Pharmaceutical nanotechnology

A highly sustainable and versatile granulation method of nanodrugs via their electrostatic adsorption onto chitosan microparticles as the granulation substrates

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ABSTRACT

Nanodrugs play important roles in enhancing the sustainability of pharmaceutical manufacturing via their ability to enhance the bioavailability of poorly soluble drugs, resulting in less drug wastage and less mass/energy consumed in their manufacturing. Despite their sustainability enhancement capability, solid dosage form manufacturing of nanodrugs remains lacking from the sustainability perspective. One example is the granulation of nanodrugs prior to tablet preparation, where existing methods (e.g. wet granulation, spray granulation, spray drying) require high energy and time expenses, or are highly intricate often leading to product inconsistencies. Herein we present an alternative nanodrug granulation method via electrostatic adsorption of the nanodrugs onto chitosan microparticles acting as granulation substrates. The method is sustainable involving only mixing of aqueous suspensions of the nanodrugs and substrates under ambient conditions, followed by washing and drying. We investigate the effects of substrate's physical characteristics and nanodrug to substrate ratio on the nanodrug loading in the granules, content uniformity, nanodrug recovery, and granule flowability. Ciprofloxacin and curcumin nanoplexes prepared by drug–polyelectrolyte complexation are used as the model nanodrugs with neutrally, positively, and negatively charged chitosan microparticles as the substrates. Granules having 25% (w/w) nanodrug loading at 50% (w/w) recovery with good flowability have been successfully prepared.

1. Introduction

The role of drug nanoparticles – nanodrugs in short – in the sustainability enhancement of pharmaceutical manufacturing has been well recognized, which can be attributed to their ability in enhancing the bioavailability of poorly soluble drugs categorized as Biopharmaceutics Classification System (BCS) Class II and IV molecules (Shegokar and Muller, 2010). The high bioavailability afforded by the nanodrugs is attributed primarily to the high drug dissolution velocity resulted from their high surface area to volume ratio. The high bioavailability leads to a lower dose requirement, which in turn reduces drug wastage and overall mass/energy consumption in the solid dosage form manufacturing, resulting in a more sustainable manufacturing practice.

Ironically, despite their important role in the sustainability enhancement, the current state of nanodrug manufacturing remains far from being sustainable, where the industrially practiced methods are dominated by those exhibiting high energy expense, heavy use of solvents, low mass efficiency, and batch-to-batch consistency (e.g. wet milling, antisolvent nanocrystallization, spray drying) (Chan and Kwok, 2011; Matteucci et al., 2006; Van Eerdenbrugh et al., 2008). To address this issue, we have developed a truly sustainable nanodrug preparation method via self-assembly drug–polyelectrolyte complexation process, which is rapid, energy minimal, solvent free, and produces high yield and drug content (Cheow and Hadinoto, 2012). The next challenge that we aim to tackle here is to enhance the solid dosage form manufacturing sustainability of nanodrugs.

To prepare their solid dosage forms (e.g. tablets, capsules), nanodrugs must first be transformed into highly flowable and uniformly sized granules that are made up of agglomerates of the nanodrugs. Conventionally, the nanodrugs are transformed into granules by one of the following methods – (1) spray drying with water-soluble excipients, whose primary function is to prevent irreversible agglomeration of the nanodrugs (Kho et al., 2010; Li

Abbreviations: AA, acetic acid; BCS, biopharmaceutics classification system; CCM, curcumin; CIP, ciprofloxacin; CS, chitosan; DSC, differential scanning calorimetry; DXT, dextran sulfate; FE-SEM, field emission scanning electron microscopy; GRAS, generally regarded as safe; HMW, high molecular weight; LMW, low molecular weight; MW, molecular weight; PAH, poly(allylamine hydrochloride); PCS, photon correlation spectroscopy; PE, polyelectrolyte; PBS, phosphate buffer saline; PLGA, poly(lactic-co-glycolic) acid; TGA, thermal gravimetric analysis; TPP, triply phosphate.

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et al., 2011), (2) low-shear wet granulation in the presence of binder and filler materials, followed by wet milling or sieving to obtain the desired size (Friedrich et al., 2010; Schmidt and Bodmeier, 1999), and (3) spray granulation in which the nanodrugs are sprayed onto fluidized microparticle substrates (e.g. lactose, mannitol) aided by binder materials (Basa et al., 2008; Bose et al., 2012). In this regard, the fluid-bed granulation method, which has been proven effective for drug microparticles, has not been attempted for nanodrugs due to their minute size that renders their fluidization extremely challenging (van Ommeren et al., 2012).

Even though spray drying is a straightforward method capable of producing granules with the desired characteristics, from the sustainable manufacturing perspective, it is far from ideal as it requires high energy expense and often results in low production yield due to the significant nanodrug loss in the harsh environment of the drying chamber (Kho et al., 2010). Likewise, the low-shear wet granulation method is lengthy and energy expensive due to its multiple processing steps. Moreover, using this method, the nanodrugs are often trapped inside the filler materials resulting in ineffective drug release that leads to drug wastage (Friedrich et al., 2010).

Unlike spray drying and low-shear wet granulation, spray granulation is not characterized by the abovementioned unsustainable manufacturing characteristics. However, spray granulation is a highly intricate process in which the granule characteristics produced (e.g. size, drug content) are dependent on numerous parameters, in particular, fluidization velocity (Basa et al., 2008; Bose et al., 2012). As high fluidization velocity is needed to prevent agglomeration of the granulation substrates, and to obtain good layering of the nanodrugs on the substrates, breakage and attrition are unavoidable (Poutiainen et al., 2012). This in turn results in wide granule size distributions, non-uniformity in the drug content, and batch-to-batch product inconsistencies, which would lead to high product rejection rates, resulting in unsustainably low mass efficiency (Schmidt and Bodmeier, 1999).

Herein we present an alternative nanodrug granulation method via electrostatic adsorption of the nanodrugs to oppositely charged granulation substrates as illustrated in Fig. 1. This alternative granulation method is highly sustainable because it can be performed in short time under ambient conditions resulting in low energy expense. The method is simple involving only mixing of aqueous suspensions of the nanodrugs and the granulation substrate, followed by washing and drying steps. Therefore, this method can be effortlessly incorporated into an existing nanodrug manufacturing line. The method is also highly versatile because all nanodrugs inherently carry charges as part of their colloidal stabilization mechanism, while substrates having components exhibiting the opposite charges from those of the nanodrugs can be easily prepared.

In the present work, chitosan microparticles are selected as the granulation substrate because of its sustainability and generally regarded as safe (GRAS) features. Chitosan is obtained from alkaline deacetylation of chitin, which is the second most abundant natural polysaccharide next to cellulose. For solid dosage form preparation, chitosan has been used as diluents in direct compression tableting, drug release modifiers in tablets, and binders in wet granulation (Chinta et al., 2009; Henriksen et al., 1993; Knapczyk, 1993). Furthermore, chitosan microparticles have been extensively used as drug delivery vehicles (Sinha et al., 2004).

We examine the effects of the charge and morphology of the chitosan microparticles, and nanodrug to substrate mass ratio on the nanodrug loading in the granules, nanodrug recovery, nanodrug content uniformity, size distribution, and flowability of the granules. Three types of granulation substrates (i.e. neutrally, negatively, and positively charged) are prepared by ionic crosslinking of chitosan with GRAS natural polyanions and polycations. Two stable amorphous nanodrugs of ciprofloxacin and curcumin – both categorized as BCS Class IV drugs (Breda et al., 2009; Wahlang et al., 2011) – prepared by the aforementioned drug–polyelectrolyte complexation process are used as the anionic and cationic nanodrug models, respectively.

2. Materials and methods

2.1. Materials

High molecular weight (HMW) chitosan (CS) (MW = 310–375 kDa, 75–85% deacetylation), low molecular weight (LMW) CS (MW = 50–190 kDa, 75–85% deacetylation), sodium tripolyphosphate (TPP), ciprofloxacin (CIP), sodium chloride (NaCl), potassium hydroxide (KOH), glacial acetic acid (AA), rhodamine 6G, Pluronic F68, and phosphate buffer saline (PBS) are purchased from Sigma–Aldrich (USA). Poly(allylamine hydrochloride) (PAH) (MW = 120–200 kDa) – a synthetic cationic polyelectrolyte (PE) widely used in drug and gene deliveries – and curcumin (CCM) are purchased from Alfa Aesar (USA). Dextran sulfate (DXT) (MW = 5 kDa) – an anionic PE – is purchased from Wako Pure Chemical Industries (Japan). Poly(lactic-co-glycolic) acid (PLGA) (Purasorb 5004A) is received as a gift from PURAC Biomaterials (Netherlands).
2.2. Methods

2.2.1. Preparation and characterization of the nanodrugs

The anionic CIP and cationic CCM nanodrugs are prepared in the form of drug–PE nanoparticle complex (i.e. nanoplex) by the self-assembly drug–PE complexation method detailed in (Cheow and Hadinoto, 2012). In principle, CIP being an amphoteric drug forms cations when it is dissolved in aqueous AA solution at pH lower than its pK_{a1} (=6.1), whereas acidic drug CCM forms anions when it is dissolved in aqueous KOH solution at pH larger than its pK_{a} (=10.2). The ionized drug solution is subsequently added to oppositely charged aqueous PE solution (i.e. anionic DXT for CIP and cationic PAH for CCM) in the presence of NaCl, upon which drug–PE electrostatic interactions take place resulting in the nanoplex formation. The drug content of the nanoplex is quantified using UV–Vis spectrophotometer at absorbance wavelength of 463 and 263 nm for CIP and CCM, respectively. The size and zeta potential of the nanoplex are characterized by photon correlation spectroscopy (PCS) using Brookhaven 90Plus (Brookhaven Instruments, USA). In addition to the nanoplexes, for comparison purposes, cationic PLGA nanoparticles are prepared by emulsification-solvent-evaporation.

2.2.2. Preparation and characterization of the granulation substrates

The neutrally charged granulation substrate is prepared by ionic crosslinking of CS with TPP as illustrated in Fig. 2A. Briefly, HMW CS is dissolved in 10 mL 1.0% (v/v) aqueous AA solution at 2.0% (w/v). The aqueous CS solution is atomized using a two-fluid atomizer (BÜCHI B-290, Switzerland) into 500 mL 2.5% (w/v) aqueous TPP solution under constant stirring, resulting in the formation of CS/TPP crosslinked microparticles. The distance between the atomizer and the TPP solution is kept at 40 cm. The atomizer feed rate is fixed at 180 mL/h, while the compressed air rate is varied between 160 ± 30 L/h to produce substrates of different sizes. 0.2% (w/v) Pluronic F68 is added in the TPP solution to prevent agglomeration of the CS/TPP microparticles on the liquid–air interface. In addition, pH of the TPP solution is adjusted to 5 by adding AA to optimize
crosslinking density, hence strengthening physical integrity of the CS/TPP microparticles (Bhumkar and Pokharkar, 2006).

The CS/TPP microparticles are aged in the solution for 1 h after which the suspension is filtered and washed thrice with deionized water. Their net charge density, which consists of contributions from the free (i.e., not crosslinked) charges of the cationic CS and the anionic TPP, is determined by charge titration following the method of (Kam and Gregory, 1999). The step-by-step procedures of the charge titration are presented in the Supplementary Information’s Section 1. The negatively charged granulation substrate is prepared by the same procedures using 1.0% (w/v) HMW CS, but by replacing the crosslinker solution with aqueous solution of 1.0% (w/v) TPP and 1.0% (w/v) DXT, resulting in the formation of DXT-layered CS/TPP microparticles (Fig. 2B).

The positively-charged granulation substrate is prepared by having an additional CS layer, either HMW CS or LMW CS, ionically crosslinked to preformed CS/TPP microparticles (Fig. 2C). Briefly, 1 mL of the washed CS/TPP microparticle suspension is mixed with 1 mL of 0.4% (w/v) aqueous CS solution (i.e., the second crosslinking medium). The pH of the mixed suspension is adjusted to 3.5 after which it is placed in a shaking incubator for 90 min. The resulting CS-layered CS/TPP microparticles are filtered and washed thrice with deionized water. The effect of the ionic strength is studied by performing the second crosslinking step in 0, 0.1, and 0.5 M salt medium (i.e., NaCl). In total, six variations of the CS-layered CS/TPP microparticles are prepared (i.e., three salt concentrations each for the LMW and HMW CS layers).

2.2.3. Nanodrug granulation by electrostatic adsorption onto the granulation substrate

The feasibility of employing the neutrally charged CS/TPP microparticles, which possess both positive and negative charge components, as granulation substrates is examined using both the CIP and CCM nanoplexes. Whereas the positively charged CS-layered CS/TPP microparticles and the negatively charged DXT-layered CS/TPP microparticles are used for the adsorptions of the anionic CIP and cationic CCM nanoplex, respectively.

Briefly, fixing the final volume at 1 mL, aqueous nanoplex suspension is added to aqueous suspension of the granulation substrate at different nanoplex to substrate mass ratios (R). The mixed suspension is placed in a shaking incubator for 30 min, followed by three cycles of centrifugation and washing to remove the nonadsorbed nanoplex. On this note, the effect of two washing methods (i.e., invasive versus noninvasive) on the nanoplex adsorption is investigated using the PLGA nanoplexes. The details of the investigation from which the invasive method is determined to be better are provided in the Supplementary Information’s Section 2. The washed nanodrug granules are freeze dried after which they are stored in dry cabinet for at least 48 h prior to their characterization.

The experimental nanoplex loading of the granules (i.e., % Nanoplex Loading in Eq. (1)) is determined by first dissolving 2 mg granules in 12 mL PBS at 37 °C followed by centrifugation, from which the amount of drug per unit mass of the granules is determined from the supernatant by UV–Vis spectroscopy. Using this information, the % Nanoplex Loading can then be calculated as the drug content in the nanoplex is known. The uniformity of the % Nanoplex Loading among the granules is characterized by its coefficient of variation (CV) obtained from twelve sampling units. The typical acceptance criteria for the % CV – defined as the relative standard deviation (i.e., standard deviation over mean) – for uniform content is approximately 7.8% (Senderak, 2009).

The efficiency of the adsorption process – defined as the ratio of the mass of nanoplex adsorbed to the mass of nanoplex in the feed (i.e., % Nanoplex Recovery in Eq. (2)) – can be calculated from R and % Nanoplex Loading as shown in Eq. (2). The step-by-step derivation of Eq. (2) is provided in the Supplementary Information's Section 3. A low value for the % Nanoplex Recovery signifies a large deviation between the experimental loading and the theoretical loading (i.e., at 100% Nanoplex Recovery), which is given as R/(1 + R). The % Nanoplex Loading and % Nanoplex Recovery reported here are based on a minimum of three replicates.

\[
\% \text{ Nanoplex Loading} = \frac{(\text{Mass of nanoplex})_{\text{adsorbed}}}{\text{Mass of granule}}
\]

\[
\% \text{ Nanoplex Recovery} = \frac{\% \text{ Nanoplex Loading}}{R \times (100 - \% \text{ Nanoplex Loading})}
\]

2.2.4. Physical characterizations of the nanodrug granules

The granule morphology is examined by field emission scanning electron microscopy (FE-SEM) (JSM-6700F, JEOL, USA), where the granule size is determined from the FE-SEM images with a minimum of 200 particle counts. For non-spherical granules, the size is characterized in terms of the maximum and minimum Feret diameters, where the Feret diameter represents the distance between two parallel lines tangent to the particle boundary measured at different angles. The tap density (\(\rho_{\text{tap}}\)) of the granules is determined after 2000 taps using tap densitometer (Quantachromme, USA), whereas the bulk density (\(\rho_{\text{bulk}}\)) is obtained from the volume of a known mass of the granules without tapping. The granule flowability is
characterized by the Carr’s Index (Eq. (3)) using two replicates, where the granules are deemed as highly flowable when Carr’s Index \( \leq 21 \) and poorly flowable when Carr’s Index \( \geq 33 \) (Zheng, 2009).

\[
\text{Carr's Index} = \left( 1 - \frac{\rho_{\text{bulk}}}{\rho_{\text{tab}}} \right) \times 100\% \quad (3)
\]

The moisture content of the granules prepared by the optimal formulation is determined by thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) using TGA/DSC 1 (Mettler Toledo, USA). 5 mg of the granules was filled into an alumina pan and heated from 25 °C to 400 °C at 10 °C/min. The in vitro drug release rate from the granules is examined in comparison to that of the original nanoplex powders. For this purpose, 300 µg of the nanoplex and 3 mg of the nanoplex granules are suspended in 2 mL PBS at 37 °C. At fixed time intervals, 0.2 mL aliquot is withdrawn, centrifuged, and the supernatant was filtered and diluted 10-fold after which the drug concentration in the supernatant is measured by UV–Vis spectrophotometer.

3. Results and discussion

3.1. Physical characteristics of the nanodrugs

The CIP nanoplex is highly spherical (Fig. 3A) with an average size of \( \approx 340 \) nm and zeta potential of \( \approx -44 \) mV owed to the presence of the anionic DXT on its surface acting as a stabilizer. The cationic CCM nanoplex is considerably smaller with an average size of \( \approx 70–100 \) nm (Fig. 3B) and zeta potential of \( \approx +40 \) mV owed to the stabilization provided by the cationic PAH. The CIP and CCM contents in the nanoplexes are, respectively, \( \approx 92\% \) (w/w) (or 8% DXT) and 45% (w/w) (or 55% PAH). The PLGA nanoparticles are also spherical (image not shown) in the size range of \( \approx 120–150 \) nm and with zeta potential of \( \approx +34 \) mV.

3.2. Physical characteristics of the granulation substrates

The charge density and size of the bare (i.e. no adsorbed nanoplex) granulation substrates, in both their wet suspension and dry powder states, are presented in Table 1, whereas the morphology, including the FE-SEM and light microscope images, and size distribution of the bare substrates are presented in the Supplemental Information’s Sections 4 and 5.

<table>
<thead>
<tr>
<th>Granulation substrate</th>
<th>Charge density (mequiv/g)</th>
<th>Wet size (µm)</th>
<th>Dry size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXT-layered CS/TPP microparticles</td>
<td>(-)</td>
<td>420</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td>0.51</td>
<td>290</td>
<td>140</td>
</tr>
<tr>
<td>CS/TPP microparticles</td>
<td>( \approx 0 )</td>
<td>600</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>480</td>
<td>260</td>
</tr>
<tr>
<td>CS-layered CS/TPP microparticles</td>
<td>(+) 0.03</td>
<td>500–1000</td>
<td>200–450</td>
</tr>
</tbody>
</table>

Similar morphology is observed for the neutrally charged granulation substrates (i.e. the CS/TPP microparticles), with the only difference is that they exhibit smooth surfaces after drying.

Table 1 Physical characteristics of the three granulation substrates used.
substrates, the particle size distributions are significantly improved toward monodispersity upon drying. On this note, the neutral net charge of the CS/TPP microparticles is as a result of the charge contribution from the free cationic CS being canceled out by that of the free anionic TPP.

In contrast, the positively charged granulation substrates (i.e., the CS-layered CS/TPP microparticles) are not spherical in shape. In fact, their shapes can vary from **highly dimpled spheres to concave rectangles** to irregular forms depending on (1) MW of the layered CS and (2) salt concentration in the second crosslinking medium. The shape transformation from the spherical shape is likely due to the interaction between CS in the second crosslinking medium and the free TPP in the preformed CS/TPP microparticles. The effects of the MW of the layered CS and the salt concentration of the second crosslinking medium on the morphology of the CS-layered CS/TPP microparticles are presented in the Supplementary Information’s Section 5.

In brief, the use of HMW CS as the layered CS leads to the formation of highly dimpled spherical particles that turn into irregularly shaped particles as the salt concentration in the second crosslinking medium is increased. The same effect, albeit smaller particles are produced, is observed using LMW CS as the layered CS, where concave rectangular particles are produced at high salt concentrations. In this regard, the higher salt concentration results in greater charge screening effects that in turn increase the interactions between the CS in the second crosslinking medium and the preformed CS/TPP microparticles. Despite the change in the particle shapes, the substrate particle sizes characterized by their maximum and minimum Feret diameters do not vary widely, where a majority of them fall in the range of ≈200–450 μm for all the six substrate formulations investigated. Furthermore, the charge density remains relatively constant at ≈(+)0.02–0.03 mequiv/g independent of the formulation.

3.3. Sustainable nanoplex granulation via electrostatic adsorption

3.3.1. Using the neutrally charged granulation substrate

The effect of particle size of the substrate on the % Nanoplex Loading is investigated in the size range suitable for tableting (i.e., ≈150–300 μm after drying, or 300–600 μm before drying). Adsorptions of the anionic CIP and cationic CCM nanoplexes on the CS/TPP microparticles of three different sizes (i.e., 600, 480, 340 μm in the wet state) are performed at R = 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2.

For the CIP nanoplex, the results indicate that the % Nanoplex Loading is constant at ≈2%, regardless of the R values and the substrate size. The % Nanoplex Loading is thus much lower than the theoretical nanoplex loading (i.e., ≈16–54% for the R range investigated) denoting very poor % Nanoplex Recovery (i.e., ≤10%). Likewise, the % Nanoplex Loading for the CCM nanoplex is also constant and only slightly higher at ≈5–6%. As a result of the low % Nanoplex Loading, the morphology of the nanodrug granules presented in the FE-SEM images in Fig. 4 closely resembles that of the CS/TPP microparticles, where uniformly sized spherical granules in the size range of ≈190–330 μm are produced. The granules also exhibit high flowability with Carr’s Index in the range of ≈10–15.

The low % Nanoplex Loading is not due to insufficient nanodrug or insufficient substrate areas available for adsorption, because the % Nanoplex Loading is not improved at higher R values or smaller substrate sizes, which theoretically yield larger adsorption areas per unit volume. Therefore, the low % Nanoplex Loading must be due to poor nanodrug adsorption as a result of the lack of electrostatic driving force between the substrate and the nanodrug. The present results signify the importance of the net charge of the substrate for effective adsorption, and not the charges of the components making up the substrate. This is in contrast to previous observations in electrostatic nanoparticle adsorption onto a substrate, albeit with non-drug and significantly smaller nanoparticles (i.e., polystyrene and silica), where it was reported that effective adsorption took place as long as the substrate was made up of components having opposite charges from the nanoparticles (Rana et al., 2005). Despite the good flowability and high uniformity of the present granules, the use of substrates with non-zero net charge is pursued next to improve the % Nanoplex Loading to a more acceptable level.

3.3.2. Using the negatively charged granulation substrate

Adsorptions of the cationic CCM nanoplex to the negatively charged DXT-layered CS/TPP microparticles of two different sizes (i.e., 420 and 290 μm in the wet state) are examined at R = 0.2, 0.4, and 0.6. Even though the CCM nanoplex can be adsorbed onto the substrate as shown in Fig. 5A, a significant amount of the CCM nanoplex forms agglomerates upon its immersion in the aqueous suspension of the substrate. These agglomerates are represented by the non-spherical highly elongated particles shown in Fig. 5B, which jeopardize accurate quantification of the % Nanoplex Loading. Furthermore, the nanodrug granules are yellowish, which is the natural color of CCM crystals, in contrast to the brownish color of the CCM nanoplex (images not shown). This color change
suggests that the CCM nanoplex undergoes de-complexation from PAH back to CCM upon interaction with the DXT-layered CS/TPP microparticles, followed by agglomeration of the CCM crystals.

As the same phenomena (i.e. de-complexation and agglomeration of the CCM nanoplex) are not observed earlier in the presence of CS/TPP microparticles, DXT is postulated to be the contributing factor. To test this postulate, the CCM nanoplex is placed in aqueous DXT solution of varying concentrations. Not unexpectedly, the CCM nanoplex immediately forms agglomerates and undergoes color change (image not shown). Thus, the CCM nanoplex is not a suitable nanodrug model for the DXT-layered CS/TPP granulation substrate due to its interaction with DXT. To examine the feasibility of employing the DXT-layered CS/TPP microparticles as granulation substrates, the adsorption experiment is performed using the cationic PLGA nanoparticles at \( R = 0.9 \). The results indicate that nanoparticle granules with % Nanoplex Loading \( \approx 20\% \) and % Nanoplex Recovery \( \approx 30\% \) are successfully produced, without forming agglomerates, as shown in the Supplementary Information's Section 6. Alternatively, other anionic polyelectrolytes, such as carrageenan, can be used in place of DXT.

3.3.3. Using the positively charged granulation substrate

Adsorptions of the anionic CIP nanoplex onto the positively charged CS-layered CS/TPP microparticles are studied using the six different substrate formulations, which differ in terms of their shapes and sizes (\( \approx 500–1000 \mu m \) before drying, or \( \approx 200–450 \mu m \) after drying) as a result of whether the HMW or LMW CS is present on their surfaces. For each substrate formulation, the adsorption study is carried out at \( R \) equal to \( 0.11 \pm 0.01, 0.25 \pm 0.02, \) and \( 0.55 \pm 0.04 \) for a total of eighteen experimental runs. The slight variations in the \( R \) values are due to the variations in the suspension concentration of the different substrate formulation. A list of the eighteen experimental runs performed and their identifiers (i.e. Run XYZ) are presented in Table 2, where \( X \) corresponds to MW of the layered CS (i.e. L = Low, H = high), \( Y = 1, 2, \) or 3 corresponds to the \( R \) values (i.e. 1 = 0.11 ± 0.01, 2 = 0.25 ± 0.02, and 3 = 0.55 ± 0.04), and \( Z = a, b, \) or \( c \) corresponds to the salt concentration in the second crosslinking medium (i.e. \( a = 0 \), \( b = 0.1 \), and \( c = 0.5 \) M).

The % Nanoplex Loading and % Nanoplex Recovery of the eighteen experimental runs are presented in Fig. 6, where demarcation lines at 10% Nanoplex Loading and 50% Nanoplex Recovery are drawn to mark what we deem as the minimum acceptable loading and recovery values. Runs that do not meet these demarcation criteria are not discussed. Overall, for all the experimental runs investigated, the % Nanoplex Loading obtained from using the positively charged granulation substrate is consistently higher by at least \( \approx 3–4\% \) than that obtained using the neutrally charged substrate, hence reaffirming the importance of the substrate's

<table>
<thead>
<tr>
<th>( R )</th>
<th>LMW CS</th>
<th>Salt in the 2nd crosslinking medium</th>
<th>HMW CS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.11 ± 0.01</td>
<td>Run L1a ← No salt →</td>
<td>Run H1a</td>
<td></td>
</tr>
<tr>
<td>0.25 ± 0.02</td>
<td>Run L2a ← No salt →</td>
<td>Run H2a</td>
<td></td>
</tr>
<tr>
<td>0.55 ± 0.04</td>
<td>Run L3a ← No salt →</td>
<td>Run H3a</td>
<td></td>
</tr>
</tbody>
</table>

Table 2
A summary of the eighteen experimental runs performed for CIP nanoplex adsorption onto the CS-layered CS/TPP microparticles.
Fig. 7. FE-SEM images of (A) concave rectangular CIP nanoplex granules from Run L3b, (B) a close-up view of their surface showing the adsorbed CIP nanoplex.

net charge in the nanodrug granulation via electrostatic adsorption. Furthermore, adsorption of the nanoplex onto the CS-layered CS/TPP microparticles has a minimal effect on the morphology compared to the original substrate, as shown in Fig. 7 in which the granules from Run L3b that yields the highest % Nanoplex Loading are used as the representative sample.

Specifically, among the nine runs that use the HMW CS layer, the % Nanoplex Loading is at its highest at ≈14–15% (Fig. 6A1) in Runs H2b and H2c (R = 0.25 ± 0.02) in which irregularly shaped substrates are used. Importantly, the % Nanoplex Loading for these two runs are only slightly lower than the theoretical loading at R = 0.25 ± 0.02 (i.e. 20%), which is reflected in the high % Nanoplex Recovery in the range of ≈70% for Run H2b and ≈85% for Run H2c (Fig. 6A2). In this regard, no direct correlation is observed between the % Nanoplex Loading and R, which is likely caused by the irregular shape of the substrates.

In contrast, for the nine runs that use the LMW CS layer, the % Nanoplex Loading is shown to increase with increasing R as the substrate has regular concave rectangular shapes. The best results in terms of the % Nanoplex Loading (i.e. ≈21–24%) are observed in Runs L3a and L3b (R = 0.55 ± 0.04) (Fig. 6B1). In these two runs, the % Nanoplex Recovery is ≈50% (Fig. 6B2) resulting in % Nanoplex Loading that is approximately 10% lower than the theoretical nanoplex loading (i.e. 35%). The adsorption experiment at R > 0.55 is not performed to increase the % Nanoplex Loading because the % Nanoplex Recovery ≈50% at R = 0.55 ± 0.04 suggests that a large amount of the nanoplex is not adsorbed, hence it is not advisable to increase R further.

Next, the uniformity of the % Nanoplex Loading within the granule population and flowability of the granules from Runs H2b, H2c, L3a, and L3b are examined to identify the overall optimal formulation. In terms of the content uniformity, the granules from Run L3b exhibit the lowest % CV at ≈8.3% (Fig. 8A), which is only slightly higher than the 7.8% threshold, hence denoting reasonably uniform % Nanoplex Loading. In contrast, the granules from the other three runs exhibit % CV > 9%. In terms of the flowability, the Carr’s Index of the granules from Run L3b is ≈28 (Fig. 8B) denoting their rather poor flowability, thereby flow aid is likely needed. The poor flowability is likely due to the high aspect ratio of their concave rectangular shape. In this regard, Run H2b yields granules with the best flowability with Carr’s Index ≈20. However, in comparison to Run L3b, granules from Run H2b suffer from significantly lower % Nanoplex Loading (i.e. 14% versus 24%) and higher % CV (i.e. 9.2% versus 8.3%) due to their irregular shape. Therefore, Run L3b is deemed superior provided that its flowability can be improved.

In this regard, it is noticed that a majority of the granules remain slightly damp after freeze drying due to incomplete water removal, which is supported by the TGA/DSC analysis that reports ≈6–7% (w/w) moisture content in the freeze dried granules (data not shown). It is postulated that the granule flowability can be improved by lowering the moisture content, which in turn reduces the powder cohesiveness. For this purpose, the granules from Run L3b are placed in the oven at 50 °C for 6h after which the Carr’s Index and mass loss are quantified. The results indicate that the granules experience ≈8% mass loss, which is comparable in magnitude to their initial moisture content. Importantly, the Carr’s Index
is reduced to \( \approx 21 \) making the Run L3b granules exhibiting good flowability. Lastly, the CIP release rates from the nanodrug granules are examined in comparison to that from the original nanoplex powders in Fig. 9. On this note, antimicrobial activity of the CIP released from the nanoplex powders has been earlier tested to be not affected by the electrostatic nanoplexation process (Cheow and Hadinoto, 2012). Two types of granules i.e. the CS/TPP microparticles and the CS-layered CS/TPP microparticles are examined. The results indicate that CIP is released rapidly in a burst pattern from the raw nanoplex powders, where \( \approx 90\% \) is released over 2 h. For the granules, the initial CIP release rates are closely similar to that of the raw nanoplex powders (i.e. burst release pattern), independent of the granulation substrates. However, the amount of CIP released from the granules reaches a plateau at \( \approx 60\% \) even after 6 h.

The diminished amount of CIP released from the granules is not unexpected because not all surface areas of the nanoplexes are exposed to the dissolution medium after their adsorption onto the granulation substrates. To achieve 100% CIP release from the granules, which would make the method truly sustainable, the immediate future research direction is to engineer nanodrug granules in which the nanodrugs spontaneously desorb from the substrate, plausibly via pH responsive mechanism, upon exposure to the dissolution medium, thereby the surface area available for dissolution is not compromised. In addition to enhancing the drug release from the granules, the future research also needs to investigate how well the chitosan-based granules can be compressed into solid dosage form having the correct physical properties (e.g. hardness, friability).

### 4. Conclusion

We have successfully demonstrated the feasibility of employing electrostatic nanodrug adsorption onto a preformed granulation substrate made up of polysaccharides as an alternative granulation method of nanodrugs, which is highly sustainable owed to its minimal energy requirement, fast adsorption process, high nanodrug recovery, and uniform product characteristics. Concave rectangular granules having relatively uniform nanodrug loading of up to \( \approx 25\% \) (w/w) at \( \approx 50\% \) (w/w) nanodrug recovery, with uniform size in the range of \( \approx 200–400 \mu m \), and good flowability are successfully prepared for anionic nanodrug and positively charged substrate pairs. Our study reveals that the net charge of the granulation substrate, and not the charges of the different components making up the substrate, governs the effectiveness of the adsorption process. Without the net charges, the adsorption is ineffective as manifested in the low nanodrug loading, regardless of the amount of nanodrug and substrate surface areas available for adsorption.

Using substrate having nonzero net charges, the nanodrug loading is shown to increase with increasing nanodrug to substrate ratio. Nevertheless, the optimal nanodrug to substrate ratio should be determined at a value that leads to both high nanodrug loading and reasonable nanodrug recovery. The study also suggests that certain nanodrug may interact with the components of the granulation substrate, which could lead to undesirable phenomena, such as nanodrug agglomeration or even its transformation. Thus, appropriate pairing of nanodrug and granulation substrates must be determined first. Lastly, the amount of drug released from the granules, not unexpectedly, is found to be less compared to that of the free nanodrugs. This issue can be solved by having nanodrugs that readily desorb from the substrates upon exposure to the dissolution medium.

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### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijpharm.2013.05.045.

### References


