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Research paper

Alginate-based hybrid aerogel microparticles for mucosal drug delivery

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ABSTRACT

The application of biopolymer aerogels as drug delivery systems (DDS) has gained increased interest during the last decade since these structures have large surface area and accessible pores allowing for high drug loadings. Being biocompatible, biodegradable and presenting low toxicity, polysaccharide-based aerogels are an attractive carrier to be applied in pharmaceutical industry. Moreover, some polysaccharides (e.g. alginate and chitosan) present mucoadhesive properties, an important feature for mucosal drug delivery. This feature allows to extend the contact of DDS with biological membranes, thereby increasing the absorption of drugs through the mucosa. Alginate-based hybrid aerogels in the form of microparticles (<50 μm) were investigated in this work as carriers for mucosal administration of drugs. Low methoxyl pectin and κ-carrageenan were co-gelled with alginate and further dried with supercritical CO_2 (sc-CO_2). Spherical mesoporous aerogel microparticles were obtained for alginate, hybrid alginate/pectin and alginate/κ-carrageenan aerogels, presenting high specific surface area (370–548 $m^2\ g^{-1}$) and mucoadhesive properties. The microparticles were loaded with ketoprofen via adsorption from its solution in sc-CO_2, and with quercetin via supercritical anti-solvent precipitation. Loading of ketoprofen was in the range between 17 and 22 wt% whereas quercetin demonstrated loadings of 3.1–5.4 wt%. Both the drugs were present in amorphous state. Loading procedure allowed the preservation of antioxidant activity of quercetin. Release of both drugs from alginate/κ-carrageenan aerogel was slightly faster compared to alginate/pectin. The results indicate that alginate-based aerogel microparticles can be viewed as promising matrices for mucosal drug delivery applications.

1. Introduction

Powder formulations have several advantages over liquid ones such as simplification of the formulation, higher chemical and microbiological stability, possibility to intimately formulate the drug with functional excipients and higher local concentrations at the absorption site of the mucosa along with improved residence time [1]. In present paper we focus on highly porous biopolymer aerogel microparticles as carriers for drug delivery systems (DDS). One remarkable feature of aerogels is that they are obtained by supercritical drying of wet gels yielding solid porous structures, wherein the liquid is replaced by air with minimal damage of the gel backbone [2]. Among various possible gels, biopolymer-based ones are of special interest due to good compatibility with human tissues [3].

In the context of drug delivery, remarkable features of the biopolymer-based aerogels are as follows: (i) high specific surface area allows to carry significant amount of the confined drug [4]; (ii) drug can be stabilized in the amorphous state with high stability towards recrystallization [4–6]; (iii) aerogel backbone is as chemically stable as original biopolymer; (iv) modulated drug release profile [7,8]; and (v) compatibility with subsequent formulation steps such as tableting and coating [9,10]. Additionally, biopolymers are one of the most abundant renewable resources on earth representing a sustainable advantage compared to synthetic polymers [3,8,11].

To the best of our knowledge, the usage of biopolymer aerogels as vehicles for mucosal targeted drug delivery remains largely unexplored. Biopolymer-based aerogels with mucoadhesive properties are of special interest for pharmaceutical applications as they can be designed for administration through various mucosal delivery routes (nasal, buccal, intestinal and vaginal). This paper presents a first attempt towards aerogel microparticles for mucosal

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drug delivery. Three polysaccharides, namely alginate (with known mucoadhesive properties), pectin and κ-carrageenan are chosen for this study.

The aim of the present work was two-fold. First, to further develop the emulsion gelation method and adopt it to the production of hybrid microparticles (<50 μm) composed of alginate and a second biopolymer. Second, to assess cytotoxicity and mucoadhesive properties of original particles, and further load it with two model drugs, ketoprofen and quercetin, and to evaluate the drug load and release. Low methoxyl pectin and κ-carrageenan were chosen for blending with alginate and processed into microparticles through internal gelation combined with emulsion gelation.

2. Materials and methods

2.1. Materials

Alginic acid sodium salt was purchased from Panreac Applichem (Germany). Low methoxyl pectin and κ-carrageenan were kindly donated by Disproquima (Portugal) and FMC Biopolymer (Norway). Calcium carbonate was kindly provided by Magnesia GmbH (Germany). Sorbitan monooleate (Span 80) and polyoxyethylene sorbitan monooleate (Tween 80) were purchased from Merck (Germany). Polyglycerol polyricinoleate 4150 (PGPR) was provided by Palsgaard (Denmark). Paraffin oil, *n*-hexane and acetic acid (⩾99.8%) were purchased from Carl Roth GmbH (Germany). Anhydrous ethanol (99.9%) was purchased from H. Möller GmbH & Co.KG. Carbon dioxide (>99.9 mol% purity) was supplied by AGA Gas GmbH (Germany). Quercetin (⩾95%) and mucin from porcine stomach (type II) were purchased from Sigma-Aldrich (Steinheim, Germany). Racemic mixture of ketoprofen was kindly provided by Chemische Fabrik Kreussler & Co. GmbH (Germany). Chemicals used for antioxidant activity assay were 2′,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) from Sigma-Aldrich (St Quentin Fallavier, France) and disodium fluorescein (TCI Europe, Antwerp, Belgium). Salts used for phosphate buffer solution (PBS) preparation (sodium chloride, potassium chloride and monopotassium phosphate) were from Sigma-Aldrich (St Quentin Fallavier, France) and disodium hydrogen phosphate dihydrate from Riedel-de-Haën (Seelze, Germany). All cell culture media and supplements namely RPMI 1640, foetal bovine serum (FBS), penicillin-streptomycin (PS) and trypsin/EDTA were obtained from Invitrogen (Invitrogen Corporation, Paisley, UK). PBS powder for cytotoxicity assays was obtained from Sigma-Aldrich (St. Louis, USA) and CellTiter 96 AQueous One Solution Cell Proliferation Assay was obtained from Promega (Wisconsin, USA). For sterilization experiments tryptone soya broth (TSB) and tryptone soya agar (TSA) were purchased from Oxoid (Hampshire, England). All chemicals were used without further purification.

2.2. Production of hybrid alginate-based gel microparticles by emulsion gelation method

Alginate and hybrid alginate-based gel microparticles were prepared by the emulsion gelation combined with internal setting method (Fig. 1).

Calcium carbonate was mixed with aqueous solution of sodium alginate or sodium alginate/s biopolymer (pectin or κ-carrageenan, 1:1 w/w, overall biopolymer concentration 3 wt%) using an Ultra-Turrax homogenizer (Ika Werke, Germany). $CaCO_3$/alginate ratio of 0.365:1 (w/w) was kept throughout the study. Continuous phase was produced by mixing paraffin oil and a specific surfactant or mixture of surfactants with desirable HLB value (overall emulsifier concentration was 3 wt%). PGPR, Span 80 and Tween 80 were chosen in this study as surfactants as they are commonly used in the food industry and pharmaceutical industry [12]. To achieve HLB values between 1.5 and 4.3, a mixture of PGPR and Span 80 was used. Span 80 and Tween 80 were dissolved in paraffin oil to obtain HLB 5. The weight ratio of each surfactant was calculated according to Eq. (1):

$$HLB_{mix} = w_1 \times HLB_1 + w_2 \times HLB_2 \quad (1)$$

where HLB_{mix} is the desirable HLB value, and w_1, w_2, HLB_1 and HLB_2 are mass fractions and HLB values of the individual surfactants, respectively. The continuous phase with a desired HLB was then added to the polysaccharide/$CaCO_3$ suspension to form a w/o emulsion (1:3, v/v). Emulsification was performed using a high speed homogenizer Ultra-Turrax for a total time of 100 s divided into three homogenization steps of 20 s each with two breaks of 20 s in between. Then, freshly prepared microemulsion of acetic acid was added into the biopolymer/oil emulsion and stirred by a turbine stirrer (1000 rpm) for 1 min. The microemulsion containing acetic acid, paraffin oil and surfactants (Tween 80 and Span 80) was prepared following results by Porras et al. [13] (see Table 1).

After 1 h of stirring (200 rpm), the suspension of gel microparticles was left on the bench overnight to complete the gelation. Oil phase was removed by decantation upon centrifugation (4500 rpm, 30 min). Hexane was added to the suspension in order to dissolve the remaining oil and reduce its viscosity. The mixture was shaken and centrifuged (3000 rpm, 20 min) and hexane was decanted. For the solvent exchange, remaining suspension was rinsed with ethanol/water mixtures (30, 60, 90 and 100 vol%) followed by centrifugation (3000 rpm, 20 min) and decantation of the supernatant. To ensure water removal, the last step of the solvent exchange was repeated twice or thrice to guarantee ethanol concentration higher than 98 wt%. The ethanol concentration was measured by a densimeter DMA 4500 (Anton Paar, Austria). The resulting alcogel microparticles were placed into a filter bag soaked in pure ethanol and subjected to supercritical drying.

2.3. Supercritical drying of gel microparticles

The resulting gel microparticles were dried by extraction of ethanol with a continuous flow of supercritical CO_2 using a high pressure autoclave described elsewhere [14]. Briefly, filter paper bags containing the samples were placed into a high pressure autoclave (250 mL) and covered with ethanol in order to prevent premature solvent evaporation. The autoclave was sealed and preheated to 313 K. Preheated carbon dioxide (313 K) was supplied into the autoclave until the desired working pressure (12 MPa) was achieved. Then, the outlet valve was adjusted to achieve carbon dioxide flow rate through the autoclave of ca. 40 g min^{-1}. The outgoing flow was directed into a separator to split the fluid into ethanol and gaseous CO_2. The drying lasted for 4 h; then, the autoclave was slowly depressurized (0.5 MPa min^{-1}) and the samples were collected.

2.4. Drug loading

Two methods for drug loading are exemplified in this work. Depending on the solubility of the pharmaceutical ingredients the adsorption process may take place either in supercritical carbon dioxide (ketoprofen) or in ethanol at the last solvent exchange step (quercetin).

2.4.1. Adsorption of ketoprofen from sc-CO_2

Adsorption of ketoprofen was performed by exposing aerogel microparticles to the saturated solution of the drug in sc-CO_2. Aerogel microparticles and crystalline ketoprofen (0.2 g each) were placed into separate filter paper cartridges. This prevents contact