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Research paper

Controlling the burst release of amorphous drug–polysaccharide nanoparticle complex via crosslinking of the polysaccharide chains

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ABSTRACT

High-payload amorphous drug–polysaccharide nanoparticle complex (or nanoplex in short) represents a new class of supersaturating drug delivery systems intended for bioavailability enhancement of poorly-soluble drugs. Not unlike other nanoscale amorphous formulations, the nanoplex exhibits fast dissolution characterized by a burst drug release pattern. While the burst release is ideal for supersaturation generation in the presence of crystallization inhibitor, it is not as ideal for passive targeting drug delivery applications in which the nanoplex must be delivered by itself. Herein we developed nanoplex exhibiting controlled release via crosslinking of the polysaccharide chains onto which the drug molecules were electrostatically bound to. Curcumin and chitosan were used, respectively, as the drug and polysaccharide models with amine-reactive disuccinimidyl tartrate as the crosslinking agent. The crosslinked nanoplex exhibited improved morphology (i.e. smaller size, more spherical, and higher uniformity) that signified its more condensed structure. A twenty-fold reduction in the initial burst release rate with a threefold reduction in the overall dissolution rate was obtained after crosslinking. The slower dissolution was attributed to the more condensed structure of the crosslinked nanoplex that enhanced its dissociation stability in phosphate buffered saline. The reduction in the dissolution rate was proportional to the degree of crosslinking that was governed by the crosslinker to amine ratio. The crosslinking caused slight reductions in the payload and zeta potential of the nanoplex, but with no adverse effect on the cytotoxicity. This proof-of-concept study successfully demonstrated the use of polysaccharide crosslinking to control the drug release from high-payload amorphous drug nanoplex.

1. Introduction

Amorphous drug nanoparticles have been established as a highly effective bioavailability enhancement strategy of poorly soluble drugs by virtue of their ability to generate high apparent solubility of such drugs not obtainable in their crystalline counterparts [1]. Herein drug nanoparticles are defined as nanoparticles of the active ingredient itself, hence distinguishing them from nanocapsules in which the active ingredient is encapsulated in nanoparticle carriers typically made up of lipids or polymers. The high apparent solubility, which is higher than the thermodynamic saturation solubility, is attributed to the generation of supersaturated drug concentration afforded by the metastable state of the amorphous form of the drug nanoparticles [2].

While the conventional supersaturating drug delivery system in the form of microscale amorphous solid dispersion is also capable of generating high apparent drug solubility [3], the superiority of the supersaturation generation of the amorphous drug nanoparticles has been demonstrated in both *in vitro* [1] and *in vivo* [4–6] studies. The superior supersaturation generation of the amorphous drug nanoparticles is attributed to the fast dissolution velocity afforded by the nanoscale size, which in turn suppresses the solution-mediated crystallization of the amorphous solid phase upon dissolution [1].

Abbreviations: AUC, area under the curve; BCS, Biopharmaceutics Classification System; CHI, chitosan; CUR, curcumin; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; DR, dissolution rate; DST, disuccinimidyl tartrate; EDTA, ethylenediaminetetraacetic acid; EPR, enhanced permeability and retention; FESEM, Field Emission Scanning Electron Microscope; FTIR, Fourier Transform Infrared Spectroscopy; HPLC, High Performance Liquid Chromatography; HPMC, hydroxypropylmethylcellulose; M_{DST/NH_2}, molar ratio of DST to NH_2; MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide); NHS, N-hydroxysuccinimide; OD_{550}, optical density at 550 nm; OD_{600}, optical density at 600 nm; PBS, phosphate buffered saline; PCS, photon correlation spectroscopy; PVP, polyvinylpyrrolidone.

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Conventionally, amorphous drug nanoparticles are prepared by incorporating polymeric stabilizers having high glass transition temperatures, such as hydroxypropylmethylcellulose (HPMC) and polyvinylpyrrolidone (PVP), to occupy the high-energy sites of the drug nanoparticles immediately after their formation [7]. The roles of the polymeric stabilizers are twofold, i.e. (1) to inhibit the post-nucleation growth of the drug nanoparticles and (2) to suppress the crystallization propensity of the amorphous form during storage. Using this nanoscale amorphization strategy, various nanoparticle precipitation methods (e.g. antisolvent [1], pH-shift [6], ultrasonic [4], and evaporative [8] precipitations) have been employed in the presence of polymeric stabilizers to prepare amorphous drug nanoparticles.

Recently, we developed a new class of amorphous drug nanoparticles in the form of drug–polysaccharide nanoparticle complex (or nanoplex in short) by self-assembly electrostatically-driven complexation between drug molecules and oppositely charged polysaccharides [9]. In this nanoscale amorphization strategy, the stabilization was accomplished by restricting the molecular mobility of the drug molecules by means of physical binding with the polysaccharide chains. As the nanoplex was held together primarily by electrostatic binding, it readily dissociated in salt solution, such as phosphate buffered saline (PBS), resulting in supersaturation generation [9]. Using itraconazole as the model poorly-soluble drug, the itraconazole nanoplex was shown to exhibit superior colloidal and amorphous state stabilities, as well as prolonged supersaturation, compared to the nano-itraconazole prepared by the more conventional high-energy site occupation strategy [9].

Amorphous drug nanoparticles prepared by both strategies were known to produce the "spring and parachute" supersaturation profile characteristic of the amorphous form upon dissolution in the presence of crystallization inhibitors (e.g. HPMC, PVP) [1,9]. Initially, the drug was released in a burst pattern to produce the supersaturation peak (i.e. "spring"), followed by its gradual decline (i.e. "parachute"), resulting in a sustained supersaturation level before it eventually reached the thermodynamic saturation solubility [2]. In the absence of crystallization inhibitors, however, the rate of decline in the supersaturation level has been shown to increase with increasing burst release rates, resulting in a smaller area under the curve (AUC) for the supersaturation versus time profile, hence lower drug bioavailability *in vivo* is to be expected [10].

While the crystallization inhibitors can always be incorporated in oral solid dosage forms of amorphous drug nanoparticles intended for systemic delivery, there are therapeutic applications that would require the amorphous drug nanoparticles to be delivered locally by parenteral routes to the disease sites as stand-alone nanoparticles, instead of their granulated solid dosage form. Examples include (1) passive targeting of tumour tissues in anticancer therapies via the well-known enhanced permeability and retention (EPR) effect of nanoparticles [11], and (2) passive targeting of microbial pathogens embedded in a thick mucus (e.g. lung, eye) using mucus-penetrating antimicrobial nanoparticles [12]. For such applications, the amorphous drug nanoparticles ideally exhibit a slower burst release rate in the absence of crystallization inhibitors in order to maintain sufficiently high local bioavailability.

To the best of our knowledge, however, amorphous drug nanoparticles exhibiting controlled release functionality have not been developed before. In contrast, numerous studies have reported microscale amorphous solid dispersions exhibiting controlled drug release intended for oral systemic delivery, where the controlled release was achieved by varying the type and amount of the polymeric stabilizers used [13,14]. While there were several studies that reported controlled drug release from amorphous nanofiber drug dispersion prepared by electrospinning [15,16], the nanofibers were not classified as amorphous drug nanoparticles because only their diameters were in the nanoscale, while their lengths were in the millimetre scale.

Herein we presented the development of amorphous drug nanoplex exhibiting controlled release functionality achieved by crosslinking of the polysaccharide chains onto which the drug molecules were bound. The crosslinking was performed on the nanoplex prepared from the basic formulation immediately after its preparation. We hypothesized that the crosslinked polysaccharide chains would make the nanoplex structure more condensed, which in turn would slow down the escape of the drug molecules upon their dissociation from the polysaccharide chains in the dissolution medium.

Curcumin – a natural flavonoid isolated from turmeric plants classified as Biopharmaceutics Classification System (BCS) Class IV compound [17] – was used as the model poorly-soluble drug owed to its well-established therapeutic properties ranging from anti-inflammatory and anticancer to antimicrobial and antioxidant [18]. Moreover, curcumin as a weak acidic compound was readily ionized upon dissolution in base [19] (Fig. 1), thus making it ideal for transformation to the nanoplex by the drug–polysaccharide electrostatic complexation. Chitosan was used as the polysaccharide because (1) it was readily ionized in weak acid to produce opposite charge to that of curcumin (Fig. 1) [20], and (2) it contained amine (NH_2) functional groups that could be crosslinked upon the introduction of an amine-reactive crosslinking agent, such as disuccinimidyl tartrate (DST), which is classified as homobifunctional N-hydroxysuccinimide (NHS) esters [21].

The objective of the present work was to investigate the effects of the molar ratio of DST to the NH_2 group of chitosan in the nanoplex on the (1) physical characteristics (i.e. size, zeta potential, shape, payload) of the crosslinked nanoplex produced, (2) dissociation stability of the crosslinked nanoplex in PBS as compared to the unmodified nanoplex, and (3) *in vitro* dissolution time-profile of the crosslinked nanoplex under sink condition. In addition, the *in vitro* cytotoxicity of the crosslinked nanoplex towards human lung epithelium cells was characterized to examine whether the crosslinking had any adverse effect on the nanoplex's cytotoxicity. Lung epithelium cells were selected as the model cell because we envisioned that the amorphous curcumin–chitosan nanoplex could potentially find its application in passive-targeting therapy of respiratory infections.

2. Materials and methods

2.1. Materials

Materials for nanoplex preparation and characterization: Chitosan (CHI) having molecular weight (MW) of 50–190 kDa and 75–85% deacetylation, potassium hydroxide (KOH), sodium chloride (NaCl), sodium phosphate (Na_3PO_4) glacial acetic acid, ethanol, PBS (pH 7.4), and potassium bromide (KBr) were purchased from Sigma–Aldrich (Singapore). Curcumin (CUR) at 98% purity and disuccinimidyl tartrate (DST) were purchased from Alfa Aesar (USA) and Thermo Fisher Scientific (USA), respectively. *Materials for cytotoxicity test*: A549 adenocarcinomic human alveolar basal epithelial cells were purchased from ATCC (USA). About 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA) solution, p enicillin–streptomycin, dimethyl sulfoxide (DMSO), and (3-(4,5-d imethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT, 98% purity) were purchased from Gibco (Canada), PAA Laboratories (Austria), Sigma–Aldrich (Singapore), and Alfa Aesar (UK), respectively. Dulbecco's modified Eagle's medium (DMEM) and foetal bovine serum were purchased from HyClone (Thermo Scientific, USA).