Department of Pharmaceutics, School of Pharmacy, Fudan University, Shanghai PR China

Enhancement of dissolution of cyclosporine A using solid dispersions with polyoxyethylene (40) stearate

C. Liu, S. J. Zhu, Y. Zhou, Y. P. Wei, Y. Y. Pei

Received July 26, 2005, accepted October 18, 2005

Professor Yuan-Ying Pei, 138 Yixueyuan Road, P.O.Box 232, Fudan University, Shanghai 200032, PR China
yypei@shmu.edu.cn

Pharmazie 61: 681–684 (2006)

A solid dispersion containing cyclosporine A (CyA) and polyoxyethylene (40) stearate (PS) was prepared by the solvent-melt method and characterized by powder X-ray diffraction (PXRD), hot-stage microscopy (HSM), scanning electron microscopy (SEM) and dissolution studies. The crystalline peaks of CyA disappeared in the PXRD spectra of solid dispersions but were seen in those of physical mixtures, demonstrating the amorphous state of the drug in solid dispersions. The solubility of CyA in aqueous solutions of PS was increased linearly with increasing amount of PS in water. Dissolution of the drug from solid dispersions and physical mixtures was dramatically enhanced compared to the drug powder alone in water at 37 °C.

1. Introduction

Cyclosporine A (CyA) is a cyclic undecapeptide widely used for the inhibition of graft rejection in organ transplantations (Kahan 1989). The compound is insoluble in water and has a high octanol-water partition coefficient (log P = 2.92, Taylor et al. 1993). According to the biopharmaceutical classification system (Amidon et al. 1995), CyA is a class II compound (Chiu et al. 2003) meaning that its oral bioavailability is determined by the dissolution rate in the gastrointestinal fluid. For a poorly soluble drug, the Noyes-Whitney equation is used to describe the dissolution rate of a solid:

$$\frac{dC}{dt} = \frac{D \cdot A \cdot (C_s - C_t)}{h \cdot V} \qquad (1)$$

where the dissolution rate (dC/dt) is determined by D, the diffusion coefficient; h, the diffusion layer thickness at the solid-liquid interface; A, the specific surface area of the drug particle; V, the volume of the dissolution medium; C_s, the saturation solubility and C_t, the drug concentration at time t.

For a poorly soluble drug, the solubility enhancement is one of the effective ways to improve drug dissolution and bioavailability. The solid dispersion technology has been used extensively to enhance the solubility, dissolution and bioavailability of poorly water soluble drugs by water soluble carriers. The increased dissolution rate is achieved by the following factors (Leuner and Dressman 2000; Craig 2002): the reduction of the drug particle size to molecular level; the solubilizing effect on the drug by the water-soluble carrier and the enhancement of the wettability and dispersibility of the drug by the carrier material. The similar bioavailability of CyA dispersed in sodium lauryl sulfate-dextrin based solid microspheres to the commercial Sandimmun® in dogs was reported (Lee et al. 2001), suggesting that the solid dispersion technology is useful to deliver the poorly water-soluble CyA.

Polyoxyethylene (40) stearate (PS) is a non-ionic surfactant with a HLB value of 16.9. It is freely soluble in water. The material was used as a carrier in solid dispersion systems to improve the dissolution of griseofulvin, tolbutamide (Kaur et al. 1980) and indomethacin (Valizadeh et al. 2004). No attempt has been reported to improve the dissolution of CyA using PS.

In this study, a solid dispersion of CyA with PS was prepared by the solvent-melt method. The purpose of this investigation was to evaluate the physicochemical properties and *in vitro* dissolution of the drug from solid dispersion.

2. Investigations, results and discussion

2.1. X-ray powder diffraction (PXRD)

The X-ray diffraction patterns of samples are shown in Fig. 1 (the spectra of other ratios are similar and not shown). The diffractogram of 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 PS at 2θ values of 19.04° and 23.18° appeared in all the physical mixtures and solid dispersions. The sharp peaks of CyA were observed in the spectra of physical mixtures but were not observed in those of solid dispersions. It was suggested from the PXRD results that CyA is present in a predominantly amorphous state in all the solid dispersions. The large loss in crystallinity can be expected to enhance the dissolution and bioavailability of this water-insoluble drug.

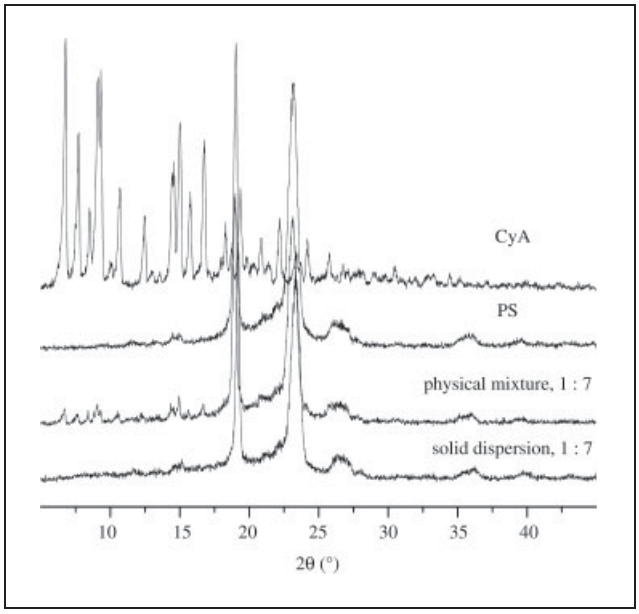

Fig. 1: X-ray powder diffraction patterns of CyA, PS, physical mixture of CyA and PS at the ratio of 1 : 7 and solid dispersion

2.2. Hot-stage microscopy (HSM)

Hot-stage microscopy (HSM) is often used to characterize the solid state form of drugs in solid dispersions (Savolainen et al. 2002). Fig. 2 shows the pictures of CyA-PS physical mixture and solid dispersion at or above the glass transition temperature of PS. CyA was crystal at the glassy transition temperature of PS (Fig. 2A). It began to melt at 151 °C and completely changed to liquid until 155 °C. The examination of the physical mixtures under the hot-stage microscope showed that the drug crystals disappeared gradually above the glassy transition temperature of PS (48 °C), and very few CyA crystals remained visible at a temperature of 154 °C (Fig. 2C). In the case of the solid dispersion, no drug crystal was observed when PS melted to liquid (Fig. 2D). These results agreed with PXRD analysis. From the results of PXRD and HSM, it was confirmed that CyA mainly exists in an amorphous state in the solid dispersions.

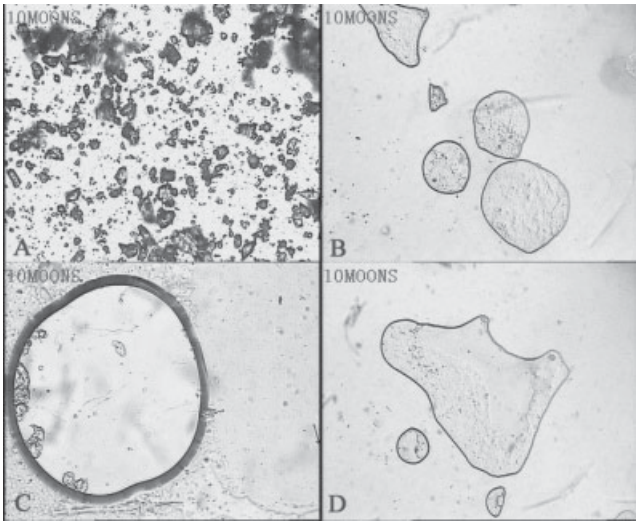

Fig. 2: HSM pictures of CyA (A), PS (B), CyA/PS physical mixture (C) and solid dispersion (D) after melting of the carrier

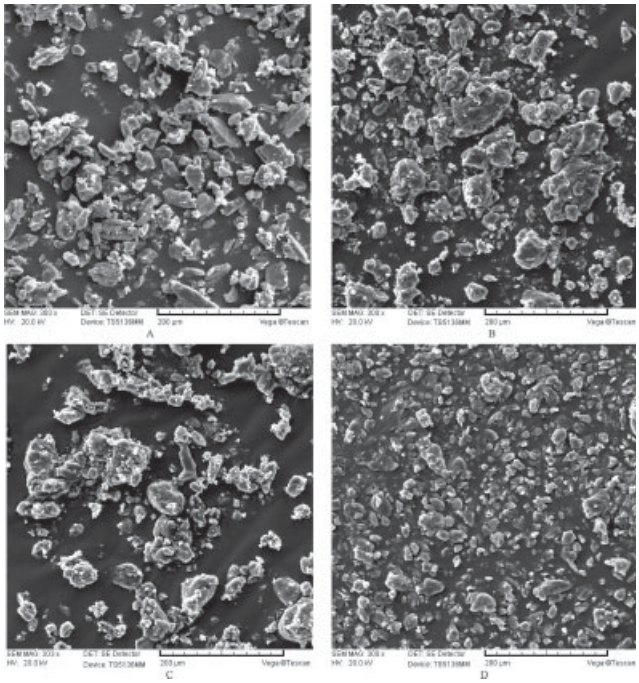

Fig. 3: Scanning electron microphotographs of CyA (A), PS (B), physical mixture (C) and solid dispersion (D)

2.3. Scanning electron microscopy (SEM)

Fig. 3 shows SEM pictures of CyA powder, PS, physical mixture and solid dispersion. CyA powder (Fig. 3A) and PS (Fig. 3B) showed the prismatic shape and the spherical shape, respectively. The appearance of the physical mixture (Fig. 3C) was similar to that of PS, indicating that the drug may be covered by the absorbed PS and dissolution may be increased by the wetting of the absorbed carrier. The appearance of the solid dispersion (Fig. 3D) was more homogenous than that of the physical mixture and the particle size was smaller, which may lead to a faster dissolution of the drug.

2.4 Solubility study

The solubility of CyA in aqueous solutions of PS is shown in Fig. 4. The solubility was increased linearly with the increasing surfactant concentration, indicating that the

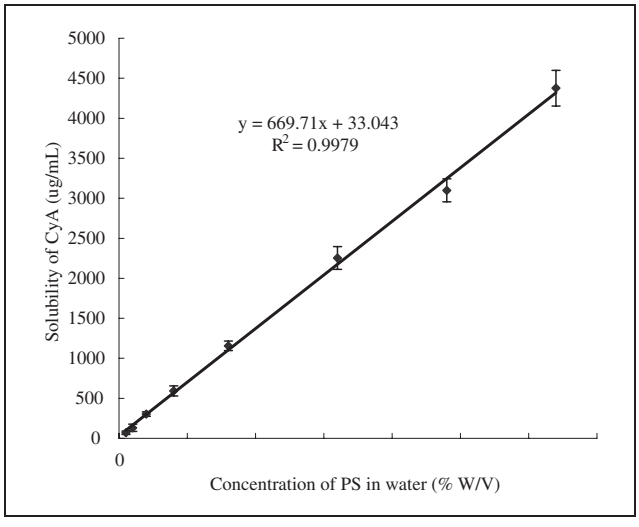

Fig. 4: Solubility of CyA in aqueous solutions of PS at 37 °C

Table 1: Gibbs free energy of transfer for the solubility process of CyA in water-carrier system at 37 °C

Concentration of PS (% W/V)	ΔG^0
0.1	−9549.30
0.2	−11170.25
0.4	−13334.32
0.8	−15063.52
1.6	−16782.37
3.2	−18504.71
4.8	−19324.45
6.4	−20214.60

micellar solubilization follows the partition model (Ahmed 2001). At 0.1% of PS, the increase in drug solubility at 37 °C was about 40 fold compared to pure drug in water. An indication of the process of transfer of CyA from water to the aqueous solutions of PS may be obtained from the values of Gibbs free energy (ΔG^0) change listed in Table 1. ΔG^0 Values were all negative, indicating the spontaneous nature of drug solubilization. The values decreased by increasing PS concentration, indicating that the transfer process became more favorable as the concentration of PS increased. Although it is not possible for one dosage unit to contain enough polymer to form such a high concentration in the dissolution medium or gastrointestinal fluid, the polymer may create a favorable microenvironment to enhance the dissolution rate if the release is carrier-controlled (Craig 2002).

2.5. Dissolution study

The amount of drug released in water increased dramatically from solid dispersions and physical mixtures compared to that of CyA powder alone (Fig. 5). The solid dispersions exhibited a higher release of about 85% of CyA within the first 20 min except the system consisting of CyA and carrier at the ratio of 1 : 5, which showed a slow drug release of 41%. Compared with physical mixtures, significantly more drug was released from solid dispersions after 20 min ($P < 0.05$), which may be due to the higher dispersion of the drug, the reduction of the particle size, the disappearance of the drug crystalline verified by PXRD, HSM and SEM and the solubilizing effect of PS. About 22–61% of CyA release from the physical mixtures within the first 20 min followed by slow dissolution of CyA into water was observed. The increase of release when the drug is physically mixed with PS is probably attributed to the wetting and solubilizing effect of the carrier. Drug release from physical mixtures reached a plateau around 50–70% at 30 min for all the physical mixtures. Solid dispersions containing drug/PS ratio lower than 1 : 7 showed little or no increase in drug release. Therefore this ratio was selected for further study, as a higher drug content is more suitable for practical use.

A model-independent parameter, the mean dissolution time (MDT), was employed to compare the dissolution profiles (Polli et al. 1997), calculated by:

$$MDT_{\text{in vitro}} = \frac{\sum_{i=1}^{n} t_{mid}\,\Delta M}{\sum_{i=1}^{n} \Delta M} \qquad (2)$$

Where i is the dissolution sample number, n is the number of dissolution sample times, t_{mid} is the time at the mid-

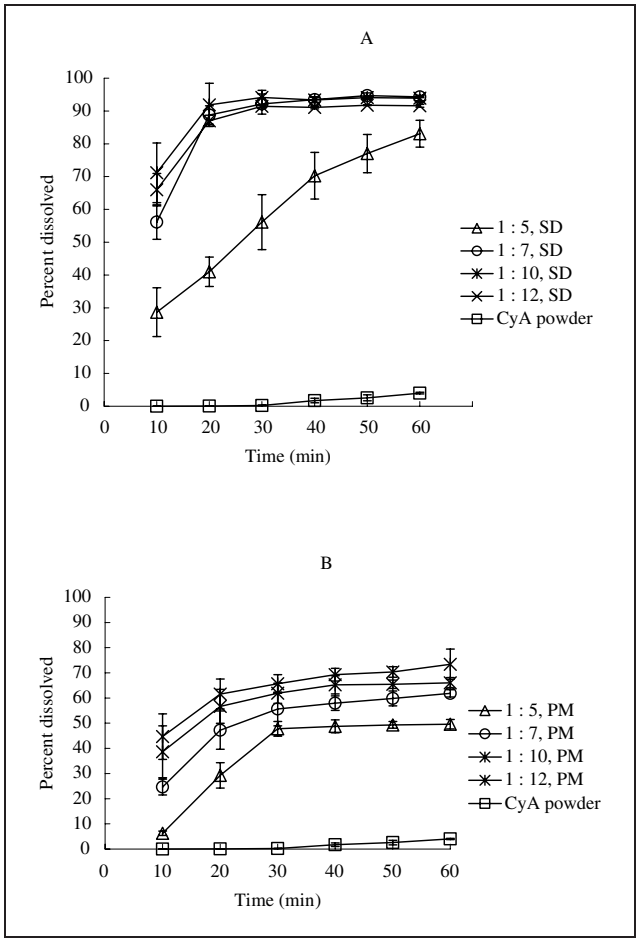

Fig. 5: Dissolution profile of CyA from solid dispersions (SD, A), physical mixtures (PM, B) and pure drug

point between times t_i and t_{i-1}, and ΔM is the amount of drug dissolved between t_i and t_{i-1}. The MDT values for physical mixtures, solid dispersions and pure drug are given in Table 2. The MDT values for solid dispersions and physical mixtures were dramatically decreased compared to those of the pure drug ($P < 0.01$). Compared to their corresponding physical mixtures, the MDT value of solid dispersion is significantly smaller except for the 1 : 5 system. The MDT of CyA was 43.6 min, then it decreased to 7.6 min after preparing solid dispersion with PS at 1 : 10 ratio (CyA : PS). The MDT of CyA decreased with increasing concentration of PS in solid dispersions.

In conclusion drug exist in the solid dispersion predominantly in the amorphous state verified by PXRD and HSM. The solubility and dissolution of CyA was enhanced by the use of solid dispersion technology. The so-

Table 2: Dissolution parameters of pure drug, physical mixtures and solid dispersions

Formulations	MDT (min)
Pure drug	43.58
Physical mixture, 1 : 5	18.42
Physical mixture, 1 : 7	15.32
Physical mixture, 1 : 10	11.41
Physical mixture, 1 : 12	12.56
Solid dispersion, 1 : 5	22.13
Solid dispersion, 1 : 7	9.92
Solid dispersion, 1 : 10	7.64
Solid dispersion, 1 : 12	8.35

lubilizing effect of PS and the absence of crystallinity might be responsible for the enhanced dissolution.
It can be concluded that the preparation of solid dispersion with PS provides a promising way to improve the solubility and dissolution of CyA.

3. Experimental

3.1. Materials

CyA (Lot#: 020801, 98.7%) was provided by Taishan city chemical pharmaceutical co., ltd (Guangdong, China). Methanol of HPLC grade was purchased from Hanbang Chemical Co. (Jiangyin, Jiangsu, China). Water was ultra filtered by passing it through a Millipore filtration system (Milli-Q®). PS of Chinese Pharmacopoeia grade was purchased from Nanjing WELL Chemical Corporation, Ltd. (Nanjing, China). All other chemicals and solvents used were of AR grade.

3.2. Preparation of solid dispersion

The solid dispersions of CyA with PS were prepared by the solvent-melt method. A varying amount of the carrier (the ratio of CyA and carrier was between 1 : 5 and 1 : 12) was heated under stirring in a water bath maintained at 65 °C. To each fused carrier, CyA in anhydrous ethanol was added under constant stirring. 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 dispersions were dried in a vacuum drier at 25 °C for 24 h and then pulverized. The powder fraction corresponding to mesh size less than 80 was collected and stored in glass desiccator at room temperature for further experiments.

3.3. Preparation of physical mixtures

Physical mixtures of CyA and PS were obtained by mixing of the accurately weighed powder of the components and sieving through a No. 80 mesh sieve. The composition of these physical mixtures corresponds to those of the solid dispersions mentioned above.

3.4. Power X-ray diffraction (PXRD)

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

3.5. Hot-stage microscopy (HSM)

CyA, PS, their physical mixture and solid dispersion were examined under a hot-stage microscope (Leica DM LP, Leica Microsystems GmbH, Germany) combined with a Linkam THMS 600 heating unit (Linkam Scientific Instruments Ltd, UK). Samples were heated at 10 °C/min until the excipients and/or CyA had melted and the temperature was then kept constant for one minute for photographing. Pictures of the samples were taken after the excipient had melted to see whether any crystals of CyA could be observed. Since the melting temperature of CyA is 155 °C and the carrier melted at much lower temperature, crystalline CyA could be seen in the microscope if CyA and the excipient had not formed a solid solution.

3.6. Scanning electron microscopy (SEM)

A scanning electron microscope (Vega TS5136MM Digital Microscopy Imaging, TESCAN, Czech) was used to examine the samples. The accelerating voltage was 20 kV at 300×. The samples were sputtered with gold for 15 min (EIKO IB-3 Ion Coater, EIKO Engineering Co. Ltd, Japan) before characterization with SEM. The current used for sputtering was 10 mA.

3.7. Determination of solubility of CyA in aqueous PS solutions

The solubility of CyA in aqueous solutions containing different concentrations of PS was determined by the shake-flask method. An excess of drug was put in the sealed glass vials with PS solutions. Then, the vials were then shaken in a water bath at 37 °C for 24 h. Samples were taken and quickly filtered through a 0.22 μm membrane filter. The concentration of CyA in the aqueous phase was diluted appropriately and assayed by HPLC. All experiments were performed in triplicate. The HPLC analysis was carried out using LC-10 AT VP and SPD-10A VP (Shimadzu co., Japan). The mobile phase (methanol/water = 90 : 10) was delivered at a flow rate of 1 mL/min through a 250 mm long C-18 column (5 μm, ODS-2 Hypersil, ThermoHypersil-Keystone, Bellefonte, PA, USA) at 55 °C. The detection wavelength was 214 nm. The injection volume was 20 μL. A linear relationship between drug concentration (C) and the peak area (A) in the range of 10–250 μg/mL was as follows:

$$C = 3 \times 10^{-5}A + 0.5652,\ r = 0.9999(n = 8). \quad (3)$$

The Gibbs free energy of transfer (ΔG^0) of CyA from pure water to the aqueous solution of PS was calculated by the following equation:

$$\Delta G^0 = -2.303RT \log S_C/S_0 \quad (4)$$

where S_c/S_0 is the ratio of the solubility of CyA in aqueous solutions of PS to that in water.

3.8. Dissolution studies

CyA powder alone, CyA-PS physical mixtures and solid dispersions equivalent to 25 mg of CyA were filled into hard gelatin capsules. The dissolution of CyA from these capsules was determined by using Apparatus No. 3 as described in the second volume of the Pharmacopoeia of the People's Republic of China (2005 Edition), which is similar to the Apparatus 2 method included in USP with smaller paddles and vessels. The dissolution studies were performed in 200 ml of water at 37 °C with stirring speed of 100 rpm. At suitable time intervals (10 min), three ml of the medium were sampled over a period of 1 h. The same volume of preheated water was added into the beaker right after each sample was withdrawn to keep a constant volume through the test. The samples were centrifuged (3000 rpm, 10 min) and the supernatant was detected by HPLC as described above. The cumulative amount of drug released was calculated and plotted versus time. Recovery of CyA from the dissolution medium containing dissolved capsule shell and the carrier was conducted by adding a known concentration of CyA in the medium and kept at 37 for 1 h. The area of CyA was detected after incubation and compared with the known concentration. The recovery of CyA was 100 ± 0.7%, calculated from the area ratio of CyA detected and added, indicating that the carrier did not disturb the analysis of CyA.

3.9. Statistics

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

References

Ahmed MO (2001) Comparison of impact of the different hydrophilic carriers on the properties of piperazine-containing drug. Eur J Pharm Biopharm 51: 221–226.

Amidon GL, Lennernäs H, Shah VP, Crison JR (1995) A theoretical basis for biopharmaceutic drug classification: the correlation of *in vitro* drug product dissolution and *in vivo* bioavailability. Pharm Res 12: 413–420.

Chiu YY, Higaki K, Neudeck BL, Barnett JL, Welage LS, Amidon GL (2003) Human jejunal permeability of cyclosporin A: influence of surfactants on P-glycoprotein efflux in Caco-2 cells. Pharm Res 20: 749–756.

Craig DQM (2002) The mechanisms of drug release from solid dispersions in water-soluble polymers. Int J Pharm 231: 131–144.

Kahan BD (1989) Cyclosporine. N Engl J Med 321: 1725–1738.

Kaur V, Grant DJW, 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.

Lee EJ, Lee SW, Choi HG, Kim CK (2001) Bioavailability of cyclosporine A dispersed in sodium lauryl sulfate-dextrin based solid microspheres. Int J Pharm 218: 125–131.

Leuner C, Dressman J (2000) Improving drug solubility for oral delivery using solid dispersions. Eur J Pharm Biopharm 50: 47–60.

Polli JE, Rekhi GS, Augsburger LL, Shah VP (1997) Methods to compare dissolution profiles and a rationale for wide dissolution specifications for metoprolol tartrate tablets. J Pharm Sci 86: 690–700.

Savolainen M, Khoo C, Glad H, Dahlqvist C, Juppo AM (2002) Evaluation of controlled-release polar lipid microparticles. Int J Pharm 244: 151–161.

Taylor NE, Mark AE, Vallat P, Brunne RM, Testa B, Van Gunteren WF (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.

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.