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Enhancing the Solubility and Oral Bioavailability of Poorly Water-Soluble Drugs using Monoolein Cubosomes

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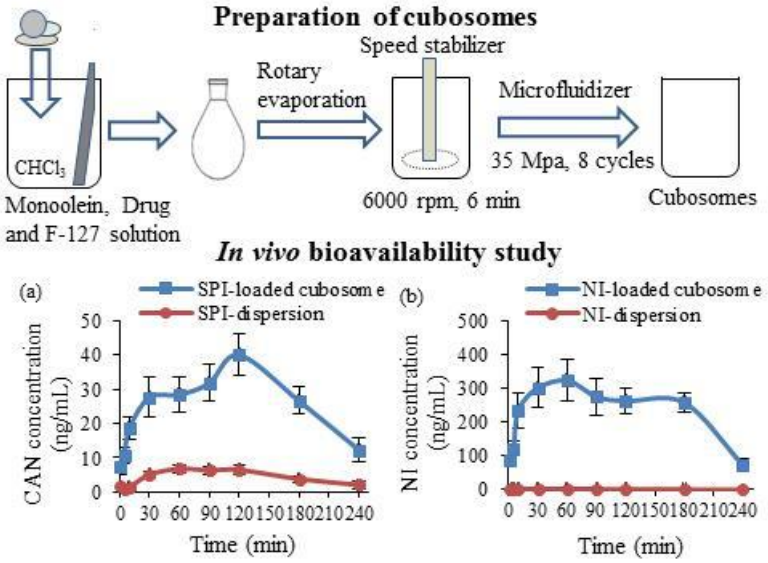
Graphical abstract



Summary

Monoolein cubosomes containing either spironolactone (SPI) or nifedipine (NI) were prepared using a high-pressure homogenization technique and characterized in terms of their solubility and oral bioavailability. The mean particle size, polydispersity index (PDI), zeta potential, solubility and encapsulation efficiency (EE) values of the SPI- and NI-loaded cubosomes were determined to be 90.4 nm, 0.187, –13.4 mV, 163 μg/mL and 90.2%, and 91.3 nm, 0.168, –12.8 mV, 189 μg/mL and 93.0%, respectively, which were almost identical to those of the blank cubosome. Small-angle X-ray scattering analyses confirmed that the SPI-loaded, NI-loaded and blank cubosomes existed in the cubic space group $Im\bar{3}m$. The lattice parameters of the SPI- and NI-loaded cubosomes were 147.6 and 151.6 Å, respectively, making them almost identical to that of blank cubosome (151.0 Å). The *in vitro* release profiles of the SPI- and NI-loaded cubosomes showed that they released less than 5% of the drugs into various media over 12–48 h, indicating that most of the drug remained encapsulated within the cubic phase of their lipid bilayer. Furthermore, the *in vivo* pharmacokinetic results suggested that these cubosomes led to a considerable increase in the systemic oral bioavailability of the drugs compared with pure dispersions of the same materials. Notably, the stability results indicated that the mean particle size and PDI values of these cubosomes were stable for at least 4 weeks. Taken together, these results demonstrate that monoolein cubosomes represent promising drug carriers for enhancing the solubility and oral bioavailability of poorly water-soluble drugs.

Keywords: cubosome; drug carrier; monoolein; nifedipine; spironolactone; high-pressure homogenization.

Introduction

The low oral bioavailability of poorly water-soluble drugs remains one of the most challenging aspects of drug development. A wide variety of formulation approaches, including lipid nanoparticles, solid dispersions, complexes with cyclodextrin or chitosan-alginates, nanoemulsions and liposomes, have been used to enhance the solubility and bioavailability of several poorly water-soluble drugs.[1–5] Cubosomes have recently attracted considerable interest from formulation scientists in terms of their potential application as drug delivery systems based on their highly ordered, compartmentalized internal structures, high lipid content and large surface area.

Cubosomes are inverse bicontinuous cubic lyotropic crystalline nanoparticles that can be loaded with poorly water-soluble drugs in their three-dimensional cubic phases, leading to pronounced increases in the solubility, stability and bioavailability of these drugs.[6–9] Cubosomes can be prepared using nontoxic, biocompatible and biodegradable ingredients, and can be readily used to encapsulate lipophilic drugs. Monoolein is a common amphiphilic building block for the preparation of cubosomes,[10] and is relatively cheap compared with other commonly used lipid excipients such as phytantriol. Nonionic triblock copolymer (Pluronic F-127) is an excellent steric stabilizer that can be used to stabilize the structures of cubosomes and prevent particle aggregation for extended periods of time.[11] Monoolein cubic nanoparticles and cubosomes have recently been proposed as potential drug carriers because they can (i) efficiently encapsulate poorly water-soluble drugs and enhance their solubility; (ii) exhibit bio-adhesive properties with sustained release properties; (iii) protect drug molecules and increase their duration of action; and (iv) improve the oral bioavailability of poorly water-soluble drugs.[9, 12–14] The results of several studies have shown that the encapsulation of 20(S)-protopanaxadiol, simvastatin and amphotericin B in monoolein cubosomes and/or cubic nanoparticles led to increases in the oral bioavailabilities of these

poorly water-soluble drugs.[15–17] However, there have been very few studies in the literature pertaining to the *in vivo* evaluation of cubosomes. Given that lipid-based formulations have been used in drug delivery to improve the oral absorption of poorly water-soluble drugs for many years,[18–20] we envisaged that cubosomes could also increase their bioavailability.

In this study, we used spironolactone (SPI) and nifedipine (NI) as model drugs to prepare SPI- and NI-loaded cubosomes using a high-pressure homogenization technique. The drug loaded cubosomes were subsequently evaluated in terms of their particle size distribution, particle stability, solubility, and encapsulation efficiency characteristics. We also evaluated the *in vitro* release rate and oral bioavailability properties of the cubosomes for the encapsulated drugs.

Experimental

Materials

Monoolein (-OL-100H) was provided as a gift from Riken Vitamin Co., Ltd (Tokyo, Japan). Pluronic F-127 was gifted from BASF (Ludwigshafen, Germany). Spironolactone and nifedipine were purchased from Tokyo Chemical Co., Ltd (Tokyo, Japan) and Sagami Kasei (Tokyo, Japan), respectively. Chloroform, methanol and formic acid were purchased from Wako Chemical Industries, Ltd (Osaka, Japan). All of the other reagents used in this study were purchased as the highest grades available from commercial sources.

Preparation of Cubosomes

The SPI- and NI-loaded cubosomes were prepared using a high-pressure homogenization technique according to a slightly modified version of a previously published method.[21] Briefly, 900 mg of monoolein, 40 mg of SPI/NI and 100 mg of pluronic F-127 were added to a beaker containing 20 mL of chloroform, and the resulting mixture was manually agitated to give a solution. All of the processes to prepare NI-loaded cubosomes were conducted in

amber glass containers because NI has high light sensitivity. The solvent was removed under reduced pressure on a rotary evaporator at 40–50 °C, and the resulting residue was dried under vacuum for 24 h at 20 °C to allow for complete removal of the organic solvent. The dried sample was dispersed in 200 mL of Milli-Q hot water with 80 °C, and premixed at 6000 rpm for 6 min using a Speed Stabilizer (Kinematica® Co., Luzern, Switzerland). The coarse dispersions were also passed through a high-pressure homogenizer (Microfluidizer® Microfluidics Corporation, Newton, MA, USA) at 35 MPa with eight pass cycles. Finally, the cubosome materials were filtered through a 0.45-μm membrane filter unit and stored in the dark at 20 °C prior to use.

Particle Size Distribution and Zeta Potential Measurements

The mean particle size, PDI and zeta potential values of the different cubosomes were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS90 (Malvern Instruments, Malvern, UK) at 25 °C. The cubosome samples were diluted with Milli-Q water by 100-fold and measurements were performed at 25 °C. Each experiment was performed in triplicate.

Small-angle X-ray Scattering Measurements

Small-angle X-ray scattering (SAXS) analyses were conducted to characterize the liquid crystalline phases of the cubosomes using a NANO-Viewer system (Rigaku, Tokyo, Japan) according to a previously reported method.[7)] Twenty microliter samples of the cubosomes were placed in a polyimide tube of 1 mm in diameter. The distance between the sample and the detector was set to 510 mm and the system was calibrated using powdered silver behenate. The temperature and exposure time were adjusted to 25 °C and 5 min, respectively. The lattice parameters of the cubic phase were calculated together with their standard error values using the Grafit 5 software (Erithacus® Software, Horley, Surrey, UK).

Quantitative Evaluation of SPI and NI by HPLC

SPI-loaded cubosome samples were diluted as necessary with acetonitrile and thoroughly mixed before being filtered through a 0.45-μm membrane filter unit. The concentration of SPI in each sample was quantified by HPLC analysis using a TSKgel ODS-80Tm® column (4.6 × 150 mm) on a Shimadzu LC-2010C HT® system (Shimadzu Corporation, Kyoto, Japan). The column was eluted with a mobile phase composed of a 7:3 (v/v) mixture of acetonitrile and water at a flow rate of 0.8 mL/min with an injection volume of 40 μL. The column heater and UV-detector were set at 40 °C and 238 nm, respectively.

NI-loaded cubosome samples were diluted as necessary with methanol and thoroughly mixed before being filtered through a 0.45-μm membrane filter unit. The concentration of NI in each sample was measured by HPLC analysis using a Cadenza CD-C18 column (3 × 150 mm) on a Shimadzu LC-2010C HT® system. The column was eluted with a mobile phase composed of a 3:2 (v/v) mixture of methanol and water containing 0.2% formic acid at a flow rate of 0.4 mL/min with an injection volume of 40 μL. The column heater and UV-detector were set at 40 °C and 236 nm, respectively. All of the samples were quantified in triplicate.

Quantitative Evaluation of SPI and NI by LC-MS/MS

The SPI concentration was determined by LC-MS/MS using an API-3000 system (Agilent Technologies, Santa Clara, CA, USA) equipped with a Develosil ODS-HG-5 column (4.6 × 150 mm). The system was eluted with a mobile phase composed of a 3:2 (v/v) mixture of methanol and water containing 0.1% formic acid at a flow rate of 0.4 mL/min. The column temperature and run time were set to 28 °C and 15 min, respectively. The conditions for the LC-MS/MS were as follows: interface, turbo spray; ionization mode, ESI in the positive ion mode; ion source, nebulizer gas, curtain gas, collision gas; ionspray voltage, 5000 V; temperature, 350 °C; measurement mode, MRM method. The analytes were detected as follows: *m*/*z* values of 417.4 and 341.4 for the precursor ion and the product ion of SPI,

respectively. For *in vivo* pharmacokinetic study, we measured the concentration of canrenone (CAN), a major metabolite of SPI, using a slightly modified version of this method, where the column temperature, flow rate and sample run time were set to 35 °C, 0.5 mL/min and 25 min, respectively. The analytes were detected as follows: *m*/*z* values of 341.3 and 107.1 for the precursor ion and the product ion of CAN, respectively.

The NI concentration was determined by LC-MS/MS analysis as above using a Cadenza CD-C18 column (3 × 150 mm) column. The system was eluted with a mobile phase composed of a 3:2 (v/v) mixture of methanol and water containing 0.1% formic acid at a flow rate of 0.4 mL/min. The column temperature and sample run time were set to 40 °C and 20 min, respectively. The conditions for the LC-MS/MS were as follows: interface, turbo spray; ionization mode, ESI in the positive ion mode; ion source, nebulizer gas, curtain gas, collision gas; ion spray voltage, 5000 V; temperature, 300 °C; measurement mode, MRM method. The analytes were detected as follows: *m*/*z* values of 347.1 and 254.2 for the precursor ion and the product ion of NI, respectively. All of the quantification experiments were performed in triplicate.

Drug Encapsulation Efficiency

The cubosome preparations consisted of a mixture of encapsulated and free drug (un-encapsulated) fractions. The drug encapsulation efficiency (EE) of the cubosomes was determined based on the amounts of encapsulated and free drug by the ultra-centrifugation method. Briefly, a 4.0-mL sample of cubosome was placed in an Amicon Ultra-4 Centrifugal Filter-100K device (Merck Millipore Ltd, Carrigtwohill, Ireland) and centrifuged at 1057 ×*g* for 30 min using an Eppendorf centrifuge (Hamburg, Germany). The concentration of free drug present in the filtrate was determined by LC-MS/MS as described above. The percentage drug encapsulation efficiency of the cubosome was calculated using the following equation:

$$\text{EE}\ (\%) = \frac{D_{\text{total}} - D_{\text{free}}}{D_{\text{total}}} \times 100$$

where D_{total} is the total drug concentration of the sample before centrifugation and D_{free} is the free drug concentration in the filtrate after centrifugation. These experiments were conducted in triplicate.

Stability Studies of the SPI- and NI-loaded Cubosomes

The SPI- and NI-loaded cubosomes were stored at 20 °C in the dark prior to use and their mean particle size and PDI values were measured at 0, 1, 2, 3 and 4 weeks using the methods described above.

***In Vitro* Release of SPI and NI from Cubosomes**

The *in vitro* release characteristics of the monoolein cubosome samples were examined using a membrane dialysis method with a variety of different release media, including 0.1 M sodium acetate buffer (pH 4.0), fasted state simulated intestinal fluid (FaSSIF) (pH 6.5) and fed state simulated intestinal fluid (FeSSIF) (pH 5.0) at 37 °C. The biorelevent FaSSIF and FeSSIF media were prepared in accordance with a previously reported protocol.[22] Briefly, a 1.0-mL sample of cubosome was added into a dialysis membrane bag (MWCO 14 kDa, Viskase Companies Inc., Darien, IL, USA), which was clamped at both ends before being submerged in 200 mL of release medium in a beaker. The beaker was then placed in a shaker bath at 37 °C and shaken horizontally at 100 strokes min^{-1}. One-milliliter aliquots of release medium were withdrawn for analysis from each beaker at 1, 2, 4, 6, 12, 24, 48, 72 and 96 h, and replaced with the same volume of fresh medium. The collected samples were instantly diluted with acetonitrile for SPI or methanol for NI and filtered through a 0.45-μm membrane filter. Finally, the concentration of drug released into the release medium was determined by HPLC as described above. These experiments were conducted in triplicate.

In Vivo Pharmacokinetic Study

Male Sprague-Dawley rats (weight: 280–310 g, age: 8–9 weeks; Japan SLC, Shizuoka, Japan) were fasted overnight for 12 h prior to the experiment. All of the procedures used in this study were performed in accordance with the guidelines approved by the Institutional Animal Care and Ethical Committee of the University of Shizuoka, Japan. SPI and NI dispersions were prepared at a concentration of 180 μg/mL by dispersing these materials in Milli-Q water as controls for the SPI- and NI-loaded cubosomes. The SPI- and NI-loaded cubosome, as well as the corresponding controls, were orally administered to the rats at doses corresponding to 1 and 0.5 mg/kg of SPI and NI, respectively. Following the administration of these formulations, blood samples were collected *via* the tail vein at 1, 5, 10, 30, 60, 90, 120, 180 and 240 min in Eppendorf tubes containing the anticoagulant heparin. The blood samples were then centrifuged at 4 °C and 4226 $\times g$ for 10 min to obtain plasma. The plasma samples were immediately treated with methanol and mixed by vortexing for 2 min, before being centrifuged at 4 °C and 4226 $\times g$ for 10 min. The supernatant was collected and filtered through a 0.20-μm membrane filter unit. We determined the concentration of CAN and NI in the filtrate by LC-MS/MS using the method described above. The main pharmacokinetic parameters, including the maximum peak plasma concentration (C_{max}), time of peak plasma concentration (T_{max}), half-life ($t_{1/2}$) and the area under the plasma concentration-time curve, were calculated using the linear trapezoidal rule from time zero to infinity ($AUC_{0\rightarrow\infty}$) and analyzed using the Win Nonlin® Pharmacokinetic program.

Statistical Analysis

Statistical analyses were performed using the Student's *t*-test. A probability value of $p<0.05$ was considered to indicate statistical significance.

Results and Discussion

Characterization of SPI- and NI-loaded Cubosomes

The results for the characterization of the SPI- and NI-loaded cubosome particles are shown in Table 1. The mean particle size, PDI and zeta potential values of the SPI- and NI-loaded cubosomes were almost identical to those of the blank cubosomes. These data show that the encapsulation of drug molecules in the monoolein cubosomes had no impact on their mean particle size, PDI or zeta potential. It is noteworthy that the PDI values of SPI-loaded, NI-loaded and blank cubosomes were less than 0.3, indicating that they were monodispersed.

The SAXS patterns and lattice parameters of the SPI-loaded, NI-loaded and blank cubosomes are shown in Fig. 1 and Table 2, respectively. The SAXS patterns of all three materials showed three Bragg peaks with relative positions at spacing ratios of $\sqrt{2}:\sqrt{4}:\sqrt{6}$. These peaks were indexed according to the Miller indices (*hkl*) = (110), (200) and (211) reflections, which were indicative of the body-centered cubic phase of the $Im\bar{3}m$ space group.[21] The encapsulation of SPI and NI molecules in the cubosomes had no discernible impact on their cubic space group. The lattice parameters of the cubic phase of the SPI-loaded cubosomes were slightly less than those of the blank cubosomes, whereas those of the NI-loaded cubosomes remained largely unchanged.

The apparent solubilities of SPI and NI increased when they were encapsulated in the monoolein cubosomes, as shown in Table 1. The encapsulation of SPI in the monoolein cubosomes led to a 6-fold increase in its solubility (28 μg/mL in water at 25 °C).[23] Furthermore, the solubility of NI increased around 9-fold (20 μg/mL in water at 25 °C) following its encapsulation in the monoolein cubosomes.[24] The observed increases in the solubilities of these drugs could be attributed to the hydrophobic region of the cubic phase of monoolein. From a structural perspective, monoolein consists of a long hydrophobic aliphatic chain and a hydrophilic glycerol moiety. In the monoolein cubosomes, the hydrophobic

aliphatic chains would form a lipid bilayer with a cubic phase, with the hydrophilic glycerol moieties forming a water channel. Poorly water-soluble drugs such as SPI and NI would therefore be most likely incorporated into the hydrophobic lipid bilayer of the cubic phase of cubosomes, leading to an increase in the drug content of the hydrophobic lipid bilayer. The encapsulation efficiencies of the SPI- and NI-loaded cubosomes were 90.2 and 93.0%, respectively, highlighting the high solubility of these drugs in the lipid phase, as well as demonstrating that most of the drug molecules were encapsulated by the cubosome. The high encapsulation efficiencies of these cubosomes could be attributed to SPI and NI being highly lipophilic.

Stability Study

The mean particle size and PDI values of the SPI- and NI-loaded cubosomes remained largely unchanged for up to 4 weeks at 20 °C (Fig. 2). The results of the stability study indicated that the SPI- and NI-loaded cubosomes were more stable than the previously reported lipid nanoparticles. For example, the mean particle size of a suspension of NI-loaded lipid nanoparticles increased by around 14–30% after 30 days at 4 °C.[25] Furthermore, the SPI-loaded liposomes were only stable at 5 °C.[26]

***In Vitro* Release of SPI and NI from Cubosomes**

The *in vitro* release profiles of SPI and NI are shown in Fig. 3. This result therefore demonstrates that NI was released at a greater rate into FaSSIF compared with the acetate buffer and FeSSIF. Although there appeared to be a decrease in the percentage of NI released from the cubosomes after 24 h, this phenomenon was attributed to the degradation of some of the NI released into the medium th rough hydrolysis or photolysis following the long incubation time. Moreover, the release profiles of the SPI- and NI-loaded cubosomes showed that no more than 5% of this drug was released into any of these media over 24 h, indicating that most of the drug particles remained encapsulated within the cubic phases of the lipid

bilayers of the monoolein cubosomes. This result also suggested that the cubosomes enhanced the solubility of SPI and NI, as stated earlier. The results obtained using the dialysis method for the *in vitro* release of these drugs from the cubosomes confirmed the drug encapsulation efficiency results determined by the ultra-centrifugation method (>90%). It has been reported that only 1.3% of the SN-38 molecules encapsulated in liposomes were released into the PBS buffer over 30 h, with the value reaching only 1.9% after 120 h.[27] In contrast, the results of a later study showed that around 7% of the SN-38 molecules encapsulated in lipid nanocapsules were released into the PBS buffer over 24 h.[28] Baskaran et al. (2014) reported that only 2.04% of the curcumin molecules encapsulated in monoolein cubic liquid crystalline nanoparticles were released into phosphate buffer saline at pH 7.4, with the rest of the drug molecules remaining unchanged within the nanoparticles.[13]

***In Vivo* Pharmacokinetic Study**

The oral bioavailabilities of the SPI- and NI-loaded cubosomes were investigated in rats and the results were compared with those of the corresponding pure drug dispersions. The pharmacokinetic parameters of these materials are shown in Table 3. The concentration of CAN, a major metabolite of SPI and NI, was determined at different time intervals in all four of these systems, and the results were plotted against time (Fig. 4). These results therefore implied that the oral bioavailabilities of SPI and NI increased considerably following the encapsulation of these drugs in the cubosomes. The mean $AUC_{0-240min}$ and $AUC_{0-\infty}$ values of cubosome-encapsulated drugs increased significantly compared with the corresponding cubosome-free dispersions (Table 3). The oral administration of a solid dispersion of NI (1 mg/kg equivalent of NI) to rats was previously reported to give an $AUC_{0-\infty}$ value of 31,676 ng min/mL. In this study, the oral administration of the NI-loaded cubosomes (0.5 mg/kg equivalent of NI) gave an $AUC_{0-\infty}$ value of 58,540 ng min/mL, indicating that the formulation of NI with monoolein cubosomes led to a 4-fold increase in the $AUC_{0-\infty}$ compared with a

solid dispersion of NI.[29] Furthermore, the half-lives of the SPI- and NI-loaded cubosomes increased around 1.5-fold compared with the corresponding cubosome-free dispersions. Based on these results, we speculated that several factors could be responsible for the observed increases in the absorption characteristics of these two drugs.

Several mechanisms, acting in isolation or in combination, can lead to an increase in the oral bioavailability of a drug molecule. In terms of the SPI- and NI-loaded cubosomes prepared in the current study, the observed increases in the oral bioavailability could be attributed to the small size of the cubosome nanoparticles, which would allow them to enter into the intravascular spaces and strongly adhere to the gastrointestinal membrane, leading to an increase in the absorption of the drug molecules. The high affinity of the lipid-like gastrointestinal membrane for these hydrophobic drug molecules could also explain the observed increase in the bioavailability of these compounds. In particular, lipid-like nanoparticles such as the monoolein cubosomes used in this study have mucoadhesive properties that would lead to an increase in their contact time with the gastrointestinal membrane. These properties would therefore lead to an increase in the gastrointestinal residence time of the monoolein cubosomes, resulting in an increase in their oral bioavailability.[9, 12] The plasma drug profiles of SPI and NI indicated that they were immediately absorbed, which could be attributed to the adsorption of the monoolein cubosomes onto the intestinal membranes. Last, the presence of lipids in the gastrointestinal tract such as the monoolein cubosomes can stimulate the secretion of bile into the small intestine from the gall bladder. The cubosomes could then interact with the bile salts to form mixed micelles, which could be absorbed together with the drugs into systemic circulation.[30] Furthermore, the SPI- and NI-loaded cubosomes could favor lymphatic transport from the small intestine in a similar manner to that reported for other lipid-based formulations such as liposomes.[18–20]

Although only small amounts of SPI and NI were released (<5%) from the monoolein cubosomes into the different release medium *in vitro*, the absorption of these drugs increased considerably compared with pure dispersions of the same materials. This disparity between the *in vitro* release profiles and the *in vivo* drug absorption characteristics could be attributed to the differences between the *in vitro* and *in vivo* environments, the latter of which is much more complex than the former. Furthermore, it is possible that the cubic phase of the monoolein cubosomes could be converted to a different phase (e.g., hexagonal phase) by one of the many compounds found in the gastrointestinal tract.[31] This would result in the rapid release of SPI and NI, leading to an increase in their bioavailability. Furthermore, the absorption of these materials would be regulated by gastric emptying and intestinal transit time, which could explain the differences observed in the *in vitro* release profiles and *in vivo* drug absorption characteristics of these materials.[32] We also observed small deviations in the absorption peaks of SPI and NI at 30 and 180 min, respectively, which could be attributed to the complex nature of the *in vivo* environment. Although the mechanisms responsible for the observed increases in bioavailability of SPI and NI from the cubosomes remain unclear, it is envisaged, based on the results of this study, that these cubosomes could potentially be used as suitable carriers for improving the oral bioavailabilities of SPI and NI.

Conclusion

The results of this study show that monoolein cubosomes containing SPI and NI in the cubic phase of their lipid bilayer enhanced the solubility and oral bioavailability characteristics of both of these drugs. SAXS analyses confirmed that these cubosomes existed in the cubic $Im\bar{3}m$ space group and that they retained their structure after the addition of the drugs. In terms of their physiochemical properties, the mean particle sizes of these cubosomes were less than 100 nm and their PDI values were less than 0.3, which indicated

that they were monodispersed. The cubosomes also had zeta potentials in the range of –10 to –16 mV. The *in vitro* release profiles of the SPI- and NI-loaded cubosomes showed that they lost less than 5% of their encapsulated drugs into a variety of different media over 12–48 h. *In vivo* pharmacokinetic results also suggested that these systems exhibited sustained plasma drug levels and enhanced oral bioavailability. The results of a stability study suggested that the particle size and PDI values of the SPI- and NI-loaded cubosomes remained stable for at least 4 weeks. The SPI- and NI-loaded cubosomes developed in this study therefore represent promising carrier systems for the efficient delivery of drugs for the treatment of hypertension and related diseases.

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Conflict of Interest

The authors declare no conflict of interest.

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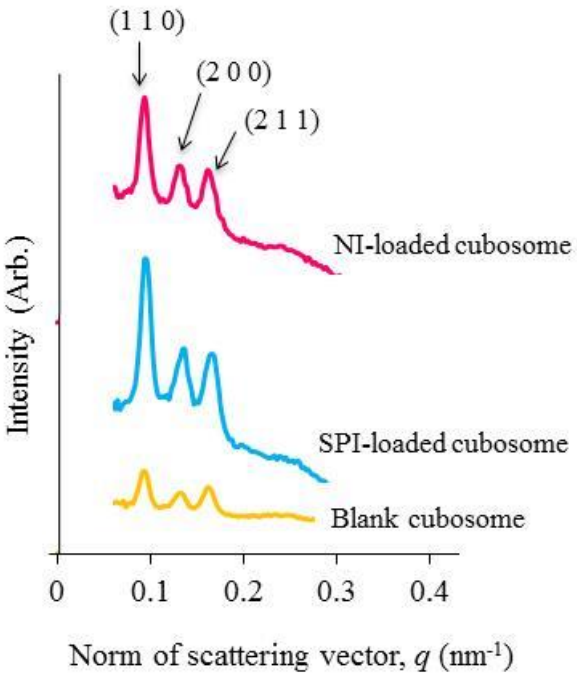
Figure Legends

Fig. 1. Intensity vs. the norm of the scattering vector obtained by SAXS measurements for the SPI-loaded, NI-loaded and blank cubosomes (without model drug). $q=2\sin\theta/\lambda$, where θ is the Bragg angle and λ is the wavelength of X-ray.

Fig. 2. Changes in the mean particle size (line) and PDI (bar) values of the SPI-loaded cubosome (a) and the NI-loaded cubosome (b) up to 4 weeks at 20 °C. These data represent the mean values ± S.D. (n=3).

Fig. 3. *In vitro* release profiles of SPI (a) and NI (b) from the monoolein cubosomes in acetate buffer, FaSSIF and FeSSIF over 96 h at 37 °C. These data represent the mean values ± S.D. (n=3).

Fig. 4. *In vivo* drug release, a) plasma CAN concentration-time plot after an oral dose of 1.0 mg/kg of the SPI-loaded cubosome and an SPI dispersion, b) plasma NI concentration-time plot after an oral dose of 0.5 mg/kg of the NI-loaded cubosome and an NI dispersion. These data represent the mean values ± S.D. (n=5).



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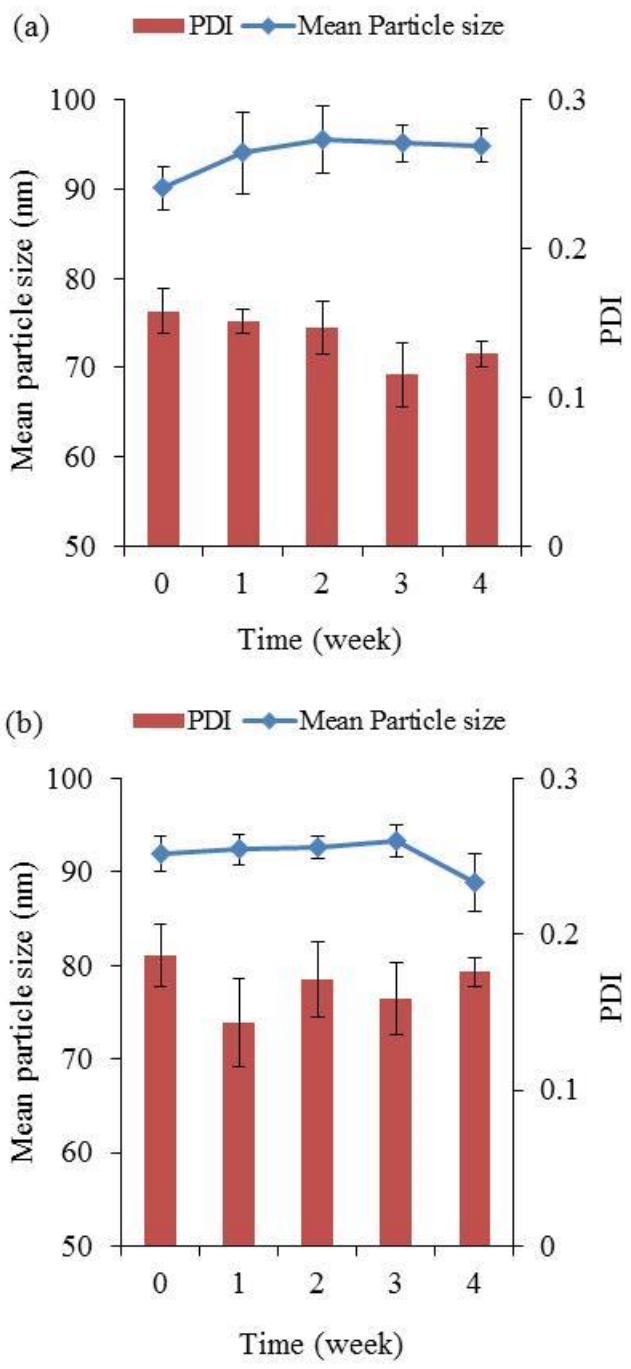

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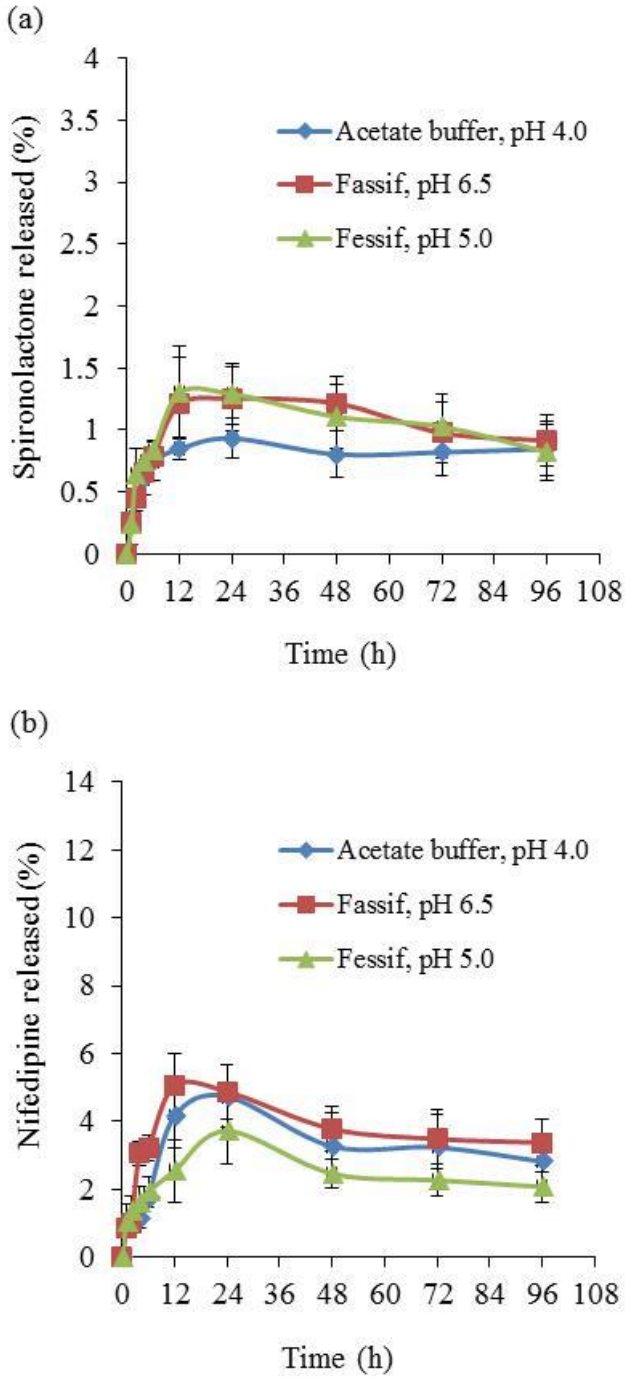

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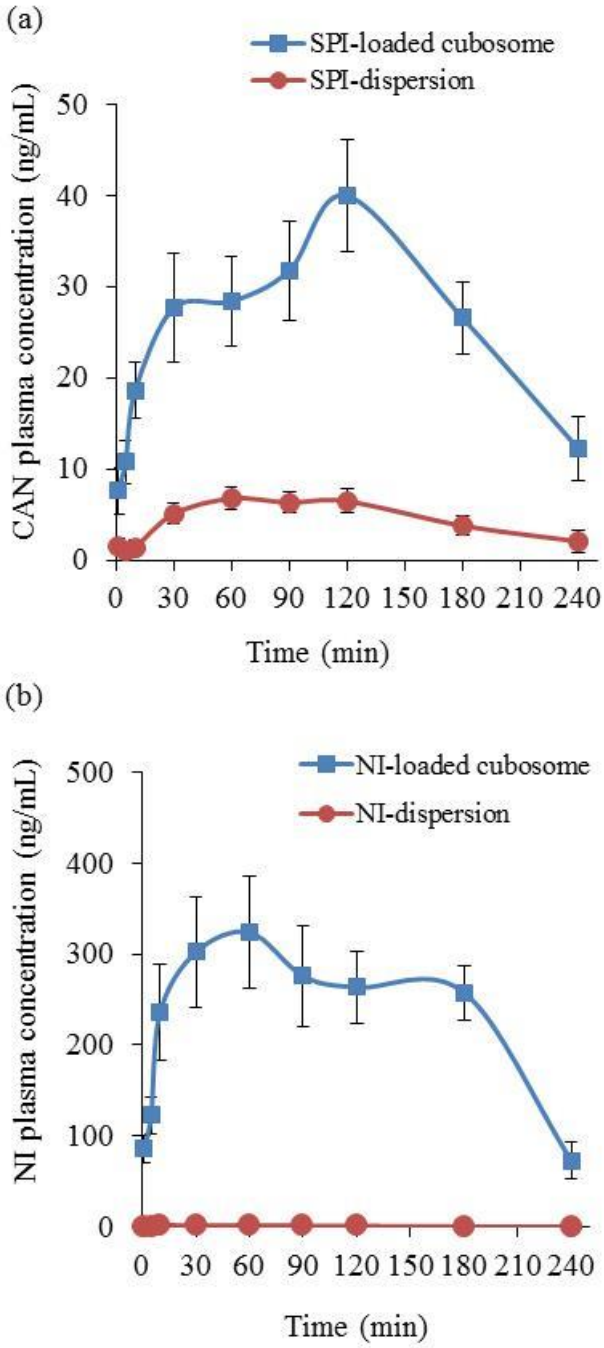

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