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Preparation and characterization of nickel chelating functionalized poly (lactic-co-glycolic acid) microspheres

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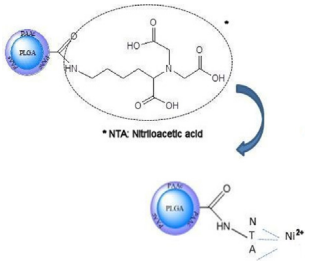
HIGHLIGHTS

- Surface modified poly (lactic-co-glycolic acid) microspheres have been easily prepared by an emulsion method.
- Polyacrylic acid employed as stabilizing agent has been modified with nitrilotriacetic acid.
- Surface modified poly (lactic-co-glycolic acid) microspheres are able to chelate nickel ions.

GRAPHICAL ABSTRACT

Poly (lactic-co-glycolic acid) particles were prepared by a double-emulsion procedure employing modified polyacrylic acid as the stabilizing agent. These particles are able to chelate nickel which is key for future biomedical applications.



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ABSTRACT

The preparation of poly (lactic-co-glycolic acid) spheres functionalized with tri-(nitrilotriacetic acid) (NTA) employing a double-emulsion and evaporation method is described. First, polyacrylic acid (PAA) employed as emulsion stabilizer was chemically modified with NTA to yield modified PAA (PAA-NTA). PAA-NTA was characterized by ^{1}H-NMR, ATR-FTIR spectroscopy and thermogravimetric analysis (TGA). Scanning electron microscopy (SEM) was employed to characterize the morphology and particle size distribution of PLGA spheres obtained with both type of surfactants, PAA and PAA-NTA. Finally, we proved that PLGA particles obtained with modified PAA are able to chelate nickel ions, key for the development of future biomedical applications for these materials.

1. Introduction

During the last years, microparticles of poly (lactic-co-glycolic acid) (PLGA) have been widely investigated due to their biocompatibility and their biodegradability as materials for controlled drug release and tissue engineering [1–4]. There are several techniques useful for the preparation of biodegradable polymeric particles such as: emulsion-based methods, evaporation or extraction of solvent, nanoprecipitation, salting out, spray-drying method, etc. [5,6]. The selected method determines the characteristics of spheres, and, according to literature, emulsion-based methods appear as the most widely used technique in the fabrication of PLGA microparticles aimed for biomedical applications [7,8]. The typical W/O/W double emulsion process consists of four steps: (1) primary emulsification: an aqueous solution of the active agent (internal water phase, W) is emulsified into an organic solution containing the biodegradable polymer (oil phase, O); (2) re-emulsification:

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the primary emulsion (W/O) is further emulsified into a second aqueous phase containing a stabilizer (external water phase, W) to form a W/O/W double emulsion; (3) solidification: the organic solvent is removed by evaporation or extraction and then solid microparticles are formed; and (4) separation and purification: microparticles are collected by centrifugation or filtration and subsequently lyophilized [9–12].

There is currently great interest in the development of PLGA particles for the delivery of proteins; however its encapsulation is not straightforward since the structure of proteins is sensitive to processing conditions such as temperature and exposure to organic solvents. In the case of PLGA particles obtained by an emulsion and evaporation process, the protein is exposed to organic solvents which might damage its structure/activity. An alternative approach for the development of protein delivery systems is the functionalization of the polymer matrix with nitrilotriacetic acid (NTA). Nitrilotriacetic acid (NTA)-ligand chemistry is extensively employed for the surface immobilization of proteins [13–16]. The functionalization of a polymeric material with NTA allows to selectively bind histidine amino acids (His6-tag) without altering the function of the protein [14]. The surface modification protocol is cost-effective and leads to a nearly quantitative reversibility of the protein binding. The resulting materials are of paramount importance in several applications such as protein purification using affinity chromatography [17,18], analysis with antibody microarrays [19], general agents to separate and transport proteins [20] and sensor platforms [21,22]. The affinity of the His6-tag to chelated metal ions has been exploited to attach proteins or peptides to various micro or nanoparticles in order to obtain colloidally stable materials for applications in efficient protein capture and delivery processes. Some examples include polystyrene particles [23], polyketal particles [24], magnetic nanoparticles [25] and poly (N-isopropylacrylamide) microgel particles [26]. In the case of poly (lactic-co-glycolic acid), nickel ions were incorporated using a metal chelating lipid [27].

In the present study we propose modifying polyacrylic acid with NTA using well-known activation methods including treatments with *N*,*N*′-dicyclohexylcarbodiimide (DCC) and *N*-hydroxysuccinimide (NHS) [28]. The modified PAA is employed in the preparation of PLGA particles by a double-emulsion and evaporation technique so that a novel route of preparation of functionalized PLGA particles is developed. Finally, the ability of the modified PLGA particles to chelate nickel ions is demonstrated as a preliminary step to develop potential biomedical applications for these materials.

2. Experimental

2.1. Materials

Poly (DL-lactide-co-glycolide), PLGA (I.V. 0.95–1.20 dL/g) (Mw 91,600–120,000 Da) ether terminated, with a 50/50 ratio (PLA/PGA) from Absorbables Polymers/Lactel (Durect Corporation, UK), was used as biodegradable polymer. Poly (acrylic acid) (PAA) with an average molecular weight of ~5000 g/mol was purchased from Scientific Polymer Products, Inc (Sp2). *N*-hydroxysuccinimide 98% (NHS), *N*,*N*′-dicyclohexylcarbodiimide, 99% (DCC), N_α,N_α-bis(carboxymethyl)-L-lysine hydrate ≥97.0% (NTA), nickel chloride ($NiCl_2$) and *N*,*N*-dimethylformamide anhydrous 99.8% solvent (DMF) were purchased from Sigma–Aldrich.

2.2. Modification of poly (acrylic acid) with N_α,N_α-bis(carboxymethyl)-L-lysine hydrate

2.5 g of poly (acrylic acid) (PAA) were dissolved in 15 mL of dimethylformamide (DMF) at room temperature under inert atmosphere (N_2). Next, 1.43 g of DCC and 0.8 g of NHS dissolved in 5 mL of DMF were added to the PAA solution at room temperature and under nitrogen atmosphere. The resulting solution was stirred during 1 h (PAA/DCC/NHS stoichiometric molar ratio: 1/0.2/0.2). Afterwards, 1.84 g of NTA was added and after 24 h, a white precipitate was observed and the solution was centrifuged (4500 rpm, 8 min) to remove it. The supernatant was precipitated with a NaOH–water solution at 40% v/v, and centrifuged at (4500 rpm, 8 min). The resulting precipitate from the supernatant was completely dried under vacuum at 160 °C. Next, the sample was dialyzed (dialyze membrane MWCO: 1000, wet in 0.05% sodium azide) during 2 weeks and then lyophilized to be characterized. The sample was denoted as PAA-NTA. The degree of modification of PAA-NTA was determined by proton nuclear magnetic resonance analysis (^{1}H NMR Bruker at 300 MHz of frequency). Samples were dissolved in Deuterium oxide (99.9 atom% D, Water-d_2) purchased by Sigma–Aldrich.

2.3. Preparation of PLGA particles by double-emulsion and evaporation method

PLGA particles were prepared by double-emulsion and evaporation technique. The methodology in brief goes as follows: 0.125 g of PLGA were dissolved in 5 mL chloroform and mixed by tip sonication with 2 mL of Milli-Q water in ice bath. For the formation of the second emulsion, 10 mg of the emulsion stabilizer (PAA or PAA-NTA) were dissolved in 20 mL of distilled water (0.05% wt.). The aqueous phase containing the emulsion stabilizer was sonicated with the first emulsion during 30 min. The water-oil-water emulsion was stirred overnight to allow solvent evaporation (chloroform). When the organic solvent was evaporated the particles were collected by centrifugation at 4500 rpm during 10 min. The supernatant was removed and the precipitate was washed with water and centrifuged again. Samples were washed four times with distilled water and finally they were freeze-dried and stored. Samples were denoted as PLGA_PAA and PLGA_PAA-NTA which corresponds to the particles obtained with raw PAA and modified PAA respectively.

For Ni (II) loading, PLGA_PAA and PLGA_PAA-NTA particles were dispersed at a concentration of 1 mg particles/mL and incubated overnight in an aqueous $NiCl_2$ solution (0.05 M). Unbound nickel was removed by dialysis against 1 L of deionized water overnight at room temperature. The dialysis process was repeated three times.

2.4. Morphological characterization and measurement of z-potential

The morphology of the polymer particles was analyzed by scanning electron microscope (ESEM, XL30 Philps). Each sample was dispersed with deionized water at 0.1 mg/mL and a drop of the sample was deposited onto a cover glass. The cover glass was kept at room temperature during 24 h to dry the sample evaporating the water. Subsequently the dried sample was coated with an ultrathin coating of gold deposited on the sample by high-vacuum metallization. The mean diameter and the particle size distribution were obtained from SEM images using the Image J software counting at least 100 individual particles.

Dynamic light scattering (DLS, Malvern Nanosizer Nano S) was employed for the determination of the electrophoretic mobility of PLGA particles dispersed in Milli-Q water at 25 °C and pH was varied between 1 and 11. The electrophoretic mobility was transformed into zeta potential using the Smoluchowski equation. All measurements were repeated three times and the average of three runs was taken as the result.