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A supersaturating delivery system of silibinin exhibiting high payload achieved by amorphous nano-complexation with chitosan

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ABSTRACT

The therapeutic potentials of silibinin – a phytochemical isolated from milk thistle plants – have not been fully realized due to its poor oral bioavailability caused by the low aqueous solubility. Existing solubility enhancement strategies of silibinin by nanonization were limited by their low payload. Herein we developed a supersaturating delivery system of silibinin exhibiting a high payload (≈76%) in the form of amorphous silibinin–chitosan nanoparticle complex (or silibinin nanoplex in short) prepared by self-assembly drug-polysaccharide complexation. The effects of (1) pH and (2) charge ratio of chitosan to silibinin on the nanoplex's physical characteristics (i.e. size, zeta potential, and payload) and preparation efficiency (i.e. silibinin utilization, overall yield) were investigated. The formation of nanoplex (≈240 nm) was feasible only in a narrow pH range (5.1–5.8) and favored charge ratio below unity. At the optimal condition (pH 5.8 and charge ratio of 0.30), the nanoplex preparation exhibited 87% silibinin utilization rate and 63% yield signifying its high efficiency. The amorphous state and colloidal stabilities of the nanoplex during storage, and prolonged supersaturation generation (3 h) at more than 10× of the saturation solubility were successfully demonstrated.

1. Introduction

The hepatoprotective effect of silibinin – a natural biologically active flavonolignans isolated from milk thistle plants – has been well established (Fraschini et al., 2002). Silibinin has also been demonstrated to possess antioxidant (Naso et al., 2011), anti-inflammatory (Gupta et al., 2000), and anticancer properties (Deep and Agarwal, 2010). The oral bioavailability of silibinin, however, is extremely low (<1%) caused primarily by its poor gut absorption and phase II metabolism in the liver (Theodosiou et al., 2014; Wu et al., 2007). Complexation of silibinin with phospholipids was shown to improve the gut absorption and in turn the bioavailability of silibinin attributed to the higher lipophilicity of the silibinin–phospholipid complex, resulting in better gut permeability (Kidd and Head, 2005; Xiao et al., 2006). A separate study, however, determined that the gut permeability was not the rate-limiting step in the gut absorption of silibinin, instead it was the slow dissolution rate of silibinin due to its poor solubility in the gastrointestinal fluid (Wang et al., 2010).

Hence, not coincidentally, a majority of studies on bioavailability enhancement of silibinin set their aims at improving the dissolution rate by means of nanonization to take advantage of the large specific surface areas afforded by nanoparticles. Various nanoformulation platforms ranging from liposomes (El-Samaligy et al., 2006), solid lipid nanoparticles (Zhang et al., 2007), polymer nanoparticles (Pooja et al., 2014) to porous silica nanoparticles (Cao et al., 2013) and nano-emulsions (Wu et al., 2006) have been employed as delivery vehicles for silibinin. Recently, a combined approach in which silibinin–phospholipid complex was encapsulated in liposomes was also pursued (Angelico et al., 2014).

These nanoformulation strategies, however, possess a major drawback in their low silibinin payloads (<15 wt.%). The low payload leads to a high dosing requirement to achieve the therapeutic effect in which a large fraction of the administered dose is made up of carrier materials that not only end up wasted, but also possibly have adverse health effects due to their large amount. Moreover, the high dosing requirement would make therapy regimen of silibinin too costly for most patients, hence limiting its potential for widespread clinical applications. To address this drawback, Wang et al. (2010) avoided the use of carriers altogether and developed carrier-free silibinin crystalline nanoparticles exhibiting high payload (75%). However, their nano-silibinin preparation was lengthy and energy-intensive involving multiple cycles of high-pressure homogenizations.

Furthermore, even though the above-mentioned nanoformulation strategies could enhance the dissolution rate of silibinin, the amount

Abbreviations: AA, acetic acid; C, supersaturated concentration; C_{sat}, saturated solubility; CE, complexation efficiency; CHI, chitosan; DSC, differential scanning calorimetry; FESEM, field emission scanning electron microscopy; FTIR, Fourier transform infrared spectroscopy; HPLC, high performance liquid chromatography; HPMC, hydroxypropyl methylcellulose; PBS, phosphate buffered saline; PCS, photon correlation spectroscopy; PXRD, powder x-ray diffraction; $R_{CHI/SLB}$, charge ratio of chitosan to silibinin; SGJ, simulated gastric juice; SIJ, simulated intestinal juice; SLB, silibinin; T_g, glass transition temperature; TGA, thermogravimetric analysis; UV–Vis, ultraviolet visible spectroscopy.

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of silibinin available for absorption remained limited due to its low thermodynamic saturation solubility (<0.1 mg/mL) (Bai et al., 2006). A supersaturating drug delivery system that can generate a highly supersaturated drug concentration upon dissolution is therefore ideal for silibinin as the said system can produce an apparent solubility that is multifold higher than the thermodynamic saturation solubility (Brouwers et al., 2009). Enhanced bioavailability would then ensue provided that the high apparent solubility is maintained for duration sufficient for gut absorption. This is achieved by incorporating crystallization inhibitors, such as hydroxypropyl methylcellulose (HPMC), in the dosage formulations (Tajarobi et al., 2011).

For this purpose, several studies have developed supersaturating delivery systems of silibinin in the form of microscale amorphous solid dispersions (Li and Hu, 2004; Qiu et al., 2005; Sun et al., 2008). These amorphous silibinin formulations, however, also exhibited low payloads due to the large amount of polymer excipient required in solid dispersions to stabilize the metastable amorphous form (Laitinen et al., 2013). Herein we developed a high-payload supersaturating delivery system of silibinin in the form of amorphous silibinin–chitosan nanoparticle complex (or nanoplex in short).

The amorphous silibinin nanoplex was prepared by the self-assembly electrostatically-driven drug–polysaccharide complexation developed previously by our group (Cheow et al., 2014; Nguyen et al., 2015). The method was simple, rapid, and having a low energy requirement involving only ambient mixing of the drug and polysaccharide solutions. Chitosan was used as the polysaccharide because (1) it could be readily ionized in base to produce charge opposite to silibinin, and (2) its inclusion in dosage formulation has been shown to improve intestinal absorption of the drug attributed to the chitosan-mediated epithelial tight junction opening (Sonaje et al., 2012; Yeh et al., 2011).

In this method, negatively charged silibinin (after its deprotonation in base) were mixed with oppositely charged chitosan, resulting in the self-assembly formation of soluble silibinin–chitosan complex as illustrated in Fig. 1. The soluble complex subsequently aggregated due to hydrophobic interactions among the bound silibinin molecules. Upon reaching a critical aggregate concentration, whose value was dictated by the hydrophobicity of silibinin, the complex aggregates precipitated out to form the silibinin nanoplex. The amorphous form was realized because the strong electrostatic interactions between silibinin and chitosan inhibited the former from assembling into ordered crystalline structures during precipitation.

The objectives of the present work were to investigate the effects of the two governing process variables in the drug–polysaccharide complexation (i.e. pH and charge ratio of chitosan to silibinin) on the (1) physical characteristics of the nanoplex produced (i.e. size, colloidal stability, and payload) and (2) preparation efficiency (i.e. silibinin utilization, overall yield). Subsequently, the silibinin nanoplex prepared at the optimal pH and charge ratio was characterized for its (i) colloidal stability during storage at 25 °C, (ii) supersaturation generation, and (iii) amorphous state stability during prolonged storage after drying.

2. Materials and methods

2.1. Materials

Silibinin (SLB) (98% purity), low molecular weight chitosan (CHI) (50–190 kDa), potassium hydroxide (KOH), glacial acetic acid (AA), hydroxypropyl methylcellulose (HPMC), phosphate buffered saline (PBS, pH 7.4), sodium chloride (NaCl), hydrogen chloride (HCl), potassium phosphate monobasic (KH_2PO_4), potassium bromide (KBr), and ethanol were purchased from Sigma-Aldrich (USA).

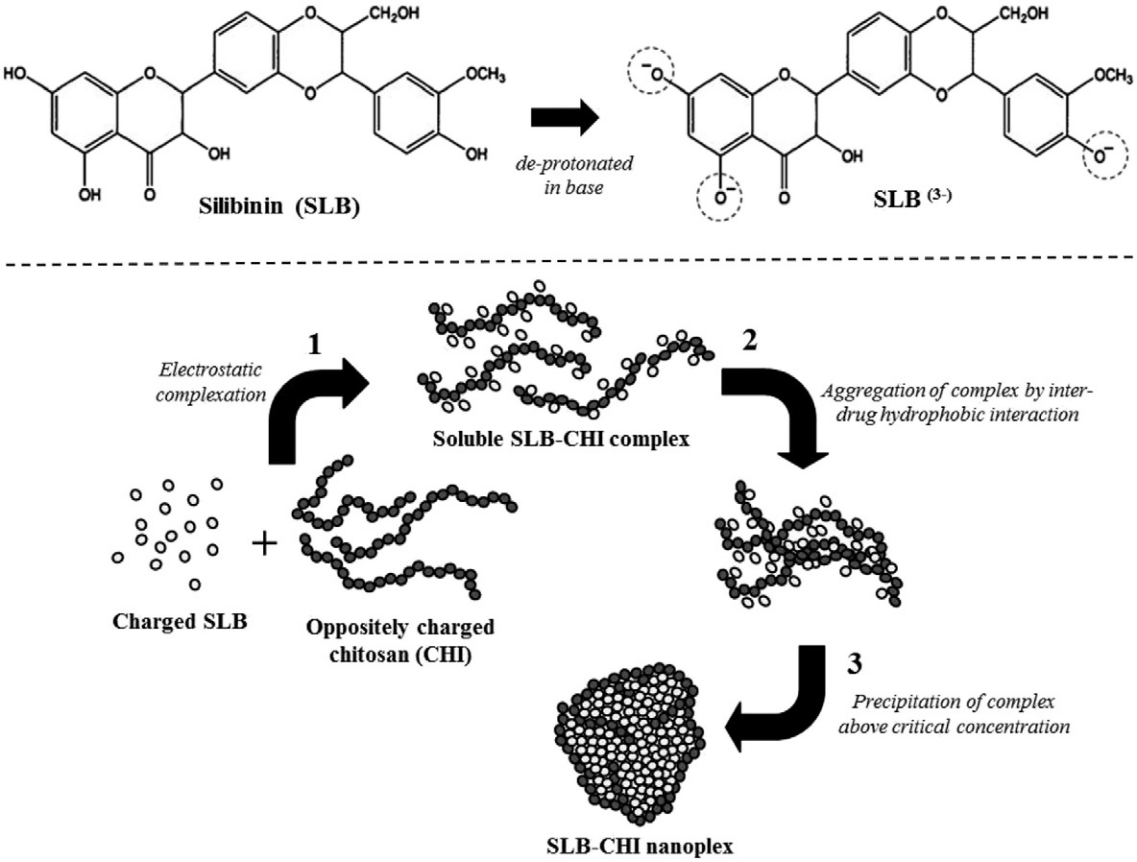

Fig. 1. Complexation of silibinin (SLB) with oppositely charged chitosan (CHI) to form amorphous silibinin–chitosan nanoparticle complex (or SLB–CHI nanoplex in short).