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Research paper

Mucus permeating carriers: Formulation and characterization of highly densely charged nanoparticles

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ABSTRACT

The GI mucus layer represents a significant block to drug carriers absorption. Taking an example from nature, virus-mimicking nanoparticles (NPs) with highly densely charged surface were designed with the aim to improve their mucus permeation ability. NPs were formulated by combining chitosan with chondroitin sulfate and were characterized by particle size, ζ-potential and hydrophobicity. The interaction occurring between NPs and diluted porcine intestinal mucus was investigated by a new method. Furthermore, the rotating tube technique was exploited to evaluate the NP permeation ability in fresh undiluted porcine intestinal mucus. NPs (400–500 nm) presenting a slightly positive (4.02 mV) and slightly negative (−3.55 mV) ζ-potential resulted to be hydrophobic and hydrophilic, respectively. On the one hand the hydrophobic NPs undergo physico-chemical changes when incubated with mucus, namely the size increased and the ζ-potential decreased. On the other hand, the hydrophilic NPs did not significantly change size and net charge during incubation with mucus. Both types of NPs showed a 3-fold higher diffusion ability compared to the reference 50/50 DL-lactide/glycolide copolymer NPs (136 nm, −23 mV, hydrophilic). Based on these results, this work gives valuable information for the further design of mucus-penetrating NPs.

1. Introduction

Oral administration is the most extensively used drug administration path with the best patient compliance [1]. However, the GI-tract still represents a formidable barrier for drug carriers absorption making more challenging and more essential formulation of new oral drug delivery systems (DDSs) able to overcome this stumbling block [2,3]. Among various barriers being encountered with the GI-tract, the mucus gel layer covering its surface has major impact on the efficacy of DDSs. This layer, with a thickness between 37 and 170 μm [4–6], covers a pH range from 1–2 in the stomach to 7–8 in the colon and, from the lumen to the epithelium, covers a pH gradient reaching pH 7.4 [7,8]. Mucus protects the underlying epithelium from toxic substances by its continuous secretion and by forming polyvalent adhesive interactions, which in part involve the lipids in its composition (20% of the dry weight) [7]. Moreover, this viscous gel presents sialic and sulfonic acid substructures that supply a net negative charge. Accordingly, to overcome this barrier DDSs should be tailored following certain guidelines, for example a high positive charge and/or a hydrophobic shell are characteristics that should be avoided. Potential DDSs for oral administration are nanoparticulate systems [9–11] that can be designed to possess these features. However, conventional nanoparticles (NPs) are quickly transported through the GI-tract making the mucus permeation unfeasible [12,13]. On the contrary, particulate systems with efficient permeation ability already exist in nature. For example, viruses are capable of diffusing through the mucus as fast as in saline [14]. The most representative viruses able to overcome the mucus are poliovirus, a sense-strand RNA, nonenveloped virus belonging to the Picornaviridae family, Norwalk virus a nonenveloped, single-stranded, positive-sense RNA virion (Caliciviridae family) and the human papillomavirus a nonenveloped DNA virus [15–17]. This property is likely to be related

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to their peculiar surface characteristics. In fact viruses exhibit a highly densely charged surface due to the high concentration of cationic and anionic groups. Moreover, having a closer look at the isoelectric point of viruses, the majority of them exhibits a neutral or a negative ζ-potential in the pH range of the GI tract [18]. It was the aim of this study to design NPs with a highly densely charged surface in order to improve their mucus permeation ability. For this reason, NPs were generated via combining negatively charged chondroitin sulfate (ChS) with cationic chitosan (CS). In order to assess the influence of the particles net charge on their diffusion ability, NPs exhibiting a slightly positive and negative ζ-potential were prepared and evaluated in their mucus permeation behavior. Moreover, considering the hydrophobic characteristic of the mucus and its negative net charge, a new study was designed to investigate the physico-chemical changes which charged NPs go through when incubated with intestinal mucus.

2. Materials and methods

2.1. Materials

Chitosan 85/5 was purchased from Heppe medical chitosan GmbH (Saale, Germany). Lumogen red was purchased from Kremer pigmente GmbH & Co. KG (Aichstetten, Germany). Chondroitin sulfate A sodium salt from bovine trachea, Rose Bengal and all other salts and solvents at analytical grade were purchased from Sigma–Aldrich (Vienna, Austria). 50/50 DL-lactide/glycolide copolymer NPs (PDLG 5002 NPs) were produced by NANOMI (Oldenzaal, The Netherlands).

2.2. ChS/CS nanoparticles preparation

NPs were prepared by ionic gelation method, via complexation of ChS with CS. To prepare particles with different ζ-potential and particle size various ChS/CS weight ratios were used. CS was dissolved in 0.5% acetic acid at a concentration of 0.27 mg/mL and after total dissolution the pH was adjusted at 5.6. To 3 mL of CS solution, 1 mL of an aqueous solution of ChS at increasing concentrations was added as described in Table 1. The suspension was stirred gently for 30 min at room temperature, followed by determination of particle size and ζ-potential as described below.

2.3. PDLG 5002 nanoparticles preparation

PDLG 5002 NPs were prepared following the method described by Khayata et al. [19]. Briefly, 1% w/w Purasorb PDLG 5002 was dissolved in acetone. Then, the polymer solution was injected into stirred ultrapure water in a mass ratio of 1:3 followed by evaporation at room temperature of the solvent.

Table 1
Formulation of 4 mL NPs via combining 3 mL of CS solution and 1 mL of ChS solution at increasing concentrations to obtain different ChS/CS ratio.

ChS/CS ratio	CS concentration (mg/mL)	ChS concentration (mg/mL)	Nomenclature
0.5	0.27	0.4	ChS/CS 0.5
1	0.27	0.8	ChS/CS 1
1.2	0.27	0.96	ChS/CS 1.2
1.5	0.27	1.2	ChS/CS 1.5
1.8	0.27	1.44	ChS/CS 1.8
2	0.27	1.6	ChS/CS 2
2.2	0.27	1.76	ChS/CS 2.2

2.4. Lumogen encapsulation

In order to allow particles detection during the diffusion studies Lumogen red was encapsulated. Therefore, 200 μL of Lumogen dissolved in DMF was diluted with 3 mL of CS solution. Afterward, 1 mL of ChS solution was added dropwise. Lumogen was added in a ratio of 11.9 μg Lumogen/mg polymers. To prepare 4 mL of ChS/CS 1 NPs, 19 μg Lumogen was used and to prepare 4 mL of ChS/CS 2 NPs, 28.56 μg Lumogen was added. The obtained suspension was centrifuged at 2000 rpm for 20 min at 4 °C, the supernatant discarded and the particles washed with a DMF/water mixture. After a second centrifugation the particles were suspended in acetate buffer 0.1 M pH 5.6.

2.5. Nanoparticles characterization

Particle size and ζ-potential were determined with a NICOMP™ 380 ZLS PSS (Particle Sizing Systems, CA, USA). Each size measurement was recorded for 10 min at a scattering angle of 90° at room temperature. The ζ-potential measurements were accomplished at room temperature under electric field strength of 5 V/cm.

Samples morphology was characterized by energy filter transmission electronic microscopy (TEM) using a ZEISS Libra 120 (Zeiss AG, Oberkochen, Germany) [20].

Attenuated-total-reflectance infrared spectroscopy (ATR-IR) was utilized to characterize the samples. A Perkin Elmer Spectrum 100 ATR-IR spectrometer (Perkin Elmer, Waltham, USA) in combination with a Spectrum software version 6.3.1.0134 (Perkin Elmer, Waltham, USA) was deployed to obtain the spectra. Every sample was divided into three subsamples and every subsample was recorded with 10 scans in a wavenumber range from 4000 cm^{-1} to 650 cm^{-1} and a resolution of 1 cm^{-1}. Measurements were taken at 22 °C.

2.6. Surface hydrophobicity evaluation

The surface hydrophobicity of ChS/CS 1, ChS/CS 2 and PDLG 5002 NPs was evaluated by Rose Bengal test as previously described by Doktorova et al. [21]. Briefly, 200 μL of NP suspension, with increasing amounts of NPs from 0.05 to 1 mg/mL, was mixed with 400 μL Rose Bengal aqueous solution 100 μg/mL. All samples were incubated for 3 h, at 25 °C in a thermomixer (Thermomixer Conform; Eppendorf, Hamburg, Germany), under constant shaking, 1400 rpm. Afterward, the samples were centrifuged at 12,100 rpm for 30 min allowing the separation of the aqueous phase and the solid phase. The amount of Rose Bengal in solution (not bound to the solid phase) was calculated from the absorbance detected at 548 nm by using a microplate reader (TECAN Infinite M200, Austria GmbH). As suggested by Sahoo et al. [22], each time the test was run in parallel with the 100 μg/mL Rose Bengal solution, without sample, to give the initial concentration of Rose Bengal. Thereby, the concentration of Rose Bengal bound to the solid phase was calculated as the difference between the initial concentration and the concentration of unbound Rose Bengal. Further, the partitioning quotient, calculated in accordance with Eq. (1) reported below, was plotted against the total surface area of each sample.

$$PQ = \frac{\text{Rose bengal bound}}{\text{Rose bengal unbound}} \quad (1)$$

2.7. Intestinal mucus collection and purification

Small intestinal mucus was collected and debris removed following a protocol developed by the research group of

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