasone ≥98%, Chitosan 75–85% deacetylated, average Mw 50,000 Da, and all other chemicals were purchased from Sigma. All solvents were of analytical grade, purchased from Carlo Erba Reagents, and were used as obtained.

2.2. Preparation of drug-loaded PLGA nanoparticles

Drug-loaded PLGA nanoparticles were prepared by using a recently patented osmosis-based methodology [27]. Briefly, commercially available PLGA polymer and DXM were dissolved in DMSO. The obtained solution was transferred in a dialysis bag and immersed into a selected non-solvent of the polymer (H_2O). According to preliminary investigations, the osmotic equilibrium was reached after approximately 72 h. The precipitated polymer was recovered by centrifugation, washed several times with non-solvent solution, centrifuged and freeze-dried.

2.3. Drug loading evaluation

The drug content in DXM-loaded PLGA nanoparticles was measured using a spectrophotometric method. Drug-loaded nanoparticles were dissolved in chloroform and the drug concentration was determined by measuring the absorbance of the solution at λ= 243 nm and compared it with a calibration curve.

2.4. CS coating of DXM-loaded PLGA nanoparticles

DXM-loaded PLGA nanoparticles, prepared as described above, were coated with CS as follows: a fixed amount of DXM-loaded PLGA nanoparticles were suspended in 1% w/v chitosan in acetic acid solution under sonication and incubated overnight at room temperature, under magnetic stirring. The suspension was then centrifuged at 10,000 rpm for 30 min at 4 °C and the supernatant was removed. The pellet was then washed twice with deionized water and recovered by centrifugation.

2.5. Dielectric measurements

The dielectric properties of the polymeric nanoparticle solutions were measured in the frequency range from 1 MHz to 2 GHz, by means of a computer-controlled Impedance Analyzer Hewlett-Packard mod. 4291A coupled with a dielectric cell consisting in a short section of a cylindrical coaxial cable excited far below its cut-off frequency. The cell was calibrated with liquids of known permittivity and electrical conductivity according to a procedure previously reported [28,29].

The Analyzer, in the frequency range investigated, measures the complex reflection coefficient $\Gamma^*(\omega)$, from which the complex dielectric constant $\epsilon^*(\omega)$ is obtained following the procedure reported by Bao et al. [28], through the relationship

$$\epsilon^*(\omega) = \frac{A_1^*(\omega)\Gamma^*(\omega) - A_2^*(\omega)}{A_3^*(\omega) - \Gamma^*(\omega)} \quad (1)$$

where $A_j(\omega)$ (j = 1, 2, 3) are frequency-dependent complex constants which can be obtained from calibration procedure performed with air, short connection and Millipore water. All the measurements have been carried out in the temperature range from 20.0 °C, to 50.0 °C, controlled within 0.1 °C. The overall accuracy in the dielectric measurements is within 1% in the permittivity and within 1.5% in the total dielectric loss.

2.6. Size measurements

The average particle size and the size distribution were determined using a dynamic light scattering set-up equipped with a Brookhaven BI-9000 AT digital correlator (Brookhaven Instrum. Corp, N.Y.) at the temperature of 25 °C. The correlation functions were collected at a fixed scattering angle θ= 90 ° relative to the incident beam, and delay times from 0.8 μs to 10 s were explored. The autocorrelation function of the electric field of the scattered light has been analyzed by means of non-negative least-squares (NNLS) or CONTIN algorithms, supplied with the instrument software. The average hydrodynamic radius of the diffusing objects was calculated from the diffusion coefficient D and the Stokes–Einstein relationship, $R_H = (K_BT)/(6\pi\eta D)$, where K_BT is the thermal energy and η is the solvent viscosity.

For particles having a narrow size distribution, the index of polydispersity Q is given by $Q = \sqrt{\mu}/\overline{\Gamma}$, where $\overline{\Gamma} = \overline{D}q^2$ and μ is the variance of the distribution. Here $q = (4\pi n)/\lambda \sin(\theta/2)$ is the wavevector, with n the refractive index of the aqueous phase and λ the wavelength of the incident light. For a monodispersed sample, this index should be zero.