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Research Paper

On prilled Nanotubes-in-Microgel Oral Systems for protein delivery

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ABSTRACT

Newly discovered active macromolecules are highly promising for therapy, but poor bioavailability hinders their oral use. Microencapsulation approaches, such as protein prilling into microspheres, may enable protection from gastrointestinal (GI) enzymatic degradation. This would increase bioavailability mainly for local delivery to GI lumen or mucosa. This work's purpose was to design a novel architecture, namely a Nanotubes-in-Microgel Oral System, by prilling for protein delivery. Halloysite nanotubes (HNT) were selected as orally acceptable clay particles and their lumen was enlarged by alkaline etching. This chemical modification increased the luminal volume to a mean of 216.3 μL g^{-1} (+40.8%). After loading albumin as model drug, the HNT were entrapped in microgels by prilling. The formation of Nanoparticles-in-Microsphere Oral System (NiMOS) yielded entrapment efficiencies up to 63.2%. NiMOS shape was spherical to toroidal, with a diameter smaller than 320 μm. Release profiles depended largely on the employed system and HNT type. Protein stability was determined throughout prilling and after *in vitro* enzymatic degradation. Prilling did not harm protein structure, and NiMOS demonstrated higher enzymatic protection than pure nanotubes or microgels, since up to 82% of BSA remained unscathed after *in vitro* digestion. Therefore, prilled NiMOS was shown to be a promising and flexible multi-compartment system for oral (local) macromolecular delivery.

1. Introduction

New proteins as active pharmaceutical ingredients (API) have drawn much attention to scientists in modern pharmaceutics [1–3]. The oral delivery of these compounds is challenging in terms of bioavailability, which is substantially reduced by the conditions in the gastrointestinal (GI) tract [4,5]. The GI barriers to overcome consist mainly of enzymatic drug digestion, mucus penetration of the API or of the delivery system, and absorption of the API [6]. If primarily luminal activity is required for the therapeutic action of the macromolecule, only enzymatic protection must be achieved, which is a still challenging but realistic pharmaceutical objective. Herein, microencapsulation has shown potential to overcome this major hurdle by protecting proteins from the GI environment [7–10]. Among several other techniques, prilling can be a way to formulate proteins as microparticles [11]. The mild conditions of the process avoid thermally induced protein degradation. Prilling is also known as vibrating nozzle technique. This approach embeds the macromolecular API in a polymeric microgel by dropping a solution of both components in a hardening bath. Herein, the API-containing polymeric solution is extruded through a nozzle. The liquid stream is then broken into droplets by applying vibration. The droplets pass through a ring electrode that charges them electrostatically to avoid mid-air coalescence [12]. Finally, the droplets are collected in a hardening bath where crosslinking occurs and the API is efficiently entrapped. Both polymer and

Abbreviations: ANOVA, analysis of variance; API, active pharmaceutical ingredient; APS, ammonium persulfate; BET, Brunauer–Emmett–Teller theory; BSA, bovine serum albumin; DTT, dithiothreitol; GI, gastrointestinal; HNT, halloysite nanotube (comprising modified and non-treated halloysite nanotube); bHNT, base-modified halloysite nanotube; nHNT, non-treated halloysite nanotube; MCC, mono-N-carboxymethyl chitosan; NiMOS, Nanoparticles-in-Microsphere Oral System (comprising microgels loaded with bHNT or nHNT); bNiMOS, NiMOS loaded with base-modified HNT; nNiMOS, NiMOS loaded with non-treated HNT; PBS, phosphate buffer saline; SDS-PAGE, sodium dodecyl sulfate gel electrophoresis; SEM, scanning electron microscopy; TEM, transmission electron microscopy; TEMED, tetramethylethylenediamine.

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hardening bath may be varied to achieve a suitable formulation for the process. Several polymers have been proposed for prilling, such as alginate [13,14], pectine [15], and chitosan [16]. Chitosan is a natural linear polysaccharide that displays interesting drug delivery properties [17–19]. Many chemical modifications of this compound were suggested to enhance or modify its physicochemical properties [20]. Among chitosan derivatives, mono-N-carboxymethyl chitosan (MCC) has shown promising mucoadhesive and permeation enhancing properties, as well as improved tolerability compared to chitosan and other of its cationic derivatives [21–24]. The prilling hardening bath is generally aqueous, but lipid-based pharmaceutical compositions were recently identified as technically feasible for prilling [25]. The hardening bath can then be optimized for later steps of manufacturing, such as capsule filling of the microgel dispersion [26]. Prilled microgels have therefore shown flexibility and suitability in terms of protein formulation as a final oral dosage form.

Apart from the technology of microgels, nanoparticles have become an important field for oral delivery of macromolecules [27–29]. This formulation approach has been excellently reviewed by several authors [30–32]. It seems particularly attractive to embed a nanoparticulate system into microgels or microspheres [33]. Thus, the biopharmaceutical formulation properties may be improved by forming highly versatile multi-compartment carriers known as Nanoparticles-in-Microsphere Oral Systems (NiMOS). Bhavsar et al. pioneered this field by using gelatin nanoparticles encapsulated in poly(ε-caprolactone) microspheres for intestinal mucosal delivery of proteins and nucleotides, and were reported to be more successful for oral macromolecule administration than nanoparticles alone [34–36]. The promising multi-compartment approach has seen only a preliminary application of solid nanotubes for oral delivery of proteins. Although carbon nanotubes represent a first example of drug delivery nanostructure, their costs and toxicity strongly hinder their application in pharmaceutics [37–39]. Halloysite is, by contrast, a natural and inexpensive aluminosilicate clay with a hollow nanotubular structure. It is regarded as non-toxic, and there are preliminary findings of even anti-inflammatory properties [40–42]. Halloysite nanotubes (HNT) are extracted from clay quarries, and their characteristics vary according to the extraction site [43,44]. The use of halloysite in drug delivery was first proposed by Price et al. [45], and has since then gained increasing interest [46–49]. HNT showed the capacity of storing APIs in their lumen or to adsorb compounds on their surface. Both luminal and surface additions and modifications to HNT have been proposed to increase the loading efficiency of the tubes or to modify the drug release properties of this system [50–55]. Many of these modifications were reviewed elsewhere [56]. Most notably, chemical etching of the inner clay surface has been proposed to enlarge the luminal diameter thereby increasing the loading capacity of the tubular structure [57–59]. While HNT have been loaded with small molecules, peptides, small proteins, and nucleotides, the addition of larger proteins into the lumen may be hindered by the macromolecule size.

First steps in formation of halloysites-containing gel structures were attempted to achieve nanocomposite films [60], beads [61], magnetic microspheres [62], and mesoporous microgels [63]. There is certainly much biopharmaceutical promise in such multi-compartment systems. However, the risk of the system complexity to become a major hurdle for scale-up and manufacturing still remains. In fact, drug delivery systems are required not only to show biopharmaceutical promise, but also to be viable for later stages of galenical development regarding clinical research and finally the market [31].

The aim of this work was to design and manufacture Nanotubes-in-Microgel for protein (local) oral delivery, which falls under the umbrella of Nanoparticles-in–Microsphere Oral System (NiMOS). Local delivery of proteins targets their release for action in the lumen or at the intestinal mucosa, thus bypassing the intestinal absorption step. For this purpose, a simple microencapsulation method is introduced to form the aforementioned complex structures, namely prilling (Fig. 1). This mild process has the potential to embed protein-loaded HNTs into a microgel. HNTs were chemically etched to increase drug loading capacity and to allow protection from enzymatic protein degradation. The feasibility of the prilling approach was assessed, and the obtained NiMOS was characterized in terms of morphology, protein loading, and release. The protein stability after manufacturing was evaluated. A preliminary biopharmaceutical characterization was performed by evaluating the enzymatic digestion of the proposed NiMOS compared to HNT and microgels alone.

2. Materials and methods

2.1. Materials

Mono-N-carboxymethyl chitosan (MCC; deacetylation degree 96.1%, carboxymethylation degree 82.1%, loss on drying 11.2%, MW = 9000–13000 g mol^{-1}) was purchased from Boylechem Co., Ltd. (Shanghai, China). Ethanol (brand J.T. Baker® Chemicals) was obtained from Avantor Performance Materials BV (Deventer, The Netherlands) and hydrochloric acid solution 1 M from Scharlau SL (Sentmenat, Spain). Miglyol® 812 (triglyceryl caprylocaprate) was supplied by Hänseler AG (Herisau, Switzerland). Transcutol® HP (diethylene glycol monoethyl ether; DEGEE) was a kind gift of Gattefossé AG (Luzern, Switzerland). Acetic acid, bovine serum albumin 96%, calcium chloride anhydrous, guanidine hydrochloride, halloysite nanotubes (HNT), potassium phosphate dibasic, potassium phosphate monobasic, sodium chloride, sodium hydroxide, sodium phosphate monobasic, sodium phosphate tribasic, trypsin, trypsin inhibitor from *Glycine max*, glycerol and Tween® 80 (polyoxyethylene (20) sorbitan monooleate) were purchased from Sigma-Aldrich Chemie GmbH (Buchs, Switzerland). Acrylamide/Bis 40% solution, ammonium persulfate (APS), Coomassie Brilliant Blue G-250, bromophenol blue, dithiothreitol (DTT), sodium dodecyl sulfate (SDS), and tetramethylethylenediamine (TEMED) were purchased from Bio-Rad Laboratories AG (Cressier, Switzerland). Tricine, tris-(hydroxymethyl)-aminomethane (Tris) base and Tris hydrochloride (Tris-HCl) were purchased from Carl Roth GmbH & Co KG (Karlsruhe, Germany). The Micro BCA™ Protein Assay Kit and the molecular weight marker PageRuler™ Plus Prestained Ladder were obtained from Fisher Scientific AG (Reinach, Switzerland).

2.2. Halloysite nanotubes (HNT) activation by luminal etching

The HNT internal lumen diameter was enlarged by alkaline chemical etching according to a previously reported method [57]. Briefly, HNT were suspended in a 2 M solution of sodium hydroxide in a ratio 1:10 (*w*/*w*). The dispersion was sonicated for 50 min at 50 °C in a Sonorex Digital 10P ultrasound bath (Bandelin Electronic GmbH + CO KG, Berlin, Germany). The HNT samples were centrifuged at 4000 rpm for 15 min. Then, the supernatant was removed and 40 mL of demineralized water was added. Subsequently, both centrifugation and washing steps were repeated three times using phosphate buffer saline (PBS) pH 6.8 instead of demineralized water. A final centrifugation and washing steps were repeated with demineralized water. This base-modified HNT (bHNT) was desiccated at 105 °C until no weight variation could be detected. The pH of a 1% (*w*/*w*) dispersion bHNT and non-treated HNT (nHNT) was 7.3 and 6.8, respectively.