Physicochemical and Pharmacokinetic Characterization of a Spray-Dried Cefpodoxime Proxetil Nanosuspension

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Received February 8, 2010; accepted April 7, 2010; published online April 16, 2010

Cefpodoxime proxetil (CP) is a prodrug, the third generation cephem-type broad-spectrum antibiotic administered orally. However, CP was found to be a poorly water-soluble drug with low bioavailability when orally administered. In the present investigation, the spray-dried cefpodoxime proxetil nanosuspension (SDN) was prepared. The physicochemical properties were characterized by rheological evaluation, particle size measurement and its distribution, dynamics of reconstitution, *in-vitro* dissolution testing, surface morphology, surface area and pore size measurements. The pharmacokinetic study of SDN, in comparison to a marketed cefpodoxime proxetil for oral suspension (MS), was also performed in rabbits after a single oral dose. It was found that SDN exhibited a significant decrease in t_{max}, a 1.60-fold higher area under curve (*AUC*) and 2.33-fold higher maximum plasma concentration (C_{max}) than MS.

Key words cefpodoxime proxetil; nanosuspension; spray dried; rheological; pharmacokinetics

Cefpodoxime proxetil (CP) is a prodrug, broad spectrum, third generation cephalosporin ester. This prodrug ester is absorbed from the intestinal tract after oral administration and is hydrolyzed *in vivo* into the active cefpodoxime.[1)] Although CP is designed to improve the permeability and thus bioavailability of cefpodoxime, it still has only 50% oral bioavailability, when administered orally.[2)] The reported explanations for the low bioavailability of CP include low solubility in aqueous solution, typical gelation behavior in acidic aqueous environments and pre-absorption luminal metabolism of cefpodoxime by the action of digestive enzymes.[3,4)] Metabolism of CP into cefpodoxime inside the intestinal epithelial cell and preferential efflux of cefpodoxime into lumen is also another contribution to the low bioavailability of CP.[5)]

Recently, nanosuspensions have shown promising potential in drug delivery to offer a unique solution for the poor bioavailability of poorly water- and lipid-soluble drugs. Nanosuspensions are colloidal dispersions of nano-sized drug particles stabilized by suitable stabilizers. Nanosuspensions are unique because of their simplicity and the advantages they confer over other formulation strategies.[6)] Oral nanosuspensions have been specifically used to increase the rate and extent of the absorption of poorly water-soluble drug, to enhance the onset of action, to reduce the fed/fasted ratio, and to enhance the bioavailability. In other cases, the particulate nature of nanosuspensions can be useful in the targeting of the monocyte phagocytic system, bypassing the passage of drug through epithelial cells with improved pharmacokinetic consequences.[7)] Nanosuspensions can also be used in controlled drug delivery systems.[8)]

Nanosuspension is generally prepared to be a liquid form. In order to prepare the solid dosage form for the purpose of better physical and chemical stability, spray drying is one of the commonly used techniques for solidification. However, research literature that discusses the use of spray drying for nanosuspension is limited. Recently, the spray dried nanosuspension of itraconazole was prepared.[9)] The emphasis was to examine the impact of various formulation and processing parameters on redispersibility of the spray dried nanoparticles and *in-vitro* dissolution. The *in-vivo* study was not performed to demonstrate the bioavailability enhancement by nanosuspension.

As a solid product, solid spray-dried cefpodoxime proxetil nanosuspension (SDN) will be reconstituted in aqueous solution to form the liquid state prior to administration. It's better for SDN to regain its original state. For a heterogeneous disperse system, the rheological and thixotropic properties are important for its physical stability and clinical use. However, literature for the rheological and thixotropic properties of nanosuspensions is lacking.

The main objective of the present study is to characterize the physicochemical properties of the liquid nanosuspension prepared by high pressure homogenization and SDN. In addition, an *in vivo* pharmacokinetic study of SDN was performed, in comparison to a marketed cefpodoxime proxetil for oral suspension (MS), to demonstrate the bioavailability enhancement of SDN.

Experimental

Materials Amorphous cefpodoxime proxetil and cefpodoxime proxetil for oral Suspension (Batch No.: 071018, 50 mg amorphous cefpodoxime contained) were purchased from Sanye Pharmaceuticals Co., Ltd. (Hainan, China). Cefpodoxime was purchased from the Shanghai Institute of Organic Chemistry, Chinese Academic Sciences (Shanghai, China). Cefaclor was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Poloxamer 188 (Lutrol®F68) was obtained from the BASF Corp. (Ludwigshafen, Germany). Hydroxy-propylmethyl cellulose (HPMC) (Methocel E3-LV) was obtained from the Shanghai Colorcon Coating Technology Ltd. (Shanghai, China). Other chemicals were of HPLC or analytical grade.

Methods Preparation of CP Nanosuspension. Liquid Nanosuspension by Homogenization Jet-milled CP powder (Jetpharma, MCOne, Swiss) was dispersed in an aqueous solution poloxamer 188 (0.5%, w/v), HPMC(2%, w/v) and glycerol (5%, w/v) using an Ultra-Turrax T18 stirrer (Jahnke & Kunkel, Staufen, Germany). The obtained coarse pre-dispersion was then homogenized *via* a high pressure homogenizer (Avestin Emulsiflex-05, Avestin Inc., Ottawa, Canada). Initially, 2 cycles at 250 bar and 5 cycles at 500 bar, as a kind of premilling, were applied, and then 30 cycles at 1000 bar were run to obtain the liquid CP nanosuspension. The content of CP in liquid nanosuspension was 10% (w/v).

Preparation of SDN by Spray Drying In order to prevent the degrada-

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tion of CP in aqueous media and enhance the physical stability of liquid suspension,[10] the prepared liquid formulation was subsequently spray-dried using a mini spray-dryer equipped with a high performance cyclone (Büchi B-290: Büchi Labortechnik AG, Switzerland) with a 0.7-mm nozzle, using the following standard operating conditions: inlet temperature, 105 °C; aspirator setting, 100% (40 m^3/h); spray flow rate, 600 l/h; pump setting, 2.72 ml/min); These conditions resulted in an outlet temperature of 52—58 °C. Stability data by HPLC determination showed that no significant changes in related substances and drug content were observed, which confirmed that the homogenization and spray drying process did not cause any significant chemical degradation of the prodrug in lab-scale batch process.

Characterization of SDN. Particle Size Analysis The particle size analysis was performed by photon correlation spectroscopy (PCS) using a Zetasizer 3000 (Malvern Instruments, Malvern, U.K.). Prior to measurement, 0.5 g of SDN was diluted with 50 ml distilled water and dispersed homogeneously. Each sample was measured at 25 °C in triplicate. PCS yields the volume weighted mean particle size and the polydispersity index (PI) of the liquid nanosuspension.

Rheological Properties The static rheological property of the reconstituted liquid CP nanosuspension was investigated to demonstrate its favorable power of dispersion and therefore its physical stability and the ease of use. Prior to measurement, 0.5 g of SDN was diluted with 50 ml distilled water and dispersed homogeneously. The dispersion then was transferred into the small sample adapter of the viscosimeter (Brookfield LVDV-III Ultra, Brookfield Engineering Laboratories Inc., MA, U.S.A.), which was pre-heated to the assay temperature (25±0.3 °C). The ratio of inner radius to outer radius was 0.92 in the concentric cylinder system. The measuring element, spindle number 18 (SC4-18), was then introduced and sheared at 1.32 s^{-1} rate for 15 min in the rheometer before the measurement cycles started. The rotating speed of the spindle was programmed for control. Shear stress (τ) and viscosity (η) were measured as a function of increasing shear rate (γ) from 1.32 to 212 s^{-1} (ramp up), then decreasing the shear rate from 212 to 1.32 s^{-1} (ramp down). All rheological measurements were performed in triplicate. The rheograms obtained during the procedure of increasing shear rate was fitted to the Herschel–Bulkley model.[11] The model was verified by statistical analysis using origin 7.5 (Originlab Corp., Northampton, U.S.A.) and the rheological parameters were calculated.

Dynamic Reconstitution of SDN and Particle Size Analysis In order to get the clear observation of the dynamic reconstitution process, about 100 mg of SDN were transferred onto the water surface of a bottle containing 25 ml 0.01 mol/l hydrochloric acid. The dynamic reconstitution process of SDN in the immobile acid media was visually observed and pictured every 10 s with a digital camera. The dispersion was also observed at intervals under a Leica DMLB microscope (Leica, Wetzlar, Germany) to inspect the possible gelatinization of CP.

***In Vitro* Dissolution Testing** Dissolution tests (six replicates) of SDN were performed in 0.01 mol/l hydrochloric acid and 0.05 mol/l phosphate buffer (pH=6.5) (37 °C, V=900 ml) using the USP 2 dissolution apparatus (Sotax A7 Dissolution Apparatus: Sotax Ltd., London, U.K.) with the paddle rotating at 100 rpm. The physical mixture (PM) containing all the ingredients with the same ratio in SDN and marketed cefpodoxime proxetil for oral suspension (MS) were also tested for comparison. Samples (2 ml) were withdrawn and filtered for analysis at specified time points, and assessed for CP content by HPLC (Shimadzu Corp., Japan). For analysis, a reversed phase Shimadzu-pack VP-ODS C18 (4.6×150 mm, 5 μm particles) column in conjunction with a precolumn C18 insert was used and the peaks of interest eluted with mixtures of acetonitrile and deionized water 38 : 62 (v : v). The flow rate of 1.0 ml/min was maintained. The column effluent was monitored at 235 nm. Quantification of the compounds was carried out by measuring the peak areas in relation to those of standards chromatographed under the same conditions (R^2>0.999).

The ratio of dissolved and undissolved CP in reconstituted nanosuspension and MS was determined. Powder of SDN or MS (equivalent to 50 mg cefpodoxime) was mixed in 100 ml of distilled water and stirred magnetically for 5 min at 100 rpm (the simulation of a manual agitation). The suspension was then filtered. The content of CP in filtrate was determined using the HPLC method mentioned above. The equivalent amount of solid powder was dissolved in methanol and total CP content was determined after filtration and dilution. The ratio of dissolved and undissolved CP in reconstituted nanosuspension and MS was subsequently calculated.

Differential Scanning Calorimetry (DSC) The thermal analyses are performed using a differential scanning calorimeter (NETZSCH DSC 204, Germany) to determine the status of crystallinity of CP in SDN. The DSC runs were performed over a temperature range of 40 to 250 °C at a heating rate of 10 °C per min in an open pan using alumina as a reference material. SDN sample after a long-term stability test (25 °C, 60% RH) was also determined.

Scanning Electron Microscopy Powder samples were glued and mounted on metal sample plates. The samples were gold coated (thickness≈15—20 nm) with a sputter coater (Fison Instruments, U.K.) using an electrical potential of 2.0 kV at 25 mA for 10 min. The surface morphology of SDN and PM was examined using a Hitachi X650 scanning electron microscope (SEM) (Tokyo, Japan) operating at 20 kV.

Nitrogen Physisorption N_2 adsorption and desorption analysis was performed using a SA3100 Surface Area and Pore Size Analyzer (Beckman Coulter Inc., U.S.A.) with nitrogen gas at 77 K in order to investigate and compare the pore properties of the SDN and PM samples. Helium was used to measure the free space of tube. Prior to measurement, all the samples were degassed at 40 °C for about 5 h under a residual pressure of 5 Torr. The specific surface area (SSA) of samples was calculated on the basis of Brunauer–Emmett–Teller (BET) equation. Pore size distributions (PSD) and pore volume were determined using Barrett–Joyner–Halenda (BJH) method.[12,13]

***In-Vivo* Absorption Study. Animals** The study was approved by the Ethical Committee of China Pharmaceutical University. New Zealand male rabbits, weighting 2.5±0.3 kg, were obtained from the Laboratory Animal Center, China Pharmaceutical University (Nanjing, China). All animals were housed individually in standard cages on a 12 h light–dark cycles, were fed with standard animal chow daily, and had free access to drinking water. All animals used in this study were handled in accordance with the guidelines of the Principles of Laboratory Animal Care (State Council, revised 1988).

Experimental Protocol Bioavailability of SDN was compared with that of the MS sample. Before gavage administration to the rabbits, solid powders of SDN and MS were diluted with distilled water and dispersed homogeneously to get a dispersion at a concentration of 27.95 mg/ml.

Six male rabbits were fasted for 12 h prior to the experiment and water was available *ad libitum*. The rabbits were allocated at random to two treatment groups and administered SDN and MS in a crossover design by the oral route with a gastric catheter which was subsequently flushed with 10 ml water.[14] The washout period between the two treatments was 7 d. After gavage administration of a dose of cefpodoxime (17.14 mg/kg body weight expressed as cefpodoxime equivalents), about 1 ml of blood sample was collected through peripheral ear vein into heparinized tubes at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 12 h. Plasma was separated by centrifugation (10 °C, 10000 ***g***, 15 min) using a refrigerated table top centrifuge (Sigma 1-15K, Sigma, Germany) and kept frozen at −70 °C until analysis.

Determination of Cefpodoxime As a prodrug, cefpodoxime proxetil is hydrolyzed *in vivo* into its active metabolite cefpodoxime. The pharmacokinetic and bioavailability study of cefpodoxime proxetil is based on the determination of cefpodoxime in plasma. In this study, a modified HPLC/UV method was employed to determine the concentration of cefpodoxime in rabbit plasma using a reversed phase HPLC (Shimadzu LC 10AD, Shimadzu Corp., Kyoto, Japan).

Cefpodoxime was separated by a C18 column (Shimadzu VP-ODS column, 150 mm×4.6 mm) guarded with a precolumn (Shimadzu) and detected at 235 nm. The mobile phase consisted of 0.02 mol/l ammonium acetate solution and acetonitrile in a volume ratio of 92/8, and the pH of the mobile phase was adjusted to 4.6 by diluted acetic acid. The mobile phase was pumped at a flow rate of 1.0 ml/min. The column temperature was 30 °C.

The extraction procedure was as follows: 200 μl of rabbit plasma was mixed with 100 μl of cefaclor solution as an internal standard (44.4 μg/ml in methanol) and mixed for 1 min. Then, the plasma samples were extracted with 400 μl methanol by vortex-mixing for 10 min and centrifuged for 10 min (10 °C, 10000 ***g***). The obtained supernatant was centrifuged for another 5 min (10 °C, 10000 ***g***). Twenty microliters supernatant was injected into the HPLC system for analysis as described above.

Quantification was based on peak area ratio (Y=cefpodoxime/cefaclor). Cefpodoxime and cefaclor were separated well from impurities in plasma extracts, with retention times of 11.2 min and 13.3 min, respectively. In the concentration range of 0.495—49.5 μg/ml, peak area ratio correlated well to spiked plasma concentration (X): Y=4.2331X+0.2037, (r=0.9997, n=6). After storage for 15 d at −20 °C and freeze-thawing for three times, cefpodoxime was stable in plasma. The limit of quantification and limit of detection were 0.124 μg/ml and 0.031 μg/ml, respectively. At concentrations of 0.99, 9.90 and 34.65 μg/ml, spiked recoveries of cefpodoxime from rabbit plasma were 103.0%, 99.42%, and 100.2%; intra-day precision was 5.20%, 1.43%, and 1.95%; inter-day precision was 5.06%, 1.96%, and 1.15%.

Data Analysis Pharmacokinetic analysis was performed by means of a

model independent method using the DAS2.0 computer program (issued by the State Food and Drug Administration of China for pharmacokinetic study). The area under the plasma concentration *versus* time curve from zero to 12 h ($AUC_{0—12\,h}$) was calculated using the trapezoidal rule. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were directly obtained from plasma data.

All results were expressed as mean±S.D. The data from different formulations were compared for statistical significance by one-way analysis of variance (ANOVA). Results were considered statistically significant with p-values <0.05.

Results and Discussion

Homogenization Parameters In order to prevent the particles from blocking the gap of the homogenizer, CP powder was micronized by jet milling before homogenization. The mean size of the bulk population obtained during the high pressure homogenization process depends mainly on the power density of the homogenizer, the number of homogenization cycles, and also the hardness of the drug. The mean particle size became constant when certain cycles were carried out, that means the given power density is enough.[15)]

After several cycles with low pressure homogenization, a subsequent processing at 1000 bar was run. It was found that the particle size decreased rapidly with the increase in the number of homogenization cycles. After 20 cycles, the particle size decreased to less than 300 nm. The optimal number of cycles was found to be between 25 and 35 with a polydispersity index (PI) of 0.15 to 0.3 (Fig. 1). Finally, 30 cycles were used to prepare the nanosuspension for the following study; the particle size was determined by PCS to be 245.2± 19.7 nm.

Spray Drying and the Process of Reconstitution Considering the peroral administration of CP, and from the economical point of view, the spray drying process was deemed to be more desirable in this study, compared to the lyophilization, the main but relatively costly method used in a previous nanosuspension study.[16)]

The reconstitution of SDN into the immobile acid media was carried out to study the dynamic redispersion behavior and powder interactions in aqueous solution. The dynamic redispersion was visually observed and digitally photographed as a function of time. Once contacting with the surface of 0.01 mol/l hydrochloric acid in a glass bottle, a fast and spontaneous diffusion procedure was observed immediately (figures were not shown). The nano-sized powder was dispersed in acid media. No sign of accumulation and the typical gelatinization behavior of CP in aqueous environments was observed under microscope even after 2 h. The diameter of resulted dispersion was determined by PCS to be 266.5±25.2 nm, a similar particle size to the mean of the original liquid nanosuspension, which may indicate that the combination of polymeric surfactant, viscous polymer and glycerol is sufficient to stabilize the nanosuspensions during the spray drying process. The similar particle size shift was reported by Chaubal and Popescu who used the combination of poloxamer/sugar/charged surfactant to prevent irreversible aggregation.[9)]

Evaluation of Rheological Properties The relationships of shear rate with the viscosity and the shear stress of the reconstituted nanosuspension are shown in Fig. 2. The apparent viscosity of the nanosuspension decreased with the increase in shear rates and gradually tended towards an asymptote. Such behavior demonstrates the characteristic of shear-thinning, the property of a pseudoplastic fluid.

The most common model that describes the behavior of non-Newtonian fluids is the Herschel–Bulkley model ($\tau=\tau_0+k\gamma^n$).[11)] The polynomial coefficient of determination (R^2) being close to 1 ($R^2=0.9998$), the rheological equation of liquid CP nanosuspension can be well described by the Herschel–Bulkley model on the basis of statistical evaluation. The fitted yield stress (τ_0) is 1.34 Pa and consistency index (k) is 0.9968. The value of flow behavior index ($n=0.645$) is much less than 1, verifying the degree of shear thinning and pseudoplastic property of the nanosuspensions.

Thixotropic Behavior of Nanosuspension When the shear rate increased from 1.32 to 212 s^{-1} and then decreased at the same rate to 1.32 s^{-1}, a hysteresis loop was observed (Fig. 3). The hysteresis loop indicated that the homogenized nanosuspension had a small degree of thixotropic behavior. This might be explained as the disintegration and the recuperation of the reticular formation in the formulation.[17)] Nanosuspension particle flocculation occurs, and shearing breaks down the flocculated structures back to primary particle. The thixotropic behavior of nanosuspension would profit the physical stability of the system and the ease of use by slowing down the sedimentation velocity of drug particles.

***In Vitro* Release of CP** Standard *in vitro* SDN powder dissolution testing was used to provide a comparison with the dissolution profiles of the PM powder and MS. The SDN powder underwent very rapid dissolution in acid environment, with 100% CP released after approximately 5 min (Fig.

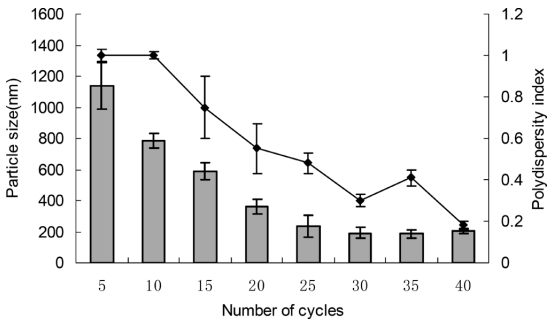


Fig. 1. PCS Diameter and Polydispersity Index of Cefpodoxime Proxetil Nanosuspension as a Function of the Number of Homogenization Cycles (Pressure=1000 bar, $n=3$)

Each value represents mean from three experiments. (■) particle size, (◆) polydispersity index.

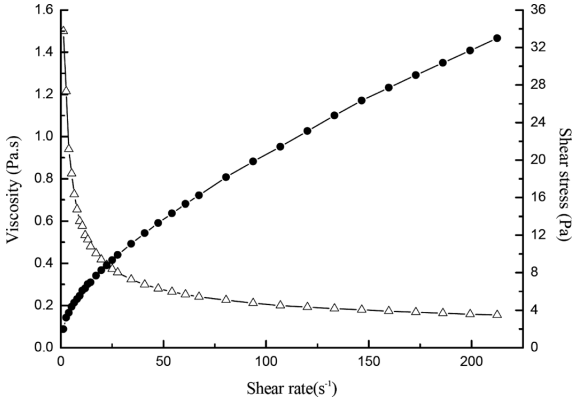


Fig. 2. Flow Curve of Cefpodoxime Proxetil Nanosuspension at 25 °C

Each value represents mean from three experiments. (△) viscosity, (●) shear stress.

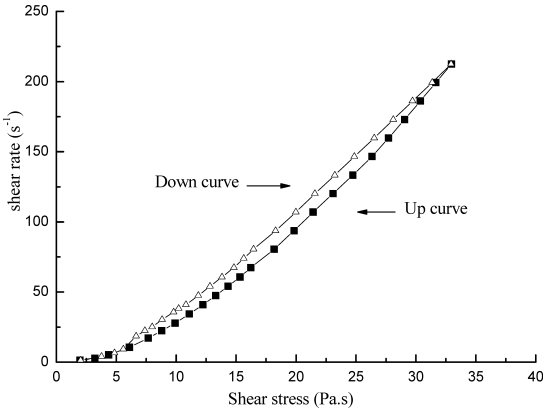


Fig. 3. Thixotropic Behavior of Cefpodoxime Proxetil Nanosuspension: Up Curve (Increasing the Shear Rate); Down Curve (Decreasing the Shear Rate)

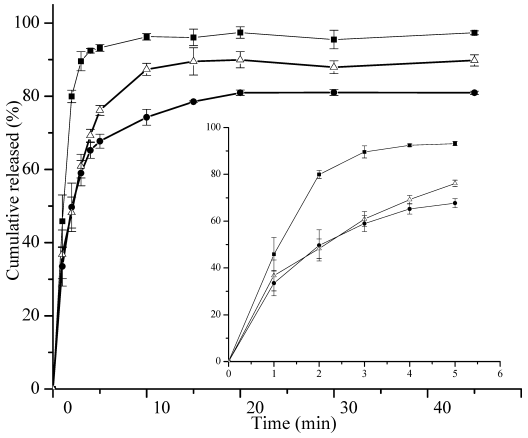


Fig. 4. Dissolution Profiles of Cefpodoxime Proxetil in 0.01 mol/l Hydrochloric Acid

Each value represents mean from six experiments. (■) spray dried cefpodoxime proxetil nanosuspension, (●) physical mixture, (△) marketed cefpodoxime proxetil for oral suspension.

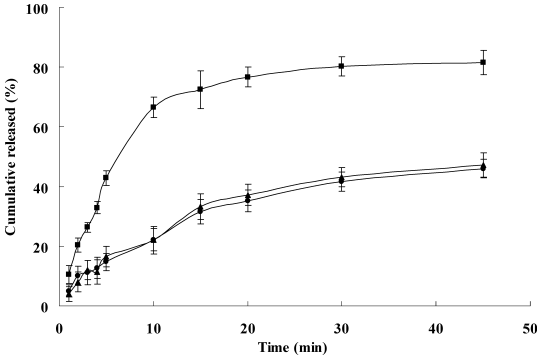


Fig. 5. Dissolution Profiles of Cefpodoxime Proxetil in 0.05 mol/l Phosphate Buffer (pH 6.5)

Each value represents mean from six experiments. (■) spray dried cefpodoxime proxetil nanosuspension, (●) physical mixture, (▲) marketed cefpodoxime proxetil for oral suspension.

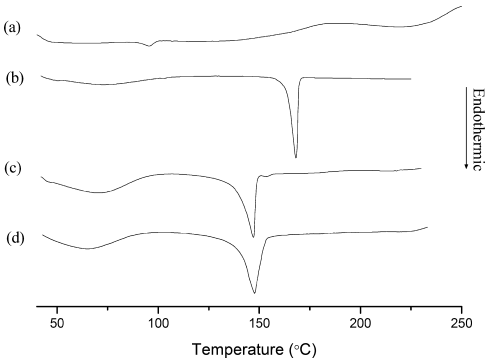


Fig. 6. DSC Graphs of Cefpodoxime Proxetil (a), Spray Dried Nanosuspension without Cefpodoxime Proxetil (b), Spray Dried Cefpodoxime Proxetil Nanosuspension (c) and Spray Dried Cefpodoxime Proxetil Nanosuspension after Storage for 12 Months (d)

The vertical axis (heat flow) is shown on an arbitrary scale.

4), while the physically mixed powder of the formulation and MS exhibited a much slower and an incomplete dissolution profile. Compared to MS, PM had a relatively rapid dissolution rate at the first 10 min of dissolution stage which may be attributed to the wetting action of poloxamer and glycerol in PM. After that, PM and MS achieved the similar incomplete dissolution amount ($p>0.05$), the additives in PM had no significant effect on the final dissolution amount of CP.

Dissolution media had evident effect on the dissolution characteristics. In pH 6.5 buffer, the dissolution curve of SDN, PM and MS all showed a significantly slower profiles compared to that in acid media (Fig. 5). However, the dissolution of SDN was still much faster and more complete than PM and MS. Almost 80% of CP was released in 45 min and about 42% of CP was rapidly released in 5 min. It had a much faster dissolution rate and much more complete dissolution amount than PM and MS. The difference was even more distinct than that in acid media.

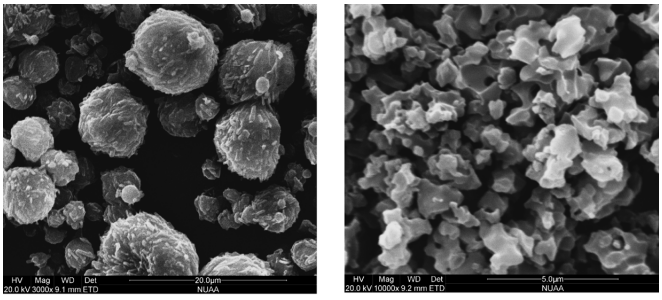
Homogenization gave CP fine particles and a resulting state of high dispersion which restricted the tendency of CP to gelatinize in acidic aqueous environments. In pH 6.5 buffer, CP gelatinization was not obviously for all samples. As observed during the process of dynamic reconstitution, no sign of gelatinization was visually observed and the fine powder diffused in acid media spontaneously and rapidly. This spontaneous dissolution was also noted in the dissolution vessels containing 0.01 mol/l hydrochloric acid. A homogeneous suspension can be generated instantaneously once the SDN sample contacts the acid medium due to the mechanical paddle rotation. As a comparison, PM and MS produced similarly obvious aggregation which was easily observed visually and was hard to separate into its original fine powder afterwards. After 2 min of agitation, gel-like structure was visually observed at the medium surface and did not vanish throughout the dissolution process. The addition of poloxamer and glycerol can not change the tendency for CP in PM to gelatinize. The gelatinization will then retard and lower the dissolution of CP from PM and MS. On the other hand, the significantly decreased particle size and weakened gelatinization of SDN may be responsible for its significantly enhanced dissolution.

DSC Studies The thermograms of the pure CP, PM, drug-free SDN and SDN containing CP were studied to determine the status of crystallinity of CP in SDN. A sharp endotherm was obtained with drug-free SDN at 164.7 °C (Fig. 6). The DSC thermogram of CP did not show any melting endotherm, demonstrating the amorphous state of CP in pure form. Both freshly prepared drug-loaded SDN and those after storage for 12 months showed only one endothemic

peak at about 148.5 °C similar to drug-free SDN. This result suggests that no crystalline nature change had taken place in SDN during preparation, and after long-term storage.

The content of CP in SDN was also determined using the HPLC method mentioned in dissolution testing. No detectable change in drug content was observed which indicating its chemical stability of CP at 25 °C.

Surface Morphology Scanning electron microscopy was used to visualize and compare the structural and surface morphology of SDN and PM (Fig. 7). The micrograph of the SDN powders indicated separate, regular and spherical particles, which had a "hairy" surface and has no adhesion to each other (Fig. 7A). The large amount of fine "hair" at the surface of the particles may be the solidified soluble excipients in the formulation produced by spray drying. Once contacting the aqueous medium, the soluble stabilizers wrapped around the spherical particle apparently dissolve rapidly to form a hydrophilic wetting environment beneficial to the physical stability of the hydrophobic drug, causing the rapid dispersion of nanosuspension in media. In contrast, the micrograph of the PM powder did not show spherical particles and showed irregular shape with a great deal of angularities along with obvious adhesion (Fig. 7B); rather, the particles appeared to have undergone fusion, which may have occurred possibly at certain stage during SEM processing.



A B

Fig. 7. Scanning Electron Micrographic Images of (A) Spray Dried Cefpodoxime Proxetil Nanosuspension, (B) Physical Mixture

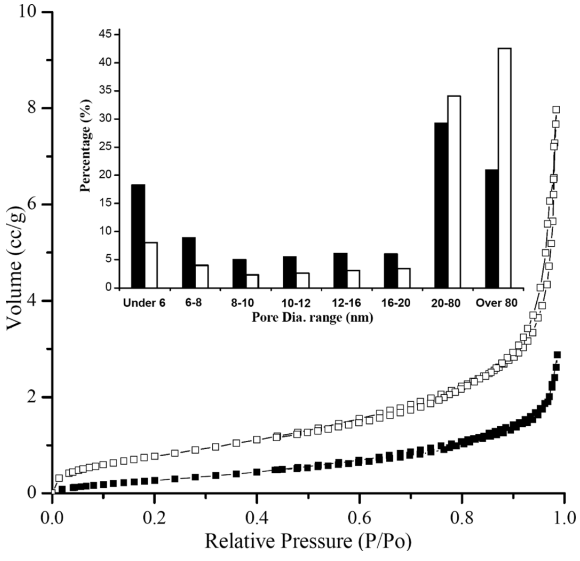


Fig. 8. N_2 Adsorption/Desorption Isotherms and the Pore Size Distributions

Each value represents mean from three experiments. (□) physical mixture, (■) spray dried cefpodoxime proxetil nanosuspension.

Surface Area and Pore Size Measurements The N_2 adsorption/desorption isotherms and the pore size distributions of SDN and PM are displayed in Fig. 8. Both nitrogen sorption isotherms correspond to type II according to the Brunauer–Deming–Deming–Teller (BDDT) classification.[18] The gradual increase in adsorption with P/P_0 from 0.2 to 0.8 is attributed to a wide range of mesopore sizes (micropore: diameter<2 nm; mesopore: 2 nm<diameter<50 nm; macropore: diameter>50 nm), and then a sudden increase in amount adsorbed at high relative pressure was observed. In comparison, the N_2 adsorption/desorption amount of PM was considerably higher than that of SDN sample at both low and high pressures. Adsorption/desorption hysteresis, attributed to capillary condensation, was observed more clearly for PM, while this is almost absent for SDN.[19] It could be shown from Fig. 8 that PM has many more macropores over 80 nm while SDN has more mesopores of less than 20 nm. The specific surface areas of SDN and PM were determined to be 2.970 m^2/g and 1.163 m^2/g using the multipoint BET method.

Enhancement of Bioavailability To confirm the usefulness of nanosuspension in improving the bioavailability of CP, an *in vivo* test was carried out in rabbits in a crossover fashion and pharmacokinetic parameters of SDN and MS were compared. The mean plasma cefpodoxime concentrations *versus* time profiles of the two formulations are shown in Fig. 9. From 15 min after gavage administration to 2 h, the cefpodoxime concentration of the nanosuspension was significantly higher than that of the conventional suspension.

Mean pharmacokinetic parameters for SDN and MS are

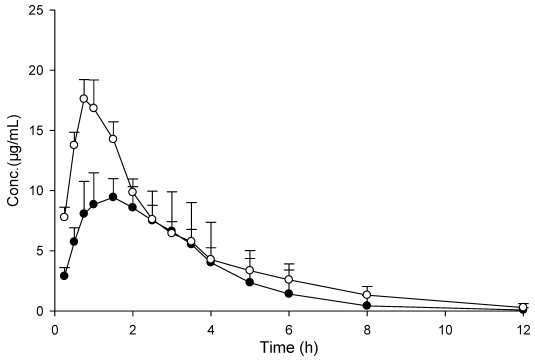


Fig. 9. Mean Cefpodoxime Concentration in Rabbit Plasma after an Oral Administration of Cefpodoxime Proxetil Formulations

Each value represents mean from six experiments. (○) spray dried cefpodoxime proxetil nanosuspension, (●) marketed cefpodoxime proxetil for oral suspension.

Table 1. Pharmacokinetic Parameters in Rabbits after Gavage Administration

Parameters	Marketed suspension	Spray dried nanosuspension
t_{max} (h)	1.75±0.68	0.75±0.11*
C_{max} (μg/ml)	10.88±1.01	18.36±2.03*
$AUC_{0—12\,h}$ (mg·h/l)	29.78±3.47	47.55±4.33*
$AUC_{0—\infty}$ (mg·h/l)	31.58±3.54	50.25±4.54*

Values are expressed as means±S.D. from six experiments. t_{max}: time to maximum concentration; C_{max}: maximum concentration; *AUC*: area under the curve of plasma concentration *versus* time from $t=0$ to $t=12$ or $t=\infty$ after gavage administration. * $p<0.05$; spray dried nanosuspension *versus* marketed suspension.

listed in Table 1. Calculated on $AUC_{0—12\,h}$, the mean relative bioavailability of SDN was 1.60-fold that of MS.

Several factors could be involved in the improvement of CP bioavailability. The decreased particle size may increase the dissolution rate by increasing surface area as elaborated in the Noyes–Whitney equation.[20] It was reported that reducing the particle size from 20—30 μm to 270 nm led to faster absorption of naproxen.[21] In addition, decreased particle size and increased surface area can lead to increased muco-adhesion, which can increase gastrointestinal transit time and lead to increased bioavailability.[4] As demonstrated in dynamic reconstitution and *in vitro* dissolution test, the restraint of typical gelatinization behavior of CP in acidic environments by nanosuspension may be another important factor beneficial to the bioavailability enhancement.

Nicolaos *et al.* reported the bioavailability enhancement of CP using an oil-in-water submicron emulsion in order to improve the oral absorption of CP in rats.[22] The mean droplet size of the submicron emulsion was 0.230 ± 0.006 μm. $AUC_{0—\infty}$ was approximately two times greater when CP was administered as submicron emulsion compared to the suspension. However, the absorption of cefpodoxime proxetil was significantly retarded due to two lipid excipients (Miglyol 812N®, Inwitor 742®) in the formulation (t_{max} increased from 78 ± 27 to 200 ± 49 min) and the values of C_{max} of suspension and submicron emulsion were also equivalent (8.1 ± 2.8 μg/ml and 9.1 ± 2.9 μg/ml).

Compared to Nicolaos's report, the mean values of C_{max} for SDN (18.36 μg/ml) were 1.69 times greater than that of CP administered as the conventional suspension (10.88 μg/ml). The nanosuspension achieved a much faster rate of drug absorption as indicated by the much shorter time to reach peak plasma levels, t_{max}, *i.e.* 0.75 ± 0.11 h *vs.* 1.75 ± 0.68 h for the suspension ($p<0.05$).

The ratio of dissolved CP and undissolved CP in reconstituted nanosuspension and MS was significantly different ($p<0.05$). The ratio is 1 to 9 for MS. Only about 10.6% of CP can dissolve in 100 ml of distilled water. While for SDN, the ratio is close to 0.5. About 33.8% of CP was in solution state after reconstitution. The high percentage of dissolved CP or the administration of more CP in solution state may be the important factor for the enhanced *AUC* and much shorter t_{max} of SDN.

Conclusion

Based on the results from physicochemical characterization in the present investigation, the novel spray dried cefpodoxime proxetil nanosuspension appeared to produce regular and spherical particles and exhibited rapid dissolution in acid media without accumulation and gelatinization. Formation of the nanosuspension maintains the poorly soluble drug at reduced particle sizes and this apparently increases the dissolution rate and therefore improves the bioavailability as described in the Noyes–Whitney equation and Ostwald–Freundlich equation.[23] After spontaneous reconstitution, the resulted nano-sized suspension was demonstrated to be pseudoplastic fluid with thixotropic behavior. Considering the significant increment of pharmacokinetic parameters (*AUC*, C_{max} and t_{max} of CP) and the thermodynamically stable solid drug delivery system, the spray dried nanosuspension may be an effective formulation strategy for the other drugs with low oral absorption.

Acknowledgment This work was funded by The Technology Platform for New Formulation and New DDS, Important National Science and Technology Specific Projects, NO: 2009ZX09310-004. The authors are grateful to Prof. George Zografi, the University of Wisconsin Madison, for valuable suggestions and helpful revisions on this article.

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