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Pharmaceutical Nanotechnology

Nanosizing of a drug/carrageenan complex to increase solubility and dissolution rate

Wei-Guo Dai *, Liang C. Dong, Yan-Qiu Song

ALZA Corporation, 1900 Charleston Road, Mountain View, CA 94039, USA

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Abstract

In this study, we present a novel approach of nanosizing a drug/polymeric complex to increase both solubility and dissolution rate of poorly water-soluble compounds. A hydrophilic polymer, lambda-carrageenan, was first complexed with a model poorly water-soluble compound to increase the compound's aqueous solubility. The compound/carrageenan complex was further nanosized by wet-milling to enhance the dissolution rate. By complexing with carrageenan, the compound became amorphous in the complex. Using additional carrageenan as a stabilizer for nanosizing, a nanosuspension of a compound/carrageenan complex with a median particle size of about 0.3 μm was successfully developed. The particle size of the nanosuspension did not increase significantly during the lyophilization process and was stable for at least 39 days at room temperature after lyophilization. This approach of nanosizing a drug/carrageenan complex increased the aqueous solubility of the compound from less than 1 μg/mL to 39 μg/mL. In addition to increasing aqueous solubility, a nanosized compound/carrageenan complex had a faster dissolution rate than the complex, the free compound, and the nanosuspension of the free compound.
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Keywords: Nanosizing; Drug/polymeric complex; Carrageenan; Solubility; Dissolution

1. Introduction

Low oral bioavailability of poorly water-soluble drugs poses a great challenge during drug development (Lipinski et al., 1997). Various approaches have been developed to improve bioavailability by increasing a drug's dissolution rate and solubility. For example, nanosizing techniques have been used to enhance dissolution rate by increasing a drug's surface area, thereby improving oral bioavailability of poorly water-soluble drugs (Liversidge and Cundy, 1995; Muller et al., 2001; Merisko-Liversidge et al., 2003; Mueller and Akkar, 2004; Rabinow, 2004; Wu et al., 2004; Jinno et al., 2006). For some drugs with very low solubility, the nanosizing approach may be limited in improving oral bioavailability due to the solubility-limited absorption of these drugs (Langguth et al., 2005). A variety of strategies such as complexing, solid solutions, pharmaceutical salts, and prodrugs have been developed to enhance drug solubility for improving bioavailability. Limitations on dissolution rate, however, may be a potential concern for solid dosage forms of the drugs (Liu, 2000). Therefore, alternative techniques for increasing both dissolution rate and drug solubility are needed for these poorly water-soluble drugs.

Carrageenan is naturally occurring anionic polysaccharide extracted from red seaweed. It consists of the sulfate esters of galactose and 3,6-anhydrogalactose copolymers. The repeating unit of lambda-carrageenan is shown in Fig. 1 (Guiseley et al., 1980). Due to the existences of anionic sulfate groups, carrageenan can interact strongly with oppositely charged drugs by ionic interaction (Aguzzi et al., 2002; Bonferoni et al., 2004a,b; Graham and Baker, 1963; Graham et al., 1963). The drug/carrageenan ionic interaction decreases the drug's diffusivity or dissolution, leading to a sustained release of water-soluble drugs. Publications describe the use of a drug/carrageenan complex for oral controlled-release tablets (Bonferoni et al., 2000, 2004b; Aguzzi et al., 2002), ophthalmic formulations (Bonferoni et al., 2004a), and nasal inserts (Bertram and Bodmeier, 2006). However, complexation with carrageenan, as reported in the literature, has been used mainly with water-soluble drugs for

* Corresponding author at: Pharmaceutical Development, ALZA Corporation, 1900 Charleston Road, Mountain View, CA 94039, USA. Tel.: +1 650 564 5265; fax: +1 650 564 5398.
E-mail address: wdai2@alzus.jnj.com (W.-G. Dai).

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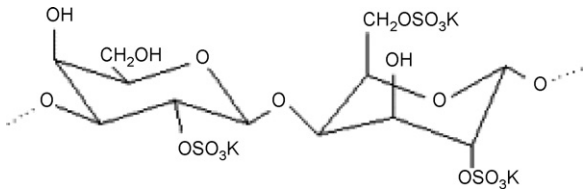

Fig. 1. Repeating units of lambda-carrageenan.

sustained release applications. Few of these studies used the drug/carrageenan complex to increase the aqueous solubility of poorly water-soluble compounds. Also, there have been relatively few publications on the preparation of a nanoparticulate drug/polymer complex using a wet-milling process.

We present a novel approach to prepare a nanoparticulate drug/polymer complex and demonstrate that this approach enables us to increase both solubility and dissolution rate for a poorly water-soluble compound. First a hydrophilic polymer, lambda-carrageenan, was complexed with a model poorly water-soluble compound to increase the compound's solubility. The compound/carrageenan complex was then nanosized by a wet-milling process to enhance the dissolution rate.

2. Materials and methods

2.1. Compound and excipients

A poorly water-soluble compound was obtained from the Johnson and Johnson Pharmaceutical Research and Development compound collection. This compound consists of C, H, F, N, O and S, with a molecular weight of 676.77 g/mol. It has a piperidine and a pyridine group.

Table 1 summarizes the molecular weight, pK_a, log P, solubility, solubility parameter, and melting point of the compound.

Lambda-carrageenan (Viscarin GP 109) was purchased from FMC Biopolymer (Philadelphia, PA). The following stabilizing excipients were used in the wet-milling for nanosizing: hydroxypropylmethylcellulose (HPMC-2910, Dow Chemical, Midland, MI), polyvinylpyrrolidone (Kollidon® K30) and Pluronic F127 (BASF, Mount Olive, NJ), dioctyl sodium sulfosuccinate (DOSS) and Tyloxapol (Sigma–Aldrich, St Louis, MO), hydroxypropyl cellulose (HPC-SL) (Nihon Soda Co., Japan), and Plasdone® S630 (ISP Technologies, Wayne, NJ).

Table 1
Physicochemical properties of the tested compound

Property	Value
Molecular weight (g/mol)	676.77
pK_a[a]	3.59, 7.8
log P[b]	2.14
Solubility	0.6 mg/mL at pH 1.2, ≤0.0002 mg/mL at pH 7.4
Solubility parameter (δ/MPa$^{1/2}$)[c]	27.77
Melting point[d] (°C)	167.4, 190.4

[a] pK_a was measured by potentiometric titration in a water/methanol mixture with UV absorption measurement.
[b] log P was determined by the shake-flask method with 1-octanol and buffer pH 10.
[c] The solubility parameter was estimated computationally using Molecular Modeling.
[d] Melting point was measured by DSC.

Simulated intestinal fluid (SIF) (pH 7.4) was prepared by adding 3.4 g of potassium phosphate monobasic (KH_2PO_4) and 0.8 g of sodium hydroxide (NaOH) to 0.50 L of purified deionized water.

2.2. Preparation of the compound/carrageenan complex

Five hundred milligrams of lambda-carrageenan were added into 50 mL of deionized water. After stirring overnight, a clear carrageenan solution at 10 mg/mL was obtained. The compound solution at 10 mg/mL was prepared by dissolving 500 mg of the compound in 50 mL of acidified water (pH 1.2). The carrageenan solution was then added slowly into the compound solution while stirring. The cloudy solution was centrifuged at 14,000 rpm for 2 min at room temperature. The compound concentration in the supernatant was measured using an HPLC gradient method with a lower quantification limit of 0.1 μg/mL, and the unbound compound fraction in the supernatants was calculated based on the initial amount of compound added. After centrifugation, the white precipitate was washed twice with acidified water (pH 1.2). After rinsed twice by deionized water, the precipitate was lyophilized, and stored at −20 °C.

2.3. X-ray diffraction (XRD)

X-ray powder diffraction was performed with a PANalytical X'Pert Pro Powder X-ray Diffraction System (PANalytical Inc., NATICK MA). The data were collected with an angular range between 3 and 35° 2Θ, using a step of 0.0167°/step with a scan rate of 0.209° 2Θ s^{-1}.

2.4. Differential scanning calorimetry (DSC)

A differential scanning calorimeter (Hyper-DSC, Perkin-Elmer, Boston, MA) was calibrated using indium. Samples (3–5 mg) were heated from 25 °C to 200 °C at 10 °C/min in aluminum pans under nitrogen atmosphere.

2.5. Nanosizing of the compound/carrageenan complex

The nanosuspensions were prepared with the NanoMill™ System (Elan, King of Prussia, PA) using Polymill 500 (polystyrene beads) as the grinding media. The excipient stock solutions (50 mg/mL) for nanosizing experiments were prepared by dissolving an appropriate amount of excipients in deionized water. The NanoMill™ System was charged with 5.43 g of grinding beads and 4.640 g of aqueous slurry containing 0.232 g (5 wt.%) of the complex and an appropriate amount of nanosizing excipients depending on the experimental design. The compound/carrageenan complex was milled at 5500 rpm for 1 h. The suspension was harvested, mixed with selected stabilizers, and lyophilized if necessary.

2.6. Particle size distribution

After milling, an aliquot of the nanosuspension was introduced into the water-filled sample cell of a Horiba-910 PS analyzer (Horiba, Irvine, CA) and the particle size distribution was measured. Values are reported for volume-weighted analyses. For the analysis of particle size of the lyophilized complex, the samples were first reconstituted in water. The appropriate volume of the complex dispersion was introduced into the sample cell for size analysis.

2.7. Solubility

The aqueous solubilities of the free compound and the compound/carrageenan complex in powder form were determined by a shake-flask method. Briefly, an excess amount of each sample was suspended in 1 mL of water, and the suspensions were shaken at 37 °C. Aliquots were withdrawn and filtered through a 0.2-μm polyvinylidene fluoride (PVDF) filter (pION, Woburn, MA). The filtered solution was diluted with acetonitrile, and the compound concentration in the filtrate was analyzed by an HPLC method. Equilibrium solubility was determined when the concentration of the compound in the suspension did not increase further with incubation time.

The aqueous solubilities of the nanosuspensions of the free compound and the compound/carrageenan complex were measured by an ultracentrifugal method. Briefly, 200 μL of nanosuspension were loaded into an ultracentrifugal tube. Samples were centrifuged at $1000 \times g$ for 45 min at 4 °C in a Beckman L-70 centrifuge using a Beckman 50.2 Ti rotor (Beckman, Palo Alto, CA). The compound concentration in a sample of the clear supernatant was measured by a gradient HPLC method.

2.8. Assay for dissolution rate

Fig. 2 shows the experimental setup for measuring dissolution rate. The samples were placed inside a SpectraPro dialysis membrane with a molecular weight cutoff of 6000–8000 Da (Spectrum Laboratories, Los Angeles, CA). The release medium outside the dialysis membrane was SIF (pH 7.4) with 2% (w/w) Pluronic F127 (average MW 12,600). The compound concentration in the release medium was measured using a gradient HPLC method at predetermined time points.

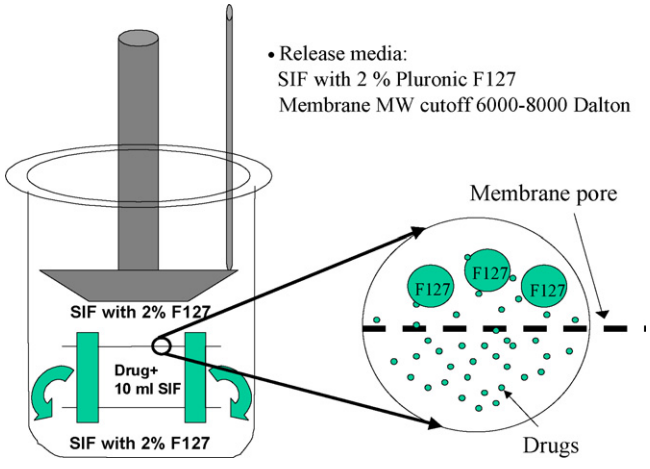

Fig. 2. Experimental setup for measuring dissolution rate.

2.9. HPLC assay

Ten microliters (10 μL) of the sample were injected into an Inertsil 5 μm ODS-2 C18 column (150 mm × 4.5 mm) (GL Sciences, Torrance, CA). The sample was eluted at 45 °C at 1 mL/min. by a gradient system consisting of 1% NaCl in 11.5 mM formic acid (mobile phase A) and acetonitrile (mobile phase B). The gradient system was programmed to decrease the proportion of mobile phase A from 80% to 20% in 5 min, to maintain 20% mobile phase A for 2 min, and finally to increase the proportion of mobile phase A back to 80% in 1 min. Compound concentration was quantitated at a UV wavelength of 254 nm. The retention time of the compound was 5.5 min during a total run time of 10 min per sample.

2.10. Statistical analysis

Results are depicted as mean ± S.D. All statistical comparisons were performed using MS Excel 2000 (Microsoft, Seattle, WA). A Student's *t*-test was conducted to determine statistical significance with a 95% confidence interval ($p < 0.05$).

3. Results and discussion

3.1. Physicochemical properties of the compound

Table 1 summarizes the molecular weight, pK_a, log *P*, solubility, solubility parameter, and melting point of the tested compound. This compound has a pH-dependent aqueous solubility: 0.6 mg/mL at pH 1.2 and ≤0.0002 mg/mL at pH 7.4. This low aqueous solubility at intestinal pH may lead to a poor oral bioavailability.

In addition, we found that even though the thermodynamic solubility of this compound was 0.6 mg/mL at pH 1.2, its kinetic solubility at that pH was as high as 10 mg/mL. The compound was also found to be chemically stable in this acidic environment for at least 2 h. This reasonably high kinetic aqueous solubility of the compound allowed us to prepare the compound/carrageenan complex in an aqueous medium.

3.2. Compound/carrageenan complex

Upon slow addition of lambda-carrageenan, the compound solution became cloudy, precipitation occurred, and the aqueous concentration of the compound decreased sharply (Fig. 3). The results indicated that the compound/carrageenan complex was formed. The compound has a piperidine (pK_a, 7.8) and a pyridine group (pK_a, 3.59), while carrageenan is a polysaccharide with anionic sulfate groups (Guiseley et al., 1980). At pH 1.2, the piperidine and pyridine groups in the compound were protonated and the sulfate groups in carrageenan were ionized. Upon slow

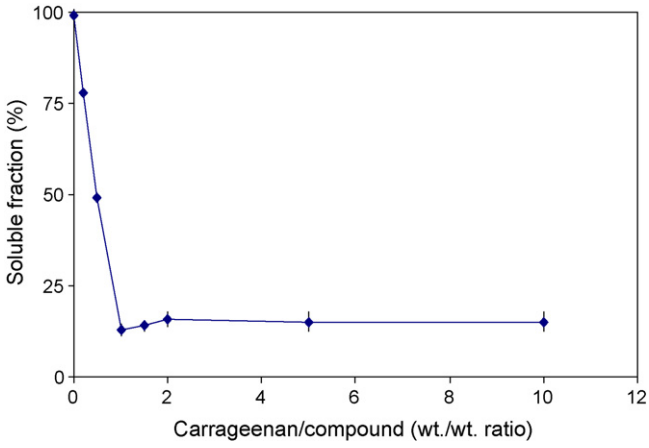

Fig. 3. Compound soluble fraction upon addition of lambda-carrageenan to the compound solution. Error bars represent standard deviations of three measurements.

addition of carrageenan to the compound solution, the positively charged compound bound with the negatively charged sulfate groups of the carrageenan. The complex formed and precipitated, leading to a sharp decrease in aqueous concentration of the compound (Fig. 3). Our results are in agreement with previous findings that carrageenan formed a complex with water-soluble drugs with basic groups (Graham and Baker, 1963; Graham et al., 1963; Aguzzi et al., 2002; Bonferoni et al., 2004b).

Evidence of complex formation was also found in the results of XRD and DSC. The free compound and its physical mixture with carrageenan did show the diffraction patterns of the crystalline structures in XRD, while the complex was found to be amorphous (Fig. 4). The amorphous structure of the complex was further confirmed by the DSC results. The complex showed a different DSC thermogram from the free compound and its physical mixture with the carrageenan. Two melting points at around 167 °C and 190 °C were found in the DSC thermograms for both free compound and the physical mixture, but not for the complex (Fig. 5). The absence of melting peaks in the DSC thermogram indicated that this complex was amorphous, which was also further confirmed by our polarized microscope experiments. These results are consistent with the findings reported by others (Bonferoni et al., 2000). We believe that the formation of an amorphous structure can be attributed to the random polymer chain structures in carrageenan. During the complex preparation, the compound was soluble in an aqueous medium and its crystalline structures were destroyed. When the compound bound with carrageenan, the polymer chains in carrageenan may have created the irregular orders/structures in the complex, leading to the formation of the amorphous structure.

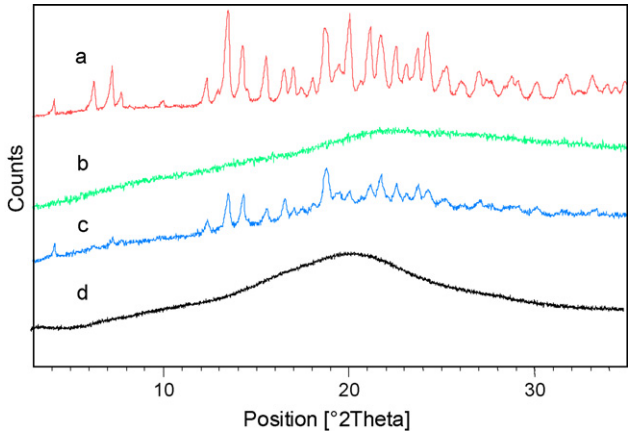

Fig. 4. XRD patterns of (a) the compound, (b) lambda-carrageenan, (c) physical mixture of compound with carrageenan, and (d) the compound/carrageenan complex.

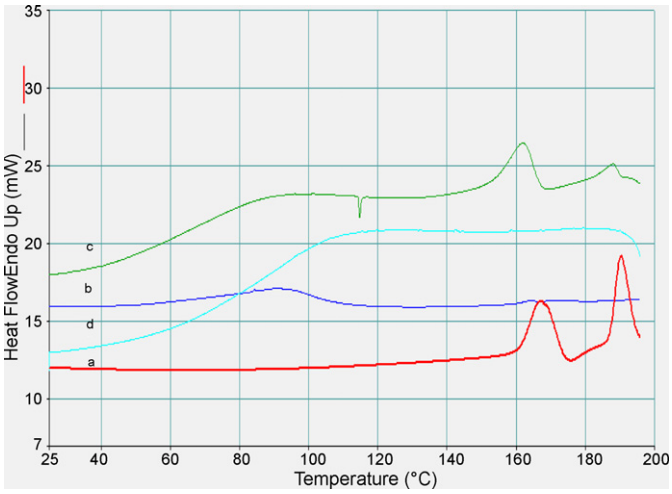

Fig. 5. DSC thermograms of (a) the compound, (b) lambda-carrageenan, (c) physical mixture of compound with carrageenan, and (d) the compound/carrageenan complex.

3.3. Nanosizing of a compound/carrageenan complex

Table 2 summarizes the particle sizes of the complex after a wet-milling process using the NanoMill™ System with seven excipients that are commonly used as stabilizers for wet-milling. The results showed that nanosized particles could not be achieved for the complex using these excipients. We investigated extensively the milling process variables and conditions

Table 2
Particle sizes of the suspensions of a compound/carrageenan complex after wet-milling with several commonly used excipients

Samples	Excipients (wt.% in the slurry)	Milling time (h)	Median particle size (μm)
1	0	1	3.8
2	Pluronic F127 (0.75%) + Kollidon® K30 (0.75%)	1	6.4
3	Kollidon® K30 (2%)	1	4.2
4	HPMC (1.5%)	1	3.1
5	HPC-SL (1.5%)	1	2.3
6	Tyloxapol (1.5%)	1	10.8
7	Plasdone® S630 (2%)	1	5.1
8	Plasdone® S630 (1.31%) + DOSS (0.15%)	1	4.7
9	HPC-SL (2%)	2	2.8
10	HPMC (2%)	2	1.8

For all nanosizing experiments, the complex content was 5 wt.% in the aqueous slurry.

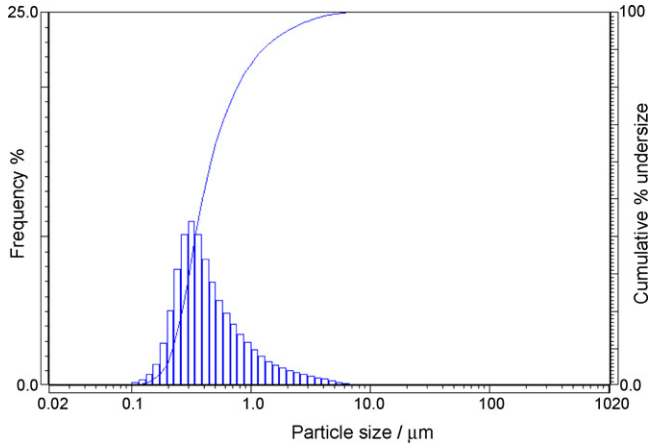

Fig. 6. A nanosized suspension of a compound/carrageenan complex using additional carrageenan as a stabilizer. Formulation: complex/carrageenan = 5/4 (w/w), milling time = 1 h, milling speed = 5500 rpm.

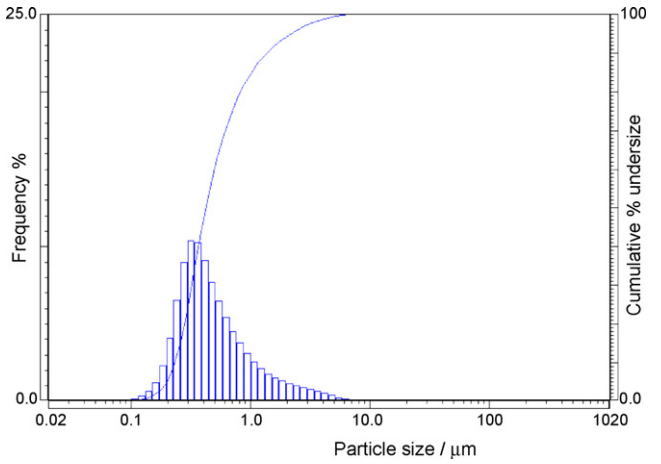

Fig. 7. The particle size distribution of the redispersion of the lyophilized nanoparticles of a compound/carrageenan complex after lyophilization. Formulation: complex/carrageenan/HPMC = 5/4/15 (w/w).

such as milling time, milling speed, complex/excipient ratio, and complex/water ratio. None of these investigations led to the formation of nanosized particles of the complex.

Since the complex in this study contained carrageenan, we used additional carrageenan as the nanosizing stabilizer. A median particle size of about 0.3 μm in the nanosuspension was successfully achieved. However, the suspension of complex nanoparticles was not stable and the particle size increased from 0.3 μm to 6.5 μm within 1 day at room temperature. We found that increasing the amount of carrageenan added during the milling process improved the stability of the nanosuspension of the complex. At a weight ratio of 5/4 (complex/carrageenan), a nanosuspension of the complex with a median particle size of about 0.3 μm was stable at room temperature for at least 1 week (Fig. 6).

Although the nanosuspension of the complex was stable at room temperature for at least 1 week, we found that the nanoparticles agglomerated/aggregated during lyophilization, and the particle size after reconstitution in water increased to 22.2 μm. To investigate this problem, a series of polymer excipients were screened to stabilize the nanosuspension of the complex after the milling process. The particle size of the lyophilized nanocomplex after reconstitution in water was monitored. The study results showed that HPMC-2910 was able to stabilize the nanosized complex during lyophilization (Fig. 7). In addition, the lyophilized complex particles retained their nano size when reconstituted in water after at least 39-day of storage at room temperature (Fig. 8). It suggested that the nanoparticle size of the lyophilized complex was stable for a period of time at room temperature after lyophilization.

Interestingly, our results showed that a nanosuspension of a compound/carrageenan complex was achieved by using additional carrageenan as a nanosizing excipient (Fig. 6) and that the excipients/stabilizers that are often used in nanosizing processes did not work (Table 2). Due to a wide molecular weight distribution of the carrageenan and a large range of sulfate weight percentages in lambda-carrageenan (30–40 wt.%), we were not able to calculate accurately the binding molar ratio of the compound to the sulfate groups in carrageenan. By HPLC analysis of the compound content in the complex, we estimate that approximately 70–90% of the piperidine and pyridine groups in the compound were bound with lambda-carrageenan. This suggests that the compound in the complex has some free basic groups. By interaction with these basic groups, additional carrageenan added during milling could readily adsorb on the surface of the nanoparticles. This adsorption decreases the surface energy of the newly created nanoparticles during the nanosizing process and thus stabilizes the nanosuspension of the complex. The adsorption of carrageenan on the nanoparticles may also result in a negative-charge layer on the nanoparticle surfaces due to the sulfate groups in the adsorbed carrageenan. Therefore, the nanosuspension of the complex may be further stabilized by electric repulsion forces (Liu, 2000). The non-ionic polymer stabilizers used in the study (Table 2), however, may not be able to adsorb readily on the surfaces of the complex nanoparticles during the milling. This hypothesis was further confirmed by using dextran sulfate (Mw ~5000) (Sigma–Aldrich, St Louis, MO), another polymer with negative charges. Using additional dextran sulfate as a stabilizer during the wet-milling, a nanosized suspension of the complex was also successfully achieved.

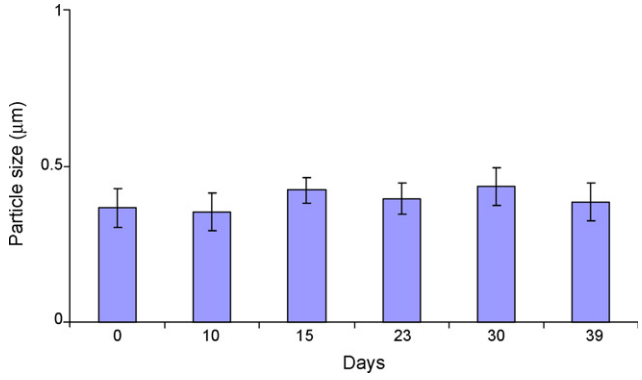

Fig. 8. The particle size of the lyophilized nanosized compound/carrageenan complex during storage at room temperature ($n = 3$). Formulation: complex/carrageenan/HPMC = 5/4/15 (w/w).

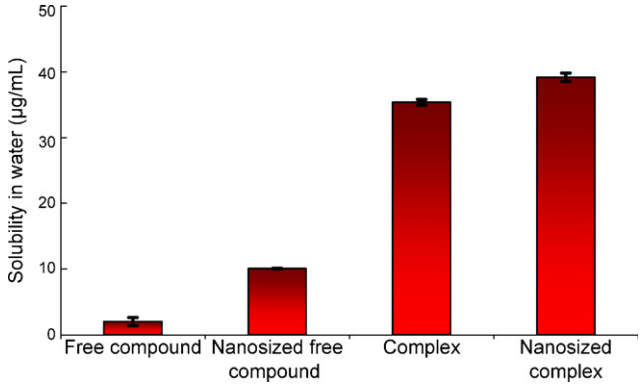

Fig. 9. Aqueous solubility of the free compound, nanosized free compound, compound/carrageenan complex, and nanosized complex. Error bars represent standard deviations of four measurements.

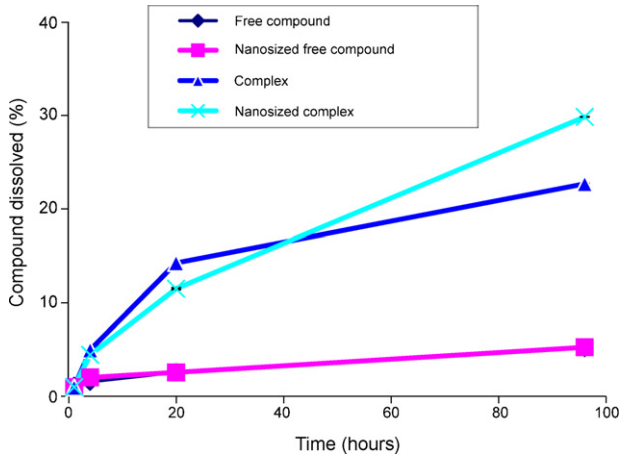

Fig. 10. The dissolution of the free compound, nanosized free compound, compound/carrageenan complex, and nanosized complex in SIF. Error bars represent standard deviations of four measurements.

3.4. Solubility and dissolution of the compound/carrageenan complex

Our solubility results showed that nanosizing the free compound increased its aqueous solubility from 1–2 μg/mL to 10 μg/mL (Fig. 9). Increasing a drug's aqueous solubility by nanosizing has been well documented in the literature and can be explained by the Ostwald–Freundlich equation (Brittain, 1995). However, increases in solubility due to nanosizing were limited (up to 10 μg/mL) because nanomilling processes did not change a drug' crystalline structures. Nanosizing only breaks down the compound into numerous nanocrystals in the suspension (Liversidge and Cundy, 1995; Merisko-Liversidge et al., 2003). As a result, the compound solubility was still primarily governed by its crystal structures.

Similar to nanosizing, complexing of the compound with carrageenan also significantly increased the compound's aqueous solubility from 1–2 μg/mL to 30 μg/mL (Fig. 9). Interestingly, the complex's aqueous solubility was also significantly higher than the aqueous solubility of free compound in the nanosuspension (10 μg/mL) (Fig. 9). As demonstrated in the results of XRD, DSC and polarized microscope studies, by complexing with hydrophilic polymers, this compound became amorphous in the complex (Figs. 4 and 5). It is well known that compounds have higher solubility in an amorphous state than in a crystalline structure (Liu, 2000). Therefore, the compound's amorphous state in the complex appears to be the predominant factor in increasing the complex's solubility.

Our approach using complexation with carrageenan differs from other published reports. The complexation with carrageenan in this study was to increase aqueous solubility of poorly water-soluble compounds by converting the drug's crystalline structure to an amorphous one. Other studies reported in the literature used a complex of high molecular weight carrageenan with water-soluble compounds to decrease the drug's diffusivity or dissolution, leading to a sustained release of the drugs (Graham and Baker, 1963; Graham et al., 1963; Aguzzi et al., 2002; Bonferoni et al., 2004b).

Not surprisingly, a nanosuspension of a compound/carrageenan complex had the highest aqueous solubility (39 μg/mL) (Fig. 9) because it combined the advantages of nanosizing and complexing with hydrophilic polymers.

In addition to increasing the drug's aqueous solubility, the complex showed faster dissolution than free compound, and the compound/carrageenan complex in nanosuspension showed the fastest dissolution rate among all the samples (Fig. 10). The dissolution rate of a solid drug is a function of drug surface (particle size) and solubility (Noyes and Whitney, 1897). Drugs with smaller particle size and higher solubility have faster dissolution rates. The increase in dissolution rate of free compound either in nanosuspension or in suspension was primarily limited by the compound's relatively low aqueous solubility (Fig. 9), which may be attributed to the compound's crystalline structure. In contrast, the complex had an increased solubility (Fig. 9) due to the amorphous structure of the compound in the complex. This enhanced solubility led to a higher dissolution rate in the complex and nanosized complex than in the free compounds. A nanosized compound/polymer complex showed the fastest dissolution rate due to the combined advantage of higher solubility and nanosizing to increase surface area.

4. Conclusions

Dissolution rate and solubility are two of several factors that affect oral bioavailability of poorly water-soluble compounds. Nanosizing techniques have been used to increase dissolution rate, which improves the low oral bioavailability of these compounds (Liversidge and Cundy, 1995; De Jaeghere et al., 2001; Muller et al., 2001; Merisko-Liversidge et al., 2003; Mueller and Akkar, 2004; Wu et al., 2004; Jinno et al., 2006). However, nanosizing could be ineffective if solubility is a limited step for drug absorption (Langguth et al., 2005). Increases in both dissolution rate and solubility are desirable to improve bioavailability.

In this study we demonstrated a novel approach of nanosizing a drug/carrageenan complex to increase both solubility and dissolution rate of a model poorly water-soluble compound, thereby potentially enhancing its bioavailability. It would be interesting to test more poorly water-soluble compounds with different

properties using this novel approach in the future studies. From our experimental results, we fully believe that the approach of combining complexation with nanosizing would has the potential to improve both the solubility and dissolution rate for other poorly water-soluble basic compounds that can form a complex with the carrageenan. Our results clearly demonstrated that the compound's aqueous solubility was increased by converting the compound's crystalline structure to an amorphous one through complexation, and nanosizing of the amorphous drug particles further improved dissolution rate.

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