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A micro- and nano-structured drug carrier based on biocompatible, hybrid polymeric nanoparticles for potential application in dry powder inhalation therapy

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ABSTRACT

We herein describe the development of a micro-scale powder containing hybrid polymeric nanoparticles, for pulmonary application. The nanoparticles, composed of chitosan, carboxy methyl-β-cyclodextrin and a gelling counterion, were prepared, by ionotropic gelation, and characterized using differential scanning calorimetry and infrared spectroscopy besides other techniques. Nanoparticles transformation into powders was achieved, by co-spray drying with thermo-protectant, resulting in microspheres with adequate aerodynamic properties. Besides morphological and aerodynamic analysis, the developed powder was subjected to in-depth profiling of surface composition, using X-ray photoelectron spectroscopy and time-of-flight secondary ion mass spectroscopy for investigating nanoparticles spatial distribution. After fluorescent labelling of both nanoparticles and microsphere matrix, confocal microscopic examination was further performed, for structure analysis. Overall, the applied analytical techniques evidence the homogeneous and almost complete encapsulation of the nanoparticles within microspheres' matrix, emphasizing the efficiency of the microencapsulation method and the potential of the hybrid nanoparticles, as a solid formulation, for lung delivery.

1. Introduction

There has been an increasing interest in the exploration of the lungs as non-invasive mucosal route for both local and systemic drug delivery [1,2]. The pulmonary route however exhibits limitations, including the complexity of its anatomical structure and its defense mechanisms, such as the mucociliary escalator and the macrophagic activity. These obstacles are known to affect the proper deposition of particles [3]. The development of an appropriate pulmonary drug formulation has been deemed one of the most important strategies to surmount such hurdles [4]. Accordingly, using safe biomaterials, like chitosan (CS) and cyclodextrins (CDs), would be helpful to the design and efficiency of drug delivery systems intended for lung application.

CS, a natural polysaccharide derived from chitin, is attractive for transmucosal drug delivery owing to its reported low toxicity, biodegradability and mucoadhesivity [5,6]. Besides its known permeation-enhancing effect for macromolecules, it has been widely employed in the development of micro-and nanocarriers and used as a dry powder dispersibility enhancer [7–9]. CDs are cyclic oligosaccharides that contain six (α-CDs), seven (β-CDs) and eight (γ) glucopyranose units, linked with α-(1,4) in a cyclized, non-polar interior and truncated cone-like structure which acts as a host, encapsulating poorly-soluble molecules, whereas the outer surface is hydrophilic [10]. They have been used to reduce bitterness and decrease tissue irritation upon dosing, enhance permeability through biological membranes [11,12], stabilize drugs and render them more soluble [13]. It has been also suggested that some CDs show mucoadhesive properties [14]. More particularly, some CDs have been explored as excipients for pulmonary delivery [13,15], acting as dispersibility enhancers as well as porogens for inhalable systems [16,17].

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Accordingly, our group has developed different CS nanoparticles including hydroxypropyl [18], sulphobutyl ether and carboxymethyl derivatives of β- or α-CDs [19,20], by ionotropic gelation technique, envisioning that such polymeric combinations should result in adequate drug carriers. More specifically, carboxy methyl-β-cyclodextrin (CMCD), complexed with CS, conferred a more controlled release profile and a good biocompatibility record with high gene expression in respiratory cell lines, as compared to classical CS nanoparticles [21–23]. When loaded with the macromolecule drug; heparin, these nanoparticles have also shown promising outcomes, in terms of asthma treatment and cellular internalization, using rat mast cells *ex vivo* [24]. Furthermore, they have demonstrated capability to overcome mucosal barriers, using rat nasal epithelium, and induce a pronounced pharmacologic effect, after being loaded with the macromolecule; insulin, and intranasally administered to rabbits [23]. Interestingly, these hybrid nanoparticles are able to encapsulate macromolecules without altering their intrinsic properties, in addition to the possibility of modulating their composition obtaining optimal physicochemical characteristics with high drug association efficiency [20–22,24].

Apart from the advantages of improved intracellular uptake, high drug loading capacity, sustained release, enhanced drug stability and absorption, nanoparticles have been studied to a large extent for lung delivery [8,22,24–26], as they particularly afford targeted lung deposition and phagocytosis escape [26–29]. Nonetheless, direct delivery of nanoparticles to the lungs is impractical in that it poses challenges related to formulation instability. This mainly arises from particle-particle interactions and exhalation of low-inertia nanoparticles, which together reduce delivery efficiency [30]. Additionally, deep lung deposition requires that particles indispensably impart adequate aerodynamic characteristics i.e. the aerodynamic diameter must be less than 5 μm [17]. Additional processing of produced nanoparticles into micro-scale powders, by the microencapsulation method, has been therefore proposed, where nanoparticles are co-spray dried with carrier particles. This method has resulted in improved handling and aerosolization of nanoparticles [8], through enhanced powder stability and acquisition of aerodynamic properties that definitively lead to efficient lung delivery [4,15,31,32]. In addition to higher stability as solid formulations [33], inhalable powders have also been demonstrated to be superior over their liquid counterparts with regard to enhanced absorption and bioavailability of active ingredients [25,31].

Biological events, triggered by interfacial interactions between biomaterials and biological environments, are governed by particles' surface chemistry [34,35]. Thus, investigating surface properties helps tailor and control delivery systems in early development and, hence their *in vivo* performance would be predictable and efficient. Surface characterization of delivery systems involves determining the composition, structure, and distribution of all components present at the surface [34]. Such characterization enabled the investigation of biodegradation kinetics of polymers, as well as the controlled release mechanisms and surface modification for targeting purposes of drug delivery systems [36,37]. More specifically, the assessment of the surface composition of dry powders could allow the control of interparticulate interactions and, in this way, enhance powder dispersion during inhalation [38,39]. Also, implementing surface-probing techniques could be useful for powder optimization, based on formulation and process parameters, with a view to enhancing aerosolization properties and, thus lung delivery.

Considering the aforementioned, interesting results produced by our group, regarding the chitosan/carboxy methyl-β-cyclodextrin hybrid nanoparticles (CS/CMCD NPs), we were encouraged to further explore their utility as a therapeutic dry powder for pulmonary delivery, through implementing the promising microencapsulation approach. In this context, we prepared, characterized the physicochemical properties of the CS/CMCD NPs, using several techniques, among which are fourier transform infrared (FTIR) and differential scanning calorimetry (DSC). Subsequently, the nanoparticles were co-spray dried with mannitol, which serves as a thermoprotectant and inert carrier excipient. A multi-technique approach was conducted to obtain comprehensive information on the distribution of nanoparticles within mannitol microspheres [40]. These analytical techniques are mainly for surface probing, and they include: X-ray photoelectron spectroscopy (XPS) and time of flight secondary ion mass spectrometry (TOF-SIMS), in conjunction with Confocal Laser Scanning Microscopy (CLSM) for structure analysis. Thus, we speculate that additional processing of nanoparticles into micro-scale powders would be beneficial to meet the specific delivery requirements of the lung environment.

2. Experimental section

2.1. Materials

Ultrapure chitosan in the form of hydrochloride salt (CS) (Protasan® UP CL 113, deacetylation degree 75–90%, viscosity < 20 mPa s, molecular weight < 150 kDa) was purchased from FMC Biopolymers [Norway], carboxy methyl-β-cyclodextrin (CMCD), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDAC), fluorescein sodium salt, sodium tripolyphosphate (TPP), D-mannitol and dialysis tubing cellulose membranes (cut-off = 12.4 kDa) were supplied by Sigma–Aldrich [Spain] and Bodipy® 630/650-X succinimidyl ester was provided by Molecular Probes [Netherlands]. Ultrapure water [MilliQ plus, Millipore Ibérica, Spain] was used throughout. All other chemicals were reagent grade.

2.2. Preparation of hybrid nanoparticles

As stated above, the CS/CMCD NPs were prepared by ionotropic gelation technique developed by our group [22]. Briefly, solutions of TPP (cross-linker) and CMCD were prepared at concentrations of 0.25–2.25 and 7.5–12 mg/mL, (w/v), respectively in ultrapure water and, then, equal volumes of both solutions were mixed together. Then, 1 mL of this mixture was added to 3 mL of 1.25–2 mg/mL CS solution, and the reaction was maintained for 10 min under mild magnetic stirring at room temperature, resulting in nanoparticles with different physicochemical properties. The formed nanoparticles were isolated for further analysis, by centrifugation on a 10 μL glycerol bed [16,000× *g*, 30 min, 15 °C, Beckmann Avanti 30, Beckmann, USA] and, then re-suspended in 100 μL of purified water, after discarding the supernatants. Nanoparticles were also prepared on a large scale up to 40 mL and, then centrifuged (18,000× *g*, 45 min, 15 °C).

For the CLSM study, the CS/CMCD NPs were formulated after CS labelling with fluorescein, following a slightly modified method described by De Campos et al. [41]. Concisely, 2.5 g of CS were dissolved in 250 mL of water, and another solution of fluorescein (100 mg) was prepared in 10 mL of ethanol. Next, both solutions were mixed together, and EDAC was added in a final concentration of 0.05 M. EDAC serves as a catalyst for the coupling reaction whereby it efficiently reacts with fluorescein carboxylic groups to form an active *O*-acylisourea intermediate that is easily displaced by nucleophilic attack from primary amino groups of CS. Subsequently, the fluorescein carboxylic groups are cross-linked with amine groups of CS, forming an amide bond and an EDAC by-product released as a soluble urea derivative. The reaction was