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Enhancing the Bioavailability of Cyclosporine A Using Solid Dispersion Containing Polyoxyethylene (40) Stearate

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ABSTRACT Solid dispersion containing polyoxyethylene (40) stearate and cyclosporine A was prepared by solvent-melt method and characterized using differential scanning calorimetry, powder X-ray diffraction, and Infrared Fourier Transform Spectroscopy (FTIR). Dissolution of the drug from solid dispersion was dramatically enhanced compared to that from the drug powder alone and physical mixture. In vivo oral bioavailability of cyclosporine A from the solid dispersion in Wistar rats was comparable to that from a commercial product, Sandimmun Neoral® ($P > 0.05$). The formulation is stable up to six months under 30°C/RH60% and one year at 25°C/RH 60% when packed in aluminum-polyethylene laminated bags.

KEYWORDS Cyclosporine A, Solid dispersion, Polyoxyethylene (40) stearate, Dissolution, Stability, Bioavailability

INTRODUCTION

Cyclosporine A (CyA), isolated from the fungus *Tolypocladium inflatum gams*, is an immunosuppressive drug widely used in the prevention and treatment of allograft rejection and autoimmune disease (Kahan, 1989). It is also used in the treatment of hematopoietic cell transplantation (Hogan & Storb, 2004), endogenous uveitis (Vitale et al., 1996), and psoriasis (Kawada et al., 2003). Cyclosporine A (CyA) is a cyclic polypeptide consisting of 11 amino acids. Although CyA has a high octanol-Ringer's partition coefficient (logP = 2.92; Taylor et al., 1993), the absorption from the gastrointestinal tract is incomplete and variable. Several factors including the relatively high molecular weight, poor solubility in water (7.3 μg/mL at 37°C, as reported by Ismailos et al., 1991), extensive metabolism by cytochrome P-450 3A4 in both liver and gut (Christians & Sewing, 1993; Watkins, 1994) and effect of P-glycoprotein (P-gp)-mediated drug efflux (Charuk et al., 1995) have been suggested as reasons for the low and variable bioavailability of CyA. Cyclosporine A (CyA) is commercially available as oral solutions and soft capsules containing microemulsion. Many researches have

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been conducted to improve its solubility and bioavailability in the form of microemulsion (Kim et al., 2002), nanoparticles (Chen et al., 2002; Ubrich et al., 2005), microspheres (Urata et al., 1999; Lee et al., 2001), and complexation with cyclodextrin (Miyake et al., 1999).

An alternative approach to improve the dissolution and bioavailability of poorly soluble drugs involves the use of solid dispersion employing water soluble polymers. Solid dispersion has been widely investigated since its emergence in 1961 (Sekiguchi & Obi, 1961) and led to a few products in market, such as a griseofulvin-in-poly (ethylene glycol) solid dispersion (Gris-PEG, Novartis) and a nabilone-in-povidone solid dispersion (Cesamet, Lilly). (Serajuddin, 1999). In recent years, numerous papers have been published by using surface-active and self-emulsifying carriers to prepare solid dispersion (Damian et al., 2002; Vippagunta et al., 2002; Chen et al., 2004; Joshi et al., 2004). The advantage of surface active vehicles over the non-surface active ones is that the former will disperse or emulsify the drug incorporated, and therefore, lead to a rapid dissolution while the latter will form a drug-rich surface layer and retard or prevent the dissolution of the drug (Serajuddin, 1999).

Polyoxyethylene (40) stearate (S40) is a nonionic surfactant with a HLB value of 16.9. It is freely soluble in water. The material was used as a carrier in the solid dispersion systems to improve the dissolution of Griseofulvin, Tolbutamide (Kaur et al., 1980), and Indomethacin (Valizadeh et al., 2004), but no in vivo bioavailability was reported so far. It is reported (Lo, 2003) that this material significantly increased apical to basolateral absorption and substantially reduced basolateral to apical efflux of epirubicin across Caco-2 monolayers, indicating its inhibition of intestinal P-gp. Therefore, the use of S40 in this formulation may improve the oral bioavailability of CyA by inhibiting the efflux of P-gp.

The preliminary experiments have shown that the solid dispersions containing S40 exhibited improving dissolution characteristics of CyA as the amount of carrier is increased (data not published). Solid dispersions containing S40/drug ratio (w/w) higher than 7:1 showed little or no further increase in drug release. Therefore, the 7:1 ratio was selected for this investigation as higher drug content is more suitable for practical use.

In this study, solid dispersions containing CyA and S40 were prepared by solvent-melt method. The purpose of this investigation is to evaluate the physical characteristics, stability, in vitro dissolution, and in vivo bioavailability of the solid dispersion.

MATERIALS AND METHODS

Materials

Cyclosporine A (CyA) (Lot#: 020801, 98.7%) was a generous gift of Taishan Pharmaceutical Company Ltd. (Guangdong, China). Cyclosporine D (CyD, Lot#: 010915, 98%) was obtained from Sichun Industrial Institute of Antibiotics (Chengdu, China). Acetonitrile UV was obtained from Burdick & Jackson (Muskegon, MI, USA). Methanol of high pressure liquid chromotography (HPLC) grade was purchased from Hanbang Chemical Co. (Jiangyin, Jiangsu, China). Methyl t-butyl ether was purchased from TEDIA (Fairfield, OH, USA). Water was ultra filtered through a Millipore filtration system (Milli-Q®). Polyoxyethylene (40) stearate (S40) of Chinese Pharmacopoeia grade was purchased from Nanjing WELL Chemical Corporation, Ltd. (Nanjing, China). Sandimmun Neoral® soft gelatin capsule (25mg strength) was the product of Novartis (Bern, Switzerland). All other chemicals and solvents used were of AR grade.

Preparation of Solid Dispersion and Physical Mixture

The solid dispersions of CyA with S40 were prepared by solvent-melt method. Cyclosporine A (CyA) in anhydrous ethanol was added under constant stirring to the fused S40 in a beaker maintained at 65°C by using a water bath. The mixture was then stirred for another 40 min at 65°C until most of ethanol evaporated. After being quenched quickly for 4 h at −18°C, the solid dispersion was dried in a vacuum drier at 25°C for 24 h and then pulverized. The powder that can pass through No. 80 mesh sieve was collected and stored in a glass desiccator at room temperature for further experiments.

Physical mixture of CyA and S40 was obtained by mixing accurately weighed powder of the components and sieving through a No. 80 mesh screen.

Powder X-ray Diffractometry (PXRD)

X-ray diffraction patterns of the pure ingredients, physical mixture, and solid dispersion were recorded using a powder diffractometer (Tokyo, Japan) with Cu-Kα radiation, operated at 40 KV, and 60 mA. The scanning speed was at 4°/min with a scanning step 0.02° 2θ. The scanning range was from 5–45° 2θ.

Differential Scanning Calorimetry (DSC)

Thermal analysis was carried out with a differential scanning calorimeter (DSC 204/1/GPhoenix®, NETZSCH-Geratebau GmbH, Selb, Bavaria, Germany). About 7 mg of CyA alone, S40, CyA-S40 physical mixture, or solid dispersion was accurately weighed into an aluminum pan and hermetically sealed. An empty pan of the same type was used as the reference. All samples were heated from 20°C to 200°C at a heating rate of 10°C/min under a dry nitrogen gas purge.

Infrared Fourier Transform Spectroscopy (FTIR)

The Fourier-transformed infrared spectra of samples were obtained after appropriate background subtraction using an Avatar™ 360 E.S.P™ FTIR spectrometer (Thermo Nicolet Corporation, Madison, WI, USA). About 2 mg of the sample was mixed with 200 mg of dry potassium bromide and compressed to a disk. The scanning range was 400–4000 cm^{-1} and the resolution was 4 per cm.

Dissolution Study

Cyclosporine A (CyA) powder alone, CyA-S40 physical mixture, and solid dispersion equivalent to 25 mg of CyA were filled into hard gelatin capsules. The dissolution of CyA from these capsules was determined by using paddle method as described in the second volume of the Pharmacopoeia of People's Republic of China (2000 Edition). The dissolution studies were performed in 900 mL of water at 37°C with stirring speed at 100 rpm. Samples were withdrawn every 10 min and centrifuged at 3000 rpm for 10 min. The supernatant of 20 μL was injected into HPLC. The mobile phase was composed of acetonitrile, methanol, and water at the ratio of 44:40:16. Flow rate was 1.5 mL/min. The oven temperature was 55°C. Cyclosporine A (CyA) in samples was detected at wavelength of 214 nm. The cumulative amount of drug released was calculated and plotted versus time. For samples subject to stability studies, the dissolution of CyA at 45 min was detected and compared to that of the freshly prepared samples.

Bioavailability Study

Male Wistar rats, ♂, weighing from 220 g to 270 g, were supplied by Shanghai SLAC Laboratory Animal Co. Ltd. [Certificate No.: SCXK (Shanghai) 2003–0003]. The rats were fasted overnight but were allowed free access to water before the experiment. The animals were randomly divided into two groups of 10 animals each. One group received 10 mg/kg of CyA in Sandimmun Neoral® used as a control, and the other was given the same dose of the solid dispersion. Both were diluted with water to 1 mg/mL before administration.

The preparations were given intragastrically to the rat through a metallic tube. Venous blood samples (about 0.6 mL) were withdrawn from the postorbital vein sinus into a polypropylene tube treated by heparin sodium at 0.5, 1, 2, 3, 4, 5, 8, 12, 24, 36, 48, and 60 h after the administration. The samples were thoroughly shaken and stored at −18°C until analysis.

The extraction procedure of CyA in whole blood was as followed (Ye et al., 1999): to an aliquot of 0.5 mL of the sample, CyD (40 μl of 20 μg/mL solution in methanol) as an internal standard, and 1 mL of HCl (0.18 mol/L) were added. The mixture was vortexed for 1 min. After that, 5 mL of methyl t-butyl ether was added, followed by horizontally shaken for 15 min and centrifuged (3000 rpm, 10 min). The ether layer was then shaken with 1 mL of NaOH (0.095 mol/L) for 5 min, centrifuged (3000 rpm, 10 min), transferred to a 5 mL-graduated centrifuge tube, and evaporated at 55°C under the purge of N_2. The residue was dissolved in 100 μL of acetonitrile/water (70:30) and washed by *n*-hexane twice (0.8 mL each time). After vortex (2 min) and centrifugation (3000 rpm, 5 min), the lower layer was separated and 20 μL was injected to a C-8 column (Kromasil, 5 μm, 25 × 0.46 cm i.d., USA). The mobile phase consisted of acetonitrile, methanol, and water at the volume ratio of 66:10:24. Flow rate was 1.5 mL/min. The oven temperature was 60°C. The wavelength was 214 nm. The peak area of CyA and CyD were recorded and the concentration of CyA was determined.

Standard calibration curves were constructed by spiking drug-free pooled whole blood with a known amount of CyA in a series of concentrations of 100, 250, 500, 1000, 2000, 3000, and 4000 ng/mL. The extraction procedure was identical with that of the samples described above. The concentrations of CyA in the calibration standards (C_{CyA}) were regressed with

the ratio of peak areas of CyA and CyD (A_{CyA}/A_{CyD}). Quantification of CyA in samples was conducted by using the linear regression line obtained from calibration standards. The calibration curve was $C_{CyA} = 327.56 \times A_{CyA}/A_{CyD} - 17.22$ ($r^2 = 0.9954$). The extraction recovery of CyA and CyD was 74.77 ± 4.65% and 73.30 ± 5.96%, respectively.

The area under the drug concentration-time curve (AUC) was calculated using the trapezoidal rule. The maximum blood concentration of drug (C_{max}) and the time to reach the maximum blood concentration (T_{max}) were obtained directly from the observation. The determination of compartment model and the derivation of the pharmacokinetic parameters were performed by using a computer program 3p97 (Chinese Society of Mathematic Pharmacology, Beijing, China). The relative bioavailability was calculated using the equation:

$$AUC_{0-60h} \text{ for solid dispersion}/AUC_{0-60h} \text{ for Neoral}^{®} \times 100\%$$

Stability Studies

Crystallization may occur upon storage if the drug is initially dispersed in a molecular state or in an amorphous state, leading to a reduced dissolution and bioavailability upon aging (Serajuddin, 1999). In order to evaluate the chemical and physical stability of the solid dispersion, stability studies were conducted at different storage conditions of temperature and relative humidity. The representative samples of three batches were filled into capsules and placed in controlled temperature cabinets at 40°C/75% RH, 30°C/60% RH (six months) and 25°C/60% RH (one year) in closed aluminum-polyethylene laminated bags. At defined intervals of time, the samples were removed and subjected to PXRD test and dissolution test to detect any change. The chemical stability was assessed by HPLC. The mobile phase was composed of acetronile, methyl t-butyl ether, and water at the ratio of 520:430:50 (0.1% phosphate was added). Flow rate was 1.5 mL/min. The detection wavelength was 210 nm. The oven temperature was 70°C.

Statistic Analysis

Data were expressed by the mean ± SD. Statistical significances of differences between the mean values were analyzed using Student's t-test.

RESULTS AND DISCUSSION

X-Ray Powder Diffraction

The x-ray diffraction (PXRD) patterns of samples are shown in Fig. 1. The diffractogram of crystalline CyA showed characteristic high-intensity diffraction peaks at 2θ values of 6.76°, 7.72°, 9.10°, 9.32°, 10.68°, 14.58°, 15.02°, 16.76°, 19.36°, and 22.20°, which reflected the crystalline nature of CyA. The characteristic peaks of S40 at 2 θ values of 19.04° and 23.18° remained in the spectra of physical mixture and solid dispersion. The sharp peaks of CyA were observed in the spectra of physical mixture. It is confirmed that the crystallinity of CyA does not change in the physical mixtures. The powder x-ray diffraction (PXRD) pattern of the solid dispersion was similar to that of the carrier, where the diffraction peaks of CyA were not observed. This result suggested CyA exists at an amorphous state in the solid dispersion. The large loss in crystallinity can be expected to enhance the dissolution and bioavailability of this water-insoluble drug.

Differential Scanning Calorimetry

Figure 2 showed the DSC thermograms of CyA, S40, their physical mixture, and solid dispersion. The spectrum of CyA showed an endothermic peak at around 155°C corresponding to the melting of CyA. The physical mixture showed no endothermic peak of CyA, even though the peaks according to CyA were

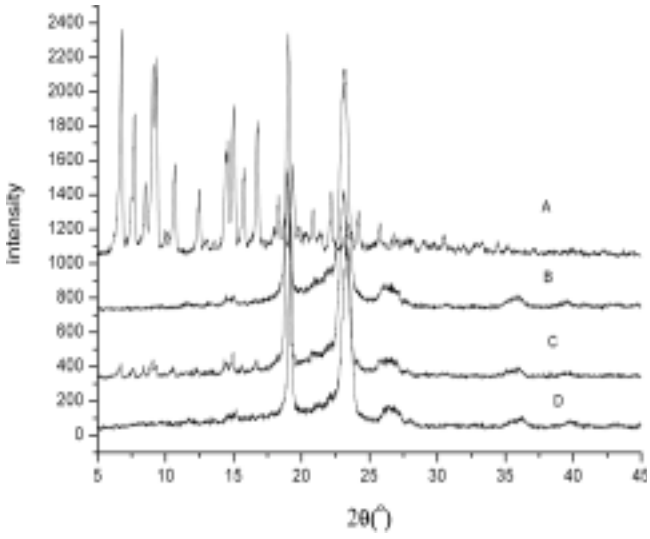

FIGURE 1 **X-ray Powder Diffraction Patterns of (A) CyA; (B) S40; (C) Physical Mixture; and (D) Solid Dispersion.**

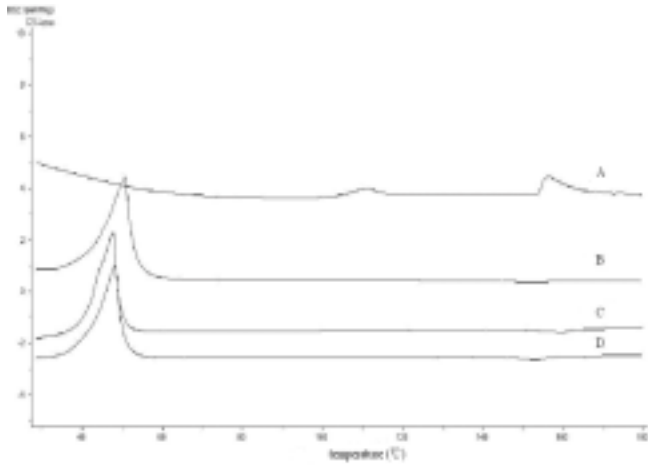

FIGURE 2 **DSC Spectra of (A) CyA ; (B) S40; (C) Physical Mixture; and (D) Solid Dispersion.**

observed in PXRD (Fig. 1). Similar phenomenon was observed for the physical mixture of tacrolimus and PEG 6000 (Yamashita et al, 2003). It is speculated that CyA may dissolve in the melted S40 during DSC measurement and only one endothermic peak at around 48°C, which corresponds to the melting of S40, was observed. Solid dispersion exhibited no endothermic peak corresponding to CyA, suggesting no crystalline of CyA in this system. From the results of PXRD and DSC studies, it was confirmed that CyA exists in an amorphous state in solid dispersion.

FTIR Study

Infrared fourier transform spectrometry (FTIR) studies were performed to detect the possible molecular interaction between CyA and S40 in the solid dispersion system. The FTIR spectra of CyA, S40, S40/CyA physical mixture, and solid dispersion are shown in Fig. 3. In the spectrum of CyA, absorption bands of N-H stretching vibration at 3322 cm^{-1}, C-H stretching vibration at 2961 cm^{-1}, and C=O stretching vibration at 1646 cm^{-1} were observed. The spectrum of S40 exhibited C=O stretching vibration at 1737 cm^{-1} and a relatively broad peak of C-H stretching vibration at 2877 cm^{-1} caused by the large molecular size of the

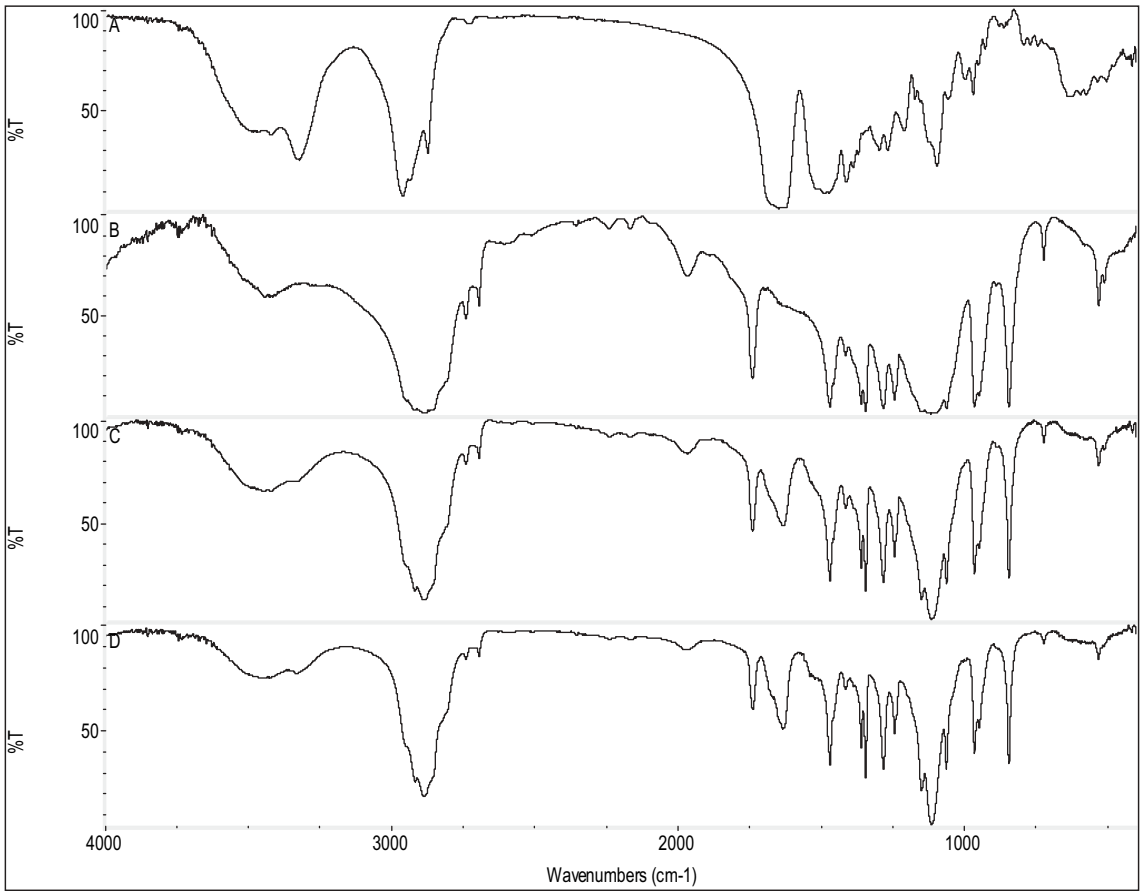

FIGURE 3 **FTIR Spectra of (A) CyA; (B) S40; (C) Physical Mixture; and (D) Solid Dispersion.**

polymer and its partially amorphous nature. The spectra of physical mixture and solid dispersion were the additive of CyA and S40. There were no new peaks or shift of peaks that appeared in the spectrum of solid dispersion compared to that of the physical mixture. This phenomenon indicated the lack of significant interactions between the drug and the carrier in the solid dispersion (Vippagunta et al., 2002; Yamashita et al., 2003).

Dissolution Test

Dissolution of CyA from capsules containing solid dispersion or physical mixture was significantly improved compared to that of CyA powder alone (Fig. 4). Compared with physical mixture, significantly more drug released from solid dispersion was observed, which may be due to the higher dispersion of the drug, the reduction of the particle size, and the disappearance of the drug crystalline verified by PXRD.

Bioavailability of Solid Dispersion

The mean CyA concentrations in whole blood for both oral administration of CyA solid dispersion and the control were shown in Fig. 5. The concentration-time curves for both preparations were best fitted to a two-compartment model with a weight of 1/C. The derived pharmacokinetic parameters were shown in Table 1. Sandimmun Neoral®, a microemulsion of CyA, was considered to deliver the drug more effectively to the systematic circulation with lower inter- and intra-subject variability (Kovarik et al., 1994). Compared to this system, the relative bioavailability of the solid dispersion preparation was 98.20%, indicating the effective absorption of this poorly water-soluble drug in gastrointestinal tract from the designed formulation. It was shown in Fig. 5 that the profiles of the CyA whole blood concentrations after single dosing of solid dispersion and Sandimmun Neoral® were quite similar. Statistical analyses revealed that there were no significant differences between the two formulations for C_{max}, AUC_{0-60}, T_{max}, K_a, K_{10}, and MRT ($P > 0.05$). The experimental results illustrated that solid dispersion is a useful approach to improve the bioavailability of this poorly water-soluble drug.

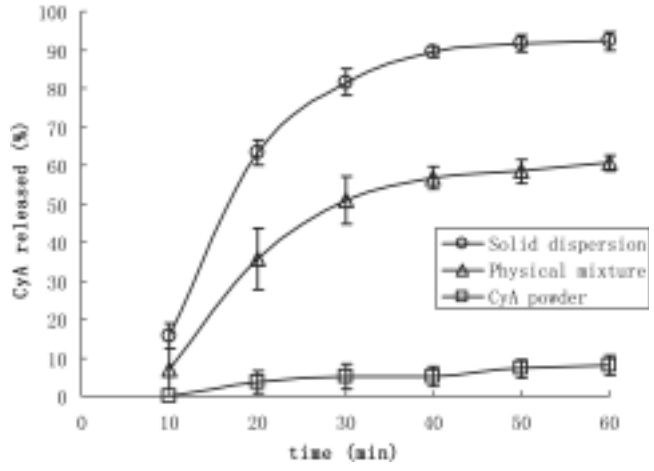

FIGURE 4 Dissolution Profile of CyA Powder (□); Physical Mixture (▲); and Solid Dispersion (○). The Error Bar Stands for the Standard Deviation.

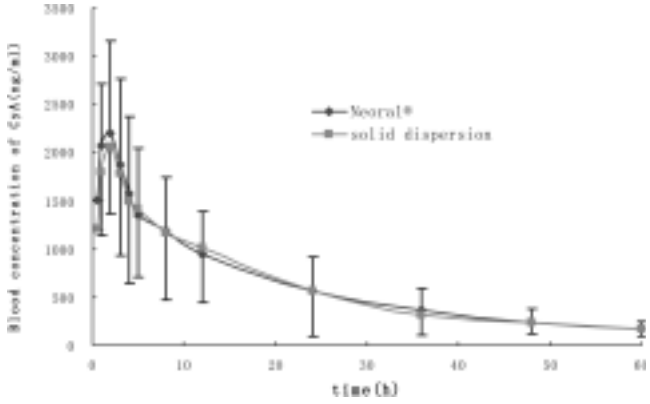

FIGURE 5 Mean Blood Concentration of CyA Versus Time Curves After Oral Administration of CyA Solid Dispersion (■) and Sandimmun Neoral® (✦) to Wistar Rats.

Stability Study

Stability problem of solid dispersion during storage is the main reason for its few marketed products compared to the numerous research papers (Ford, 1986). The amorphous form may re-crystallize out on aging. Consequently, the dissolution of the drug will be affected. Therefore, the influence of storage temperature and relative humidity on the change in dissolution properties and PXRD patterns are investigated.

The X-ray diffraction patterns of samples are shown in Fig. 6. In the freshly prepared sample, the diffraction peaks of CyA were not observed, suggesting the amorphous state of CyA in the system. It was interesting to observe that the peaks corresponding to CyA were not detected throughout the storage period for samples stored at 30°C/60% RH (six months) and 25°C/60% RH (one year), which indicated the maintenance of the amorphous state of CyA in solid dispersion

TABLE 1 **Pharmacokinetic Parameters of CyA After Oral Administration of CyA Solid Dispersion and Sandimmun Neoral® to Wistar Rats**

Pharmacokinetic parameter	CyA solid dispersion	Sandimmun Neoral®
C_{max} (ng/ml)	2348.65 ± 495.96	2557.38 ± 555.09
T_{max} (h)	2.00 ± 0.67	1.71 ± 0.67
AUC_{0-60} (ng/mlh)	40283.99 ± 5203.16	41021.10 ± 6239.87
K_a (h^{-1})	1.56 ± 0.57	1.54 ± 0.44
K_{10} (h^{-1})	0.079 ± 0.017	0.085 ± 0.020
$T_{1/2\,\beta}$ (h)	21.24 ± 5.02	21.84 ± 6.78
MRT_{0-60} (h)	17.96 ± 1.88	17.97 ± 1.29
F (%)	98.20	-

Mean ± SD (n = 10).

system under these conditions. The absence of CyA characteristic peaks can illustrate the stability of the drug in this solid dispersion system under these conditions, which is expected no change in drug dissolution during storage. The characteristic diffraction peaks of CyA appeared in samples stored at 40°C/75% RH for one month reflecting the re-crystalization of the drug. The appearance of the capsule content also changed from white powder to a lump. The agglomerate of the powder and the reappearance of the diffraction peaks of CyA indicated that the solid dispersion is unstable at higher temperature with higher relative humidity.

During the stability investigation, the content of CyA did not decrease and no other peaks appeared in the HPLC chromatogram thus providing the absence of decomposition products and, therefore, the chemical stability of the formulation under the different storage conditions.

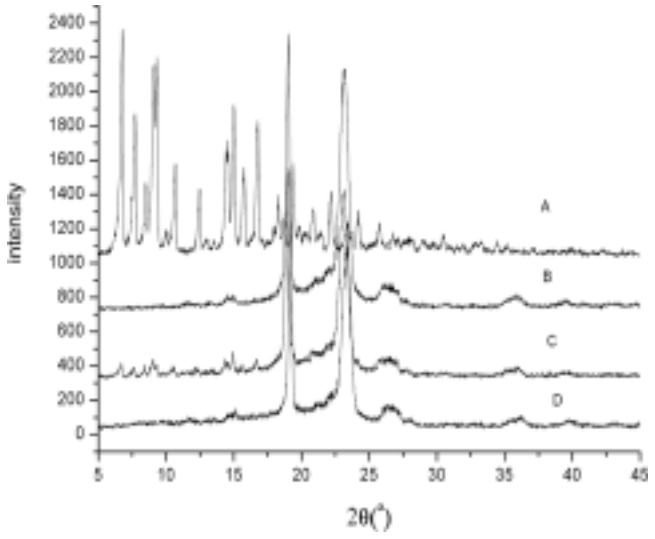

FIGURE 6 **X-ray Powder Diffraction Patterns of (A) CyA; (B) S40; (C) Freshly Prepared Sample; (D) Samples Stored at 25°C/60% RH for One Year; (E) Samples Stored at 30°C/60% RH for Six Months; and (F) Samples Stored at 40°C/75% RH for One Month.**

In order to evaluate the effect of storage conditions on the properties of solid dispersion, the dissolution was studied. Tables 2 and 3 display the dissolution of CyA from the designed formulation under different conditions at 45 min. For those capsules stored at 30°C/60% RH and 25°C/60% RH, the appearance of the powders did not change and the dissolution of CyA was similar to that at time zero during the whole period of the investigation. The dissolution results and the PXRD studies illustrated that the amorphous state of CyA in solid dispersion was kept by storing at the accelerate condition and room temperature. From these results, it was confirmed that the solid dispersion of CyA with S40 is very stable under these two conditions. For samples stored at 40°C/75% RH, conglomeration of the powder in capsules occurred and dramatic decrease of drug release (only 5.55% released at 45 min) was observed after one month. The decrease of drug dissolution may be caused by the re-crystalization of CyA confirmed by PXDR test (Fig. 6) and the lump formation. These results showed that solid dispersion was unstable at higher temperature and higher relative humidity.

CONCLUSION

The results obtained in this study indicated that solvent-melt method resulted in an amorphous solid dispersion of CyA and S40. This led to an improved dissolution compared to the physical mixture containing

TABLE 2 **Dissolution of CyA from Samples Stored at 30°C/60% RH at 45 Min**

	Lot#		
Time(month)	1	2	3
0	86.20 ± 1.56	87.54 ± 0.30	87.56 ± 1.27
1	84.22 ± 2.86	83.71 ± 3.25	84.69 ± 2.43
2	84.57 ± 3.15	84.91 ± 3.20	84.54 ± 1.86
3	85.45 ± 2.69	84.49 ± 3.49	83.56 ± 2.15
6	83.11 ± 1.68	83.90 ± 2.81	82.24 ± 2.17

Mean ± SD, $n = 6$.

TABLE 3 **Dissolution of CyA from Samples Stored at 25°C/60% RH at 45 Min**

	Lot#		
Time (month)	1	2	3
0	86.20 ± 1.56	87.54 ± 0.30	87.56 ± 1.27
3	89.87 ± 3.26	84.94 ± 4.40	88.97 ± 3.22
6	84.48 ± 1.84	84.26 ± 1.89	83.69 ± 2.46
9	87.17 ± 5.08	85.57 ± 3.42	85.13 ± 4.09
12	89.86 ± 2.08	87.26 ± 0.66	86.03 ± 1.18

Mean ± SD, $n = 6$.

crystalline drug substance. Infrared fourier transform spectrometry (FTIR) elucidated that there was no intermolecular interaction between CyA and S40. Bioavailability results showed that the absorption of CyA from solid dispersion was comparable to that of Sandimmun Neoral®. The stability results showed that the formulation is stable up to six months under accelerate conditions and one year at room temperature when packed in aluminum-polyethylene laminated bags. Based on these results, it was concluded that the solid dispersion is a useful approach to increase the bioavailability of CyA by using S40 as a carrier. Long term stability of CyA at 25°C/60% RH is the subject of an ongoing study.

REFERENCES

Charuk, J. H., Wong, P. Y., & Reithmeier, R. A. (1995). Differential interaction of human renal P-glycoprotein with various metabolites and analogues of cyclosporin A. *Am. J. Physiol.*, *269*, F31–F39.

Chen, X. X., Young, T. J., Sarkari, M., Williams, R. O., & Johnston, K. P. (2002). Preparation of cyclosporine A nanoparticles by evaporative precipitation into aqueous solution. *Int. J. Pharm.*, *242*, 3–14.

Chen, Y., Zhang, G. G. Z., Neilly, J., Marsh, K., Mawhinney, D., & Sanzgiri, Y. D. (2004). Enhancing the bioavailability of ABT-963 using solid dispersion containing Pluronic F-68. *Int. J. Pharm.*, *286*, 69–80.

Christians, U., & Sewing, K. F. (1993). Cyclosporin metabolism in transplant patients. *Pharmacol. Ther.*, *57*, 291–345.

Damian, F., Blaton, N., Kinget, R., & Van den Mooter, G. (2002). Physical stability of solid dispersions of the antiviral agent UC-781 with PEG 6000, Gelucire® 44/14 and PVP K30. *Int. J. Pharm.*, *244*, 87–98.

Ford, J. L. (1986). The current status of solid dispersions. *Pharm. Acta Helv.*, *61*, 69–88.

Hogan, W. J., & Storb, R. (2004). Use of cyclosporine in hematopoietic cell transplantation. *Transplant. Proc.*, *36*(Suppl 2S), 367S–371S.

Ismailos, G., Reppas, C., Dressman, J. B., & Macheras, P. (1991). Unusual solubility behaviour of cyclosporin A in aqueous media. *J. Pharm. Pharmacol.*, *43*, 287–289.

Joshi, H. N., Tejwani, R. W., Davidovich, M., Sahasrabudhe, V. P., Jemal, M., Bathala, M. S., Varia, S. A., & Serajuddin, A. T. M. (2004). Bioavailability enhancement of a poorly water-soluble drug by solid dispersion in polyethylene glycol-polysorbate 80 mixture. *Int. J. Pharm.*, *269*, 251–258.

Kahan, B. D. (1989). Cyclosporine. *N. Engl. J. Med.*, *321*, 1725–1738.

Kaur, V., Grant, D. J. W., & Eaves, T. (1980). Comparison of polyethylene glycol and polyoxyethylene stearate as excipients for solid dispersion systems of Griseofulvin and Tolbutamide II: dissolution and solubility studies. *J. Pharm. Sci.*, *69*, 1321–1326.

Kawada, A., Tezuka, T., Nakamizo, Y., Kimura, H., Nakagawa, H., Ohkido, M., Ozawa, A., Ohkawara, A., Kobayashi, H., Harada, S., & Igarashi, A. (2003). A survey of psoriasis patients in Japan from 1982 to 2001. *J. Dermatol. Sci.*, *31*, 59–64.

Kim, S. J., Choi, H. K., Suh, S. P., & Lee, Y. B. (2002). Pharmacolinetic and pharmacodynamic evaluation of cyclosporine A O/W-emulsion and microsphere formulations in rabbits. *Eur. J. Pharm. Sci.*, *15*, 497–502.

Kovarik, J. M., Mueller, E. A., Van Bree, J. B., Tetzloff, W., & Kutz, K. (1994). Reduced inter- and intra-individual variability in cyclosporin pharmacyokinetics from a microemulsion formulateon. *J. Pharm. Sci.*, *83*, 444–446.

Lee, E. J., Lee, S. W., Choi, H. G., & Kim, C. K. (2001). Bioavailability of cyclosporine A dispersed in sodium lauryl sulfate-dextrin based solid microspheres. *Int. J. Pharm.*, *218*, 125–131.

Lo, Y. L. (2003). Relationships between the hydrophilic–lipophilic balance values of pharmaceutical excipients and their multidrug

resistance modulating effect in Caco-2 cells and rat intestines. *J. control. Rel.*, *90*, 37–48.

Miyake, K., Arima, H., Irie, T., Hirayama, F., & Uekama, K. (1999). Enhanced absorption of cyclosporine A by complexation with dimethyl-beta-cyclodextrin in bile duct-cannulated and – noncannulated rats. *Biol. Pharm. Bull.*, *22*, 66–72.

Sekiguchi, K., & Obi, N. (1961). Studies on absorption of eutectic mixture. I. A comparison of the behavior of eutectic mixture of sulfathiazole and that of ordinary sulfathiazole in man. *Chem. Pharm. Bull.*, *9*, 866–872.

Serajuddin, A. T. M. (1999). Solid dispersion of poorly water-soluble drugs: early promises, subsequent problems, and recent breakthroughs. *J. Pharm. Sci.*, *88*, 1058–1066.

Taylor, N. E., Mark, A. E., Vallat, P., Brunne, R. M., Testa, B., & Van Gunteren, W. F. (1993). Solvent dependent conformation and hydrogen bounding capacity of cyclosporin A: evidence from partition coefficient and molecular dynamic simulations. *J. Med. Chem.*, *36*, 3753–3764.

Ubrich, N., Schmidt, C., Bodmeier, R., Hoffman, M., & Maincent, P. (2005). Oral evaluation in rabbits of cyclosporine-loaded Eudragit RS or RL nanoparticles. *Int. J. Pharm.*, *288*, 169–175.

Urata, T., Arimori, K., & Nakano, M. (1999). Modification of release rates of cyclosporine A from poly (L-lactic acid) microspheres by fatty acid esters and in-vivo evaluation of the microspheres. *J. control. Rel.*, *58*, 133–141.

Valizadeh, H., Nokhodchi, A., Qarakhani, N., Zakeri-Milani, P., Azarmi, S., Hassanzadeh, D., & Löbenberg, R. (2004). Physicochemical Characterization of Solid Dispersions of Indomethacin with PEG 6000, Myrj 52, Lactose, Sorbitol, Dextrin, and Eudragit® E100. *Drug Dev. Ind. Pharm.*, *30*, 303–317.

Vippagunta, S. R., Maul, K. A., Tallavajhala, S., & Grant, D. J. W. (2002). Solid-state characterization of nifedipine solid dispersions. *Int. J. Pharm.*, *236*, 111–123.

Vitale, A. T., Rodriguez, A., & Foster, C. S. (1996). Low-dose cyclosporin A therapy in treating chronic, noninfectious uveitis. *Ophthalmology*, *103*, 365–373.

Watkins, P. B. (1994). Noninvasive tests of CYP3A enzymes. *Pharmacogenetics*, *4*, 171–184.

Yamashita, K., Nakate, T., Ikimoto, K., Ihike, A., Tokunaga, Y., Ibuki, R., Higaki, K., & Kimura, T. (2003). Establishment of new preparation method for solid dispersion formulation of tacrolimus. *Int. J. Pharm.*, *267*, 79–91.

Ye, G. Q., Zhang, Q., & Yan, B. X. (1999). Pharmacokinetics and bioequivalence assessment of microemulsion preconcentrate solution containing cyclosporine A for oral administration. *Chin. J. Antibiot.*, *24*, 371–374.