J Incl Phenom Macrocycl Chem (2013) 75:323–332
DOI 10.1007/s10847-012-0189-6

ORIGINAL ARTICLE

Pre-formulation of an oral cyclosporine free of surfactant

Taher Boukhris · Malika Lahiani-Skiba · Damien Martin · Mohamed Skiba

Received: 10 November 2011 / Accepted: 30 May 2012 / Published online: 20 June 2012

Abstract The purpose of this study was to develop a new oral cyclosporine A (CsA) formulation free of surfactant cremophor using cyclodextrin terpolymers (P-$\alpha\beta$-CD, P-$\beta\gamma$-CD and P-$\alpha\gamma$-CD) as excipients in attempt to enhance its stability, dissolution rate and eliminate surfactant side effects. Two spray-dried dispersions (SDDs) containing poorly water-soluble CsA were prepared with either P-$\alpha\beta$-CD, P-$\beta\gamma$-CD or P-$\alpha\gamma$-CD using water (F_{H_2O}) and ethanol (F_{EOH}) via spray-drying technique and characterized by scanning electron microscopy, powder X-ray diffraction, particle size distribution, circular dichroism (CD) and nuclear magnetic resonance along with the dissolution study which was compared to Neoral® and Sandimmune®. The results showed an interaction between CsA and P-$\alpha\beta$-CD, P-$\beta\gamma$-CD and P-$\alpha\gamma$-CD without secondary structure change of CsA. The order of the CsA release from the terpolymers was ranked as follows: P-$\alpha\gamma$-CD/CsA (F_{H_2O}) = Neoral® > P-$\beta\gamma$-CD/CsA (F_{H_2O}) > P-$\alpha\beta$-CD/CsA (F_{H_2O}) > P-$\alpha\gamma$-CD/CsA (F_{EOH}) > Sandimmune® > P-$\alpha\beta$-CD/CsA (F_{EOH}) > P-$\beta\gamma$-CD/CsA (F_{EOH}). The results of (F_{H_2O}) could be explained by hydrophilisation and absence of crystallinity of CsA while maintaining part of its crystallinity in the case of formulations (F_{EOH}). In summary, developed SDD formulations P-$\alpha\gamma$-CD/CsA (F_{H_2O}) revealed same dissolution profile as Neoral® and better than Sandimmune®. These systems seem to be stable to carry cyclosporine and release it, while preserving structure and thus, potentially, also maintaining cyclosporine activity.

Keywords Cyclosporine · Spray-dried dispersion · Cyclodextrin terpolymer · New formulation · Physico-chemical characterization

T. Boukhris · M. Lahiani-Skiba · M. Skiba (✉)
Galenic Pharmaceutical Laboratory, EA4311, Faculty of Medicine and Pharmacy, Rouen University, Rouen, France
e-mail: mohamed.skiba@univ-rouen.fr

D. Martin
SMS Laboratory, UC2M2 UPRES EA3233, UFR des Sciences du Madrillet, Saint Etienne du Rouvray, France

Introduction

Cyclosporine A (CsA) is a lipophilic cyclic polypeptide composed of 11 amino acids, seven of which are N-methylated as illustrated in Fig. 1. It has been utilized clinically as a potent immunosuppressant to prevent allograft rejection in various organ transplantations and to treat systemic and local autoimmunedisorders [1, 2].

The sparing water solubility of CsA is often the cause of undesirable properties such as erratic oral absorption profile, poor oral bioavailability and complications in formulation [1]. The immunosuppressive drug CsA was first formulated as an oily emulsion (Sandimmune®) administered as liquid or in a soft gelatin capsules [3] but the uptake was characterized by poor and generally unpredictable absorption with a variation of the absolute oral bioavailability between 1 and 89 % resulting an average value of around 30 %. Meanwhile, a large number of different formulations were developed but most frequently, CsA is delivered orally as a pre-concentrate microemulsion [1] (Neoral®). The main difference between those two concepts was in the particle size distribution (PSD) of created dispersion. The droplet size in Sandimmune® and Neoral® is ranging from few nanometers to several micrometers and 100–250 nm, respectively [4]. This difference in physico-chemical characteristic resulted from a change in composition, where new surfactant like

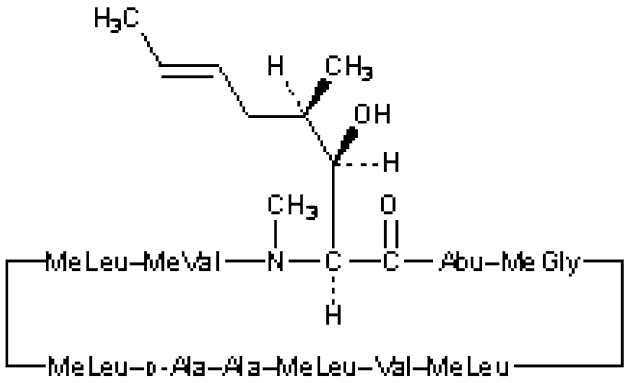

Fig. 1 *Structure* of CsA

cremophor with higher hydrophilic–lipophilic balance greater than 12 was used and was shown to improve the bioavailability of cyclosporine [5]. However, Neoral® contains a high concentration of cremophor, polyoxyethylated castor oil, which is known to exert some adverse effects, such as hypersensitivity, nephrotoxic and anaphylactoid reactions [6, 7]. Another approach such as solid dispersion (SD) was used to improve the solubility and bioavailability of lipophilic drugs. The most common polymers used were polyethylene glycol (PEG) [8], poloxamer [9], hydroxyethyl cellulose, mannitol [10], polyvinylpyrrolidone [11], and phospholipid [12] which improved the dissolution rate and oral absorption of lipophilic drugs. In regards to cyclosporine, literature stated the use of polyoxyethylene (40) stearate [13], inulin [2], dimyristoyl phosphatidylcholine [12], sodium lauryl sulfate and dextrin [14], hydroxypropylmethylcellulose phthalate and polyoxyethylene hydrogenated castor oil [15], and dimethyl β-cyclodextrin [16] for the enhancement of its intrinsic solubility, dissolution rate, absorption rate, and hence its oral bioavailability. No information was found in literature for the improvement of CsA dissolution by its SD with cyclodextrin terpolymers. However, natural cyclodextrins and their copolymers were used to improve the solubility and dissolution of other active ingredients such as nimesulide and albendazole [17, 18].

Despite the availability of various solubilization techniques, there has been a need to identify a robust, reliable, reproducible technology that can be applied broadly to structurally diverse insoluble compounds. The role of nanotechnology in drug delivery is rapidly expanding, and the ability to control the size, morphology, target selectivity, and release of drug particles is crucial for better therapeutic indices, but most of the existing methods are limited by harsh processing conditions. In this paper, we discuss the discovery and development of a spray-dried solid amorphous dispersion technology using cyclodextrin terpolymers. Such technique could accomplish the following objectives: (1) develop a CsA formulation with less excipients and reduce the side effects of surfactants used; (2) enhance the oral absorption of poorly water-soluble cyclosporine by attaining and sustaining a supersaturated concentration of drug in the gastrointestinal fluid; (3) provide a physically stable drug form (avoiding crystallization or phase separation of amorphous drug) that enables processing of the dispersion into solid dosage forms for shipment and usage; (4) provide a solid drug form that can overcome the harsh conditions of the gastrointestinal tract such as acidity and enzymatic degradation.

The present study aims at preparation of spray-dried dispersion (SDD) of cyclosporine using P-$\alpha\beta$-CD, P-$\alpha\gamma$-CD and P-$\beta\gamma$-CD mediated by spray-dryer technique. The physicochemical characterizations were investigated and dissolution studies on efficacy of the terpolymers on CsA release were carried out and compared against Neoral® and Sandimmune®. Also, cyclosporine secondary structure was evaluated by circular dichroism (CD) after it went various stress factors during spray-drying (e.g. thermal stress and/or shear stress at outlet of the spray nozzle) and its interaction with the terpolymers.

Experimental

Materials

Native cyclodextrins α, γ and β were ordered from Wacker, France. Citric acid, sodium chloride, pepsin (1:10.000, from porcine stomach mucosa) and sodium phosphate dibasic were supplied by Sigma Aldrich, France. Crystalline cyclosporine extra pure was received from Poli, Italy. *N*,*N*-dimethyldodecylamine-*N*-oxide–30 % (LDAO) in water was purchased from Molekula, United Kingdom. Ethanol, methanol, heptane and acetonitrile were obtained from VWR, France. Other reagents of analytical grade were used.

Preparation of terpolymer P-$\alpha\beta$-CD, P-$\alpha\gamma$-CD and P-$\beta\gamma$-CD

The terpolymer P-$\alpha\gamma$-CD was synthesized according to Skiba [19]. Briefly, a mixture of known amount (w/w) of cyclodextrin α, γ, citric acid and sodium phosphate dibasic was transferred into a reactor which was maintained at temperature ranging between 140 and 150 °C for 15–30 min. The obtained solid form was dissolved in water and dialyzed using polyether sulfate membrane filter with molecular weight cut off of 10,000 Da. The dialysis was controlled by measuring the conductivity of the purified water at T_0 and after 4 h of dialysis. After the dialysis, the resulted solution was spray-dried using BUCHI Mini Sprayer Dryer B-290. Spray-dryer parameters were

validated by preliminary works and were as follow: inlet temperature: 150 °C; outlet temperature: 80–90 °C; aspiration: 100 %; pump %: 20 % and pressure: (−40) mbar. The same procedure was applied to synthesize the P-$\beta\gamma$-CD and P-$\alpha\beta$-CD.

Phase-solubility study

The solubility study was conducted in volumetric flasks containing either 2 mL of increasing concentrations of cyclodextrin terpolymer P-$\alpha\beta$-CD, or P-$\alpha\gamma$-CD or P-$\beta\gamma$-CD (0–20 %w/v) where an excess amount of CsA was added. The mixture was put on horizontal shaker at 600 rpm and 25 °C for 48 h. After shaking, the samples were filtered (0.45 µm, low protein binding PVDF, Thermofisher, France). The solubilized CsA was determined by HPLC and experiment was performed in triplicate.

Preparation of SSD

A Büchi 290 nozzle type mini spray dryer (Flawil, Switzerland) was used for the preparation of the cyclosporine-loaded SDDs. Based on the solubility data, two cyclosporine-SDDs were prepared with 0.3–1 g cyclosporine and 3–10 g of either cyclodextrin terpolymers P-$\alpha\beta$-CD, P-$\alpha\gamma$-CD or P-$\beta\gamma$-CD. In the organic solvent method, cyclosporine was dissolved in 300 mL ethanol and cyclodextrin terpolymer was dispersed (F_{EOH}). Conversely, in the aqueous method each terpolymer was dissolved in 300 mL water and cyclosporine was dispersed (F_{H_2O}). They were then delivered to the nozzle with 1.4 mm diameter, flow rate of pump at 20 % and spray-dried at 150 °C inlet temperature and 80–90 °C outlet temperature. The flow rate of the drying air was maintained at the aspirator setting of 50 which indicated the pressure of the aspirator filter vessel as −40 mbar. The direction of air flow was the same as that of sprayed products.

Determination of CsA

The concentration of CsA in the resulting solution was analyzed using a USP method [20]. HPLC (Jasco PU-987) was equipped with Nova-Pack® C18 (Waters, 5 µm, 3.9 × 150 mm i.d.), UV detector (Jasco 875-UV) set at 210 nm and HPLC column temperature controller. The mobile phase consisted of acetonitrile: water: methanol: phosphoric acid (55:40:5:0.5, v/v) with flow rate of 1.0 mL/min and the column temperature was maintained at 70 °C.

Particle size distribution (PSD)

PSD was measured using Malvern Mastersizer (Malvern Hydro 2000S). Heptane was used as a dispersant with refractive index of 1.385–1.389. Each sample was dispersed in heptane and added to the sample dispersion unit containing stirrer and stirred at 2,000 rpm in order to reduce the interparticle aggregation, and laser obscuration range was maintained between 10 and 20 %. The average particle sizes were measured after performing the experiment for each batch in triplicate.

Scanning electron microscopy (SEM)

A SEM model JEOL JCM-5000 NeoScope instrument was used for the study at an accelerated voltage between 10 and 15 kV. Powder samples were stuck on SEM stub with conductive adhesive tape and coated with gold to reduce electric charges induced during analysis with a NeoCoater MP-19020NCTR.

Powder X-ray diffraction (PXRD)

PXRD analyses were carried out using D8 Discover Bruker system equipped with a software version 2.6.1. The instrument was equipped with X-ray tube containing a copper anticathode (40 kV, 40 mA, Kα1 radiation: 1.5406 Å, Kα2 radiation: 1.5444 Å) and mounted with an angular detector—Lynx eye™. The scan step was fixed at ∼0.04° with a counting time of 0.5 s/step over an angular range 3°–30°.

Nuclear magnetic resonance (NMR)

Cross polarization (CP) magic angle spinning (MAS) solid-state ^{13}C NMR spectra were recorded on a AV-400 spectrometer equipped with a probe of 4 mm MAS BB with rotation at 12,500 Hz (MAS), CP3lev with ramp up between 60 to 100 % (contact time: t_{cp} of 3.5 ms, contact strength ^{13}C of 45 Hz, contact strength ^{1}H with polarization rump between 35 to 60 kHz) and decoupled proton type spinal 64 (∼60 kHz). Powder samples of 70–80 mg of (F_{H_2O}), (F_{EOH}), cyclosporine, physical mixture of terpolymers/CsA and P-$\alpha\beta$-CD, P-$\alpha\gamma$-CD and P-$\beta\gamma$-CD were used for analysis.

Cicular dichroism (CD)

CD spectra were measured using a Jobin–Yvon-Spex CD 6 at room temperature. Far-UV spectra (190–240 nm) were recorded in a 0.05 cm-path-length cell. The spectra were recorded with a response time of 4 s, sensitivity of 10 mdeg and scan speed of 10 nm/min and converted into mean residue ellipticity in deg cm^2 $dmol^{-1}$. Crystalline CsA and SDD formulations (F_{H_2O}) and (F_{EOH}) equivalent to 0.4 mg/mL of CsA was dissolved in 55 % (acetonitrile: water) and analyzed for secondary structure where the CD

spectra were accumulated three times for data collection. Each data point was an average of three accumulations.

In vitro dissolution

The dissolution test was performed in USP type II dissolution apparatus II (Vankel, VK7000). Soft gelatin capsule (25 mg) of either Neoral® or Sandimmune® were put into a sinker, CsA-loaded SDD formulations (F_{H_2O}) and (F_{EOH}) equivalent to 25 mg of CsA was added to a vessel containing 500 mL 0.4 % v/v LDAO in water, simulated gastric fluid with pepsin (pH 1.2) at 37 ± 0.5 °C with paddle speed of 100 rpm. Each sample (1 mL) was withdrawn at 10, 20, 30, 45, 60 and 90 min. The 1 mL sample was not replaced but it was taken into account during the calculation of the CsA percent release. Concentration of cyclosporine was determined by HPLC method at a wavelength of 210 nm as described in the above method.

Results and discussion

Phase-solubility study and percent yield of cyclosporine

Solubility study revealed a progressive increase in the solubility of CsA with the terpolymers (P-$\alpha\beta$-CD, P-$\alpha\gamma$-CD and P-$\beta\gamma$-CD) concentration. According to the phase-solubility diagram classification introduced by Higuchi and Connors [21], the solubility diagrams of CsA and terpolymers at 25 °C correspond to A_n profile for P-$\alpha\beta$-CD and P-$\alpha\gamma$-CD while P-$\beta\gamma$-CD followed B_s profiles. As shown in Fig. 2. Results obtained for the solubility of CsA in water and 20 % w/v P-$\alpha\beta$-CD, P-$\alpha\gamma$-CD and P-$\beta\gamma$-CD were 17.8, 160.3, 167.2 and 68.5 (μg/mL), respectively, and that correspond to 9.0, 9.4 and 3.9-folds increase in its solubility by P-$\alpha\beta$-CD, P-$\alpha\gamma$-CD and P-$\beta\gamma$-CD, respectively. Also, the percent yield of cyclosporine from P-$\alpha\beta$-CD/CsA (F_{H_2O}), P-$\alpha\gamma$-CD/CsA (F_{H_2O}), P-$\beta\gamma$-CD/CsA (F_{H_2O}), P-$\alpha\beta$-CD/CsA (F_{EOH}), P-$\alpha\gamma$-CD/CsA (F_{EOH}), and P-$\beta\gamma$-CD/CsA (F_{EOH}) was determined and found out to be 81.9, 77.5, 91.0, 77.2, 85.7 and 85.2 %, respectively.

Dissolution study

Generally, SD formulation can be defined as a distribution of active ingredients in molecular, amorphous, and/or microcrystalline forms surrounded by inert carriers. In the present investigation, the dissolution of SDD formulations of cyclosporine-terpolymers of (F_{H_2O}) and (F_{EOH}) was conducted and compared to Sandimmune® and Neoral®. As shown in Fig. 3. The percentage of CsA dissolved after 10 min was 101.1, 101.0, 98.2, 95.1 and 89.8 % for P-$\alpha\gamma$-CD/CsA (F_{H_2O}),

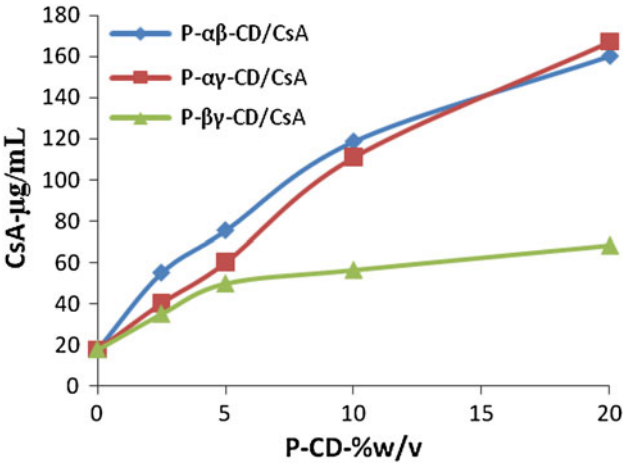

Fig. 2 Phase solubility of CyA in aqueous P-$\alpha\beta$-CD, P-$\alpha\gamma$-CD and P-$\beta\gamma$-CD at 25 °C ($n = 3$)

Neoral®, P-$\beta\gamma$-CD/CsA (F_{H_2O}), and P-$\alpha\beta$-CD/CsA (F_{H_2O}) and P-$\alpha\gamma$-CD/CsA (F_{EOH}), respectively. However, only 75.8, 66.2 and 48.7 % was released from Sandimmune®, P-$\alpha\beta$-CD/CsA (F_{EOH}) and P-$\beta\gamma$-CD/CsA (F_{EOH}), respectively followed by a progressive increase of cyclosporine release reaching up 100 % after 90 min. The depicted dissolution of formulations rank order in terms of percentage of CsA dissolved in 10 min were P-$\alpha\gamma$-CD/CsA (F_{H_2O}) = Neoral® > P-$\beta\gamma$-CD/CsA (F_{H_2O}) > P-$\alpha\beta$-CD/CsA (F_{H_2O}) > P-$\alpha\gamma$-CD/CsA (F_{EOH}) > Sandimmune® > P-$\alpha\beta$-CD/CsA (F_{EOH}) > P-$\beta\gamma$-CD/CsA (F_{EOH}). These data were superior at the one obtained with SD of CsA with hydroxypropyl cellulose (SSL) and CsA/PEG (PEG-6000) [22, 23]. Additionally, these data were in-line with previous finding of research scientists that reported an increase in the dissolution of hydrophobic drug when it is molecularly dispersed as in the case of SD [24–27].

Also, the dissolution is dependent of the rate associated with the diffusion or transport process of the solvated molecule to the solution and according to the Stokes–Einstein equation below, the diffusion coefficient is inversely proportional to the radius of a spherical drug molecule.

$$D = \frac{R \cdot T}{6\pi \cdot \eta \cdot r \cdot N}$$

where R is the molar gas constant, T is the absolute temperature, η is the apparent viscosity, r is the radius of a spherical drug molecule and N is Avogadro's number.

Therefore, diffusion coefficient was largely increased and the dissolution rate of drug became faster when the particle size is reduced as shown by the PSD data of SDD formulations (F_{H_2O}) and (F_{EOH}) which are tabulated in Table 1 and illustrated in Fig. 4 (e.g., P-$\alpha\beta$-CD/CsA [F_{EOH}]: the D_{50}, the median, is diameter where half of the population lies below 12.2 μm. Similarly, 90 % of the

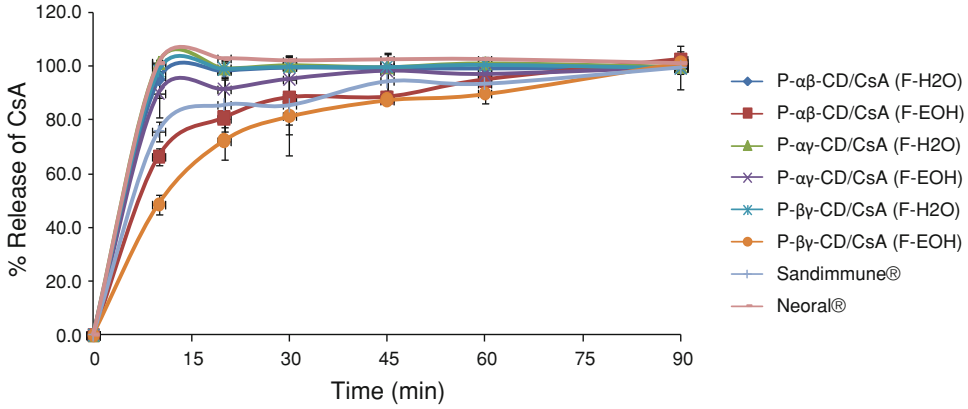

Fig. 3 Dissolution profile of cyclosporine in samples SDD (F_{H_2O}), SDD (F_{EOH}), Neoral® and Sandimmune® in a simulated gastric fluid ($n = 3$)

Table 1 PSD of cyclosporine in SDD formulations (F_{H_2O}) and (F_{EOH})

	P-$\alpha\beta$-CD/CsA (F_{EOH})	P-$\alpha\gamma$-CD/CsA (F_{EOH})	P-$\beta\gamma$-CD/CsA (F_{EOH})	P-$\alpha\beta$-CD/CsA (F_{H_2O})	P-$\alpha\gamma$-CD/CsA (F_{H_2O})	P-$\beta\gamma$-CD/CsA (F_{H_2O})
D_{10} (μm)[a]	2.0	2.3	2.0	1.3	1.3	1.3
D_{50} (μm)[b]	12.2	12.4	13.9	4.1	3.8	4.8
D_{90} (μm)[c]	22.0	22.2	24.8	8.4	7.5	9.6

[a] 10 % of the distribution lies below the D_{10}

[b] Half of the population lies below D_{50}

[c] 90 % of the distribution lies below D_{90}

distribution lies below 22.0 μm (D_{90}), and 10 % of the population lies below 2.0 μm (D_{10})).

The increase in the dissolution rate of CsA in SDD formulations (F_{H_2O}) could be explained by P-$\alpha\beta$-CD, P-$\beta\gamma$-CD and P-$\alpha\gamma$-CD hydrophilicity (aqueous solubility greater than 1 g/mL) that causes wetting of drug particle, local enhancement of drug solubility at the diffusion layer surrounding the drug particles [24] and absence of crystallinity [28]. These finding were confirmed by SEM micrographs of crystalline CsA and terpolymers based SDD formulations (F_{H_2O}) and (F_{EOH}) which revealed clear changes in the morphology of the powder particles after spray-drying to the evident formation of SD while CsA in the physical mixture kept the same morphology as cyclosporine alone. As shown in Fig. 5.

Moreover, PXRD pattern of crystalline CsA showed several intense peaks which were indicative of its tetragonal crystal form [29] while in SDD formulations CsA exhibited a halo diffraction pattern indicating its amorphous form. As shown in Fig. 6.

Although amorphous pharmaceutical materials can be readily isolated and may persist for many thousands of years, they are in fact a thermodynamically metastable state and will eventually revert to the more stable crystalline form. The quasi-equilibrium thermodynamic view of the amorphous state shows that the amorphous form has a significantly higher free energy than the crystalline form, and this why it is expected to have a much higher aqueous solubility and significantly different physical properties (e.g., density) [30, 31]. However, the matrix polymers in the SD formulations trap the drug molecule in a metastable form and prevent precipitation or crystallization from the supersaturated state, by the formation of drug–polymer assemblies or by preventing or retarding nucleation and crystal growth [30]. This latter statement explained the behavior of SDD formulations (F_{EOH}) where the terpolymers had low solubility in ethanol and were unable to prevent the crystallization of CsA (solubility in ethanol = 10 mg/mL) leading to a slow release of CsA observed in SDD formulations (F_{EOH}). In this formulation, the drug was transferred in an amorphous state, as it was soluble in the ethanol followed by re-crystallizing onto the carrier surface by the elimination of solvent [32]. On the other hand, in SDD formulations (F_{H_2O}), the dissolved terpolymers were attached to the surface of dispersed CsA and prevented its crystallization and hence resulted in an enhanced dissolution rate due to an increase in both the surface area and solubilization [33, 34].

Also, the sameness or equivalence between each two curves (i) F_{H_2O} versus Neoral®; (ii) F_{EOH} versus Neoral®,

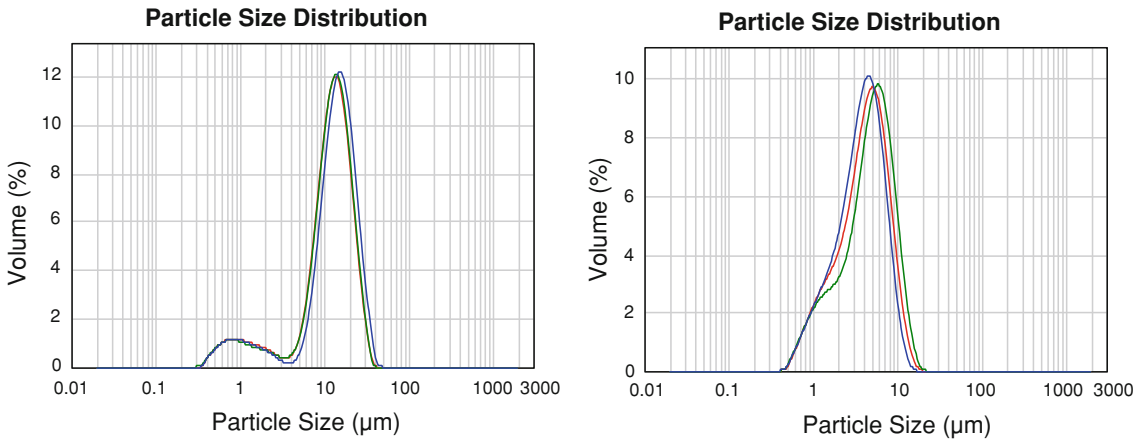

Fig. 4 PSD of CsA in samples **a**: *red* P-$\alpha\beta$-CD/CsA (F_{EOH}), *green* P-$\beta\gamma$-CD/CsA (F_{EOH}) and *blue* P-$\alpha\gamma$-CD/CsA (F_{EOH}); **b** *red* P-$\alpha\beta$-CD/CsA (F_{H_2O}), *green* P-$\alpha\gamma$-CD/CsA (F_{H_2O}) and *blue* P-$\beta\gamma$-CD/CsA (F_{H_2O}). (Color figure online)

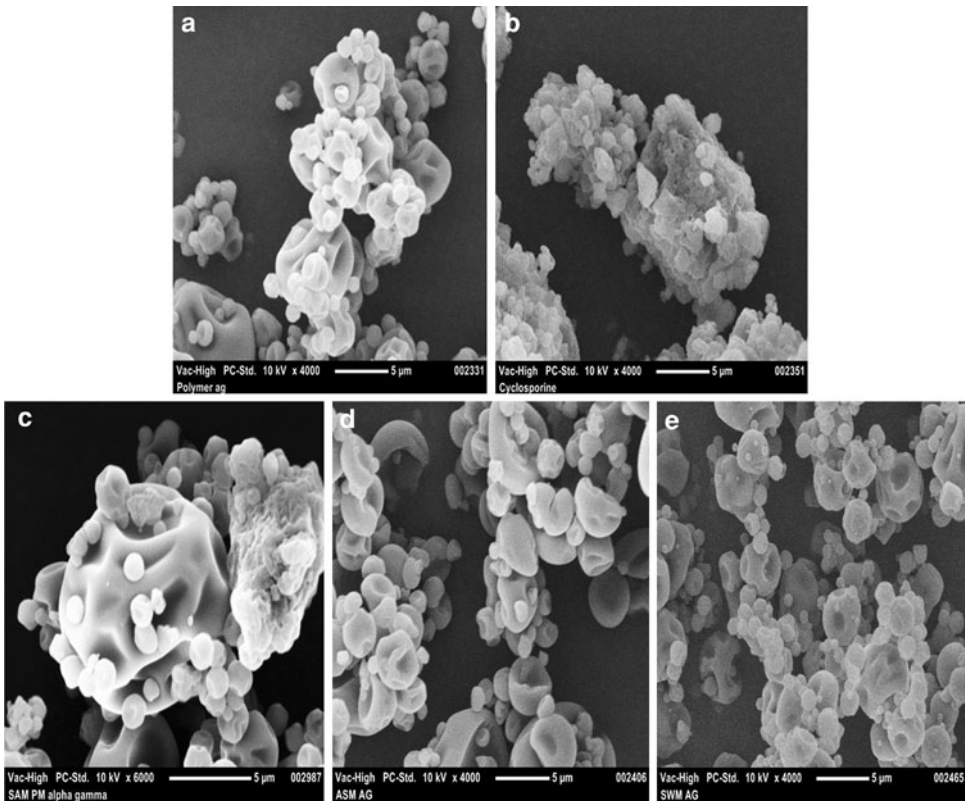

Fig. 5 SEM *photomicrographs* from cyclosporine samples, including **a** P-$\alpha\gamma$-CD, **b** crystalline CsA, **c** physical mixture of P-$\alpha\gamma$-CD: crystalline CsA, **d** SDD of CsA with P-$\alpha\gamma$-CD using water (F_{H_2O}) and **e** SDD of CsA with P-$\alpha\gamma$-CD using ethanol (F_{EOH})

(iii) F_{H_2O} versus Sandimmune® and (iv) F_{EOH} versus Sandimmune® was established by calculating a parameter called f_2 and the results are tabulated in Table 2.

$$f_2 = 50 \times \log\left\{\left[1 + (1/n)\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\}$$

where N is the number of time points, R_t is the dissolution value of the reference product (Neoral® or Sandimmune®) at time t, T_t is the dissolution value of the SSD (F_{EOH} or F_{H_2O}) at time t.

Sameness or equivalence of the two curves is declared when f_2 is between 50 and 100.

The f_2 value obtained was for F_{H_2O} [P-$\alpha\beta$-CD = 70.9 %; P-$\alpha\gamma$-CD = 80.1 %; P-$\beta\gamma$-CD = 77.1 %] and for F_{H_2O} [P-$\alpha\beta$-CD = 46.8 %; P-$\alpha\gamma$-CD = 43.1 %; P-$\beta\gamma$-CD = 44.1 %] when compared to Neoral® and Sandimmune®, respectively. However, The f_2 value obtained was for F_{EOH}

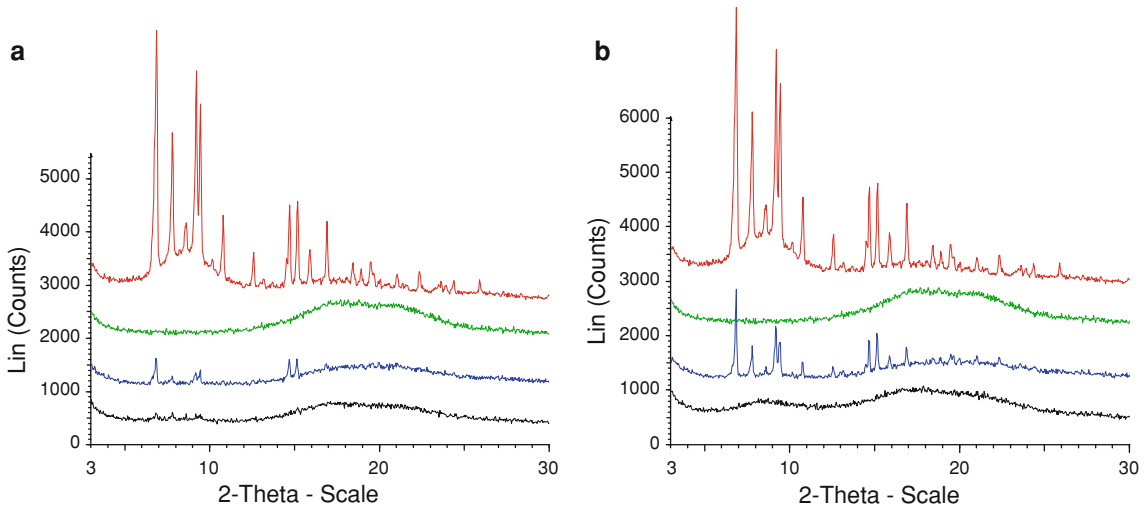

Fig. 6 PXRD patterns of cyclosporine samples (*top* to *bottom*): **a** cyclosporine, P-$\alpha\gamma$-CD, physical mixture (P-$\alpha\gamma$-CD: CsA) and SDD (F_{H_2O}); **b** cyclosporine, P-$\alpha\gamma$-CD, physical mixture (P-$\alpha\gamma$-CD: CsA) and SDD (F_{EOH})

Table 2 Similarity (f_2) values of cyclosporine in SDD formulations (F_{H_2O}) and (F_{EOH}) when compared to Neoral® and Sandimmune®

	f_2 (Neoral®)	f_2 (Sandimmune®)
P-$\alpha\beta$-CD (F_{H_2O})	70.9	46.8
P-$\alpha\beta$-CD (F_{EOH})	36.1	63.4
P-$\alpha\gamma$-CD (F_{H_2O})	80.1	43.1
P-$\alpha\gamma$-CD (F_{EOH})	55.4	55.7
P-$\beta\gamma$-CD (F_{H_2O})	77.1	44.8
P-Bγ-CD (F_{EOH})	28.1	44.5

[P-$\alpha\beta$-CD = 36.1 %; P-$\alpha\gamma$-CD = 55.4 %; P-$\beta\gamma$-CD = 28.1 %] and for F_{EOH} [P-$\alpha\beta$-CD = 63.4 %; P-$\alpha\gamma$-CD = 55.7 %; P-$\beta\gamma$-CD = 44.5 %] when compared to Neoral® and Sandimmune®, respectively. Consequently, on one hand, the SDD (F_{H_2O}) was within specification (50–100 %) when compared to Neoral® but was not with Sandimmune®. On the other hand, the F_{EOH} [P-$\alpha\beta$-CD] was within specification (50–100 %) when compared to Sandimmune® but was not with Neoral®; F_{EOH} [P-$\alpha\gamma$-CD] was within the limits when compared to both references drugs; and F_{EOH} [P-$\beta\gamma$-CD] was out of the limits when compared to Neoral® and Sandimmune®. Based on the similarity test data, Neoral®/F_{H_2O} [P-$\alpha\beta$-CD; P-$\alpha\gamma$-CD; and P-$\beta\gamma$-CD] and Neoral®/F_{EOH} (P-$\alpha\gamma$-CD) are likely to behave similarly upon oral administration and likely to exhibit similar absorption. The same statement could be applied to Sandimmune®/F_{EOH} (P-$\alpha\beta$-CD) and Sandimmune®/F_{EOH} (P-$\alpha\gamma$-CD).

Physico-chemical characterization

In the present study, the interaction between CsA and terpolymers in SDD-CsA/P-$\alpha\beta$-CD, P-$\alpha\gamma$-CD and P-$\beta\gamma$-CD was assessed by ^{13}C CPMAS NMR spectral analysis. The physical mixture P-$\alpha\gamma$-CD: cyclosporine showed the same spectra as cyclosporine alone with intense alkyl C–C peaks of chemical shift between 30 and 10; N–C=O at 174–170; C = O at 130–120; C–OH at 75–70 and C–N at 60–50 ppm which indicated that cyclosporine preserved its crystalline form as illustrated in Fig. 7. However, in both SDD formulations (F_{H_2O}) and (F_{EOH}), the intensity of alkyl C–C along with the chemical shifts were dramatically changed indicating not only a transition phase of cyclosporine from its crystalline to amorphous form but also an interaction between cyclosporine and the terpolymer P-$\alpha\gamma$-CD through hydrophobic interactions as confirmed by ^{13}C CPMAS NMR spectra. The P-$\alpha\beta$-CD/CsA and P-$\beta\gamma$-CD/CsA SDD formulations (F_{H_2O}) and (F_{EOH}) were also analyzed by ^{13}C CPMAS NMR and showed the same pattern (the results are shown).

To clarify whether the secondary structure of CsA in SDD formulations had been changed or not by either different cyclodextrin terpolymers or various stress factors during spray-drying (e.g. thermal stress and/or shear stress at the outlet of the spray nozzle), CD was performed. CD spectroscopy measures differences in the absorption of left-handed polarized versus right-handed polarized light which arise due to structural asymmetry. The absence of regular structure results in zero CD intensity, while an ordered structure results in a spectrum which contains positive and negative signals [35–37]. Alterations in the secondary structure are measured in the region of 190–260 nm, the so-called Far-UV-CD. This region is dominated by contributions of the peptide bonds, although some side chains may also be involved. The CD bands originating from aromatic amino acids and cystine in the near-UV (260–300 nm) can be utilized to determine the tertiary structure [36, 38]. Like all spectroscopic techniques, the CD signal

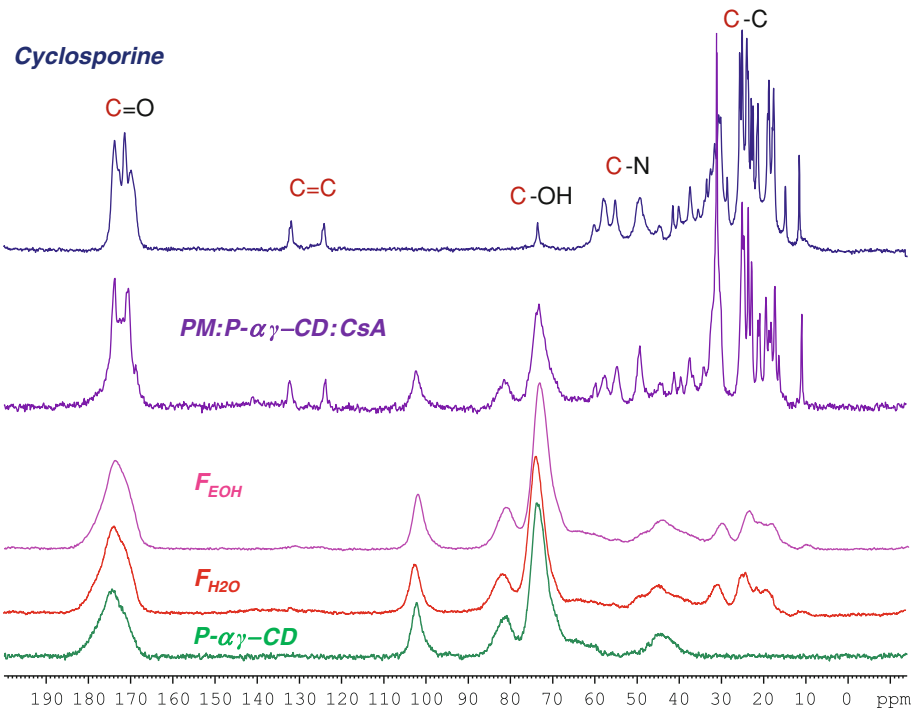

Fig. 7 ^{13}C CPMAS NMR *spectra* from *top* to *bottom*: crystalline cyclosporine, physical mixture (P-$\alpha\gamma$-CD: CsA), SDD formulation (F_{EOH}), SDD formulation (F_{H_2O}) and cyclodextrin terpolymer (P-$\alpha\gamma$-CD)

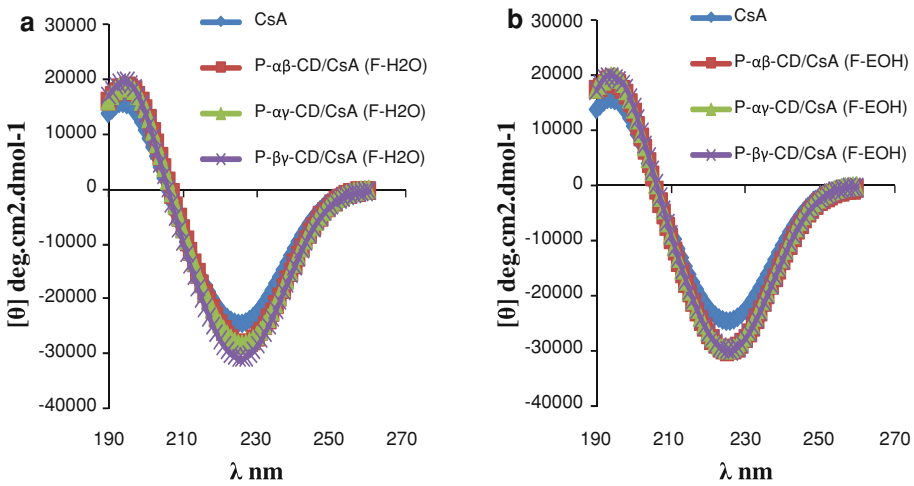

Fig. 8 Far-UV-CD *spectrum* of cyclosporine in samples **a** CsA in the diluent: 55 % ACN:H_2O (*blue*), P-$\alpha\beta$-CD/CsA (F_{H_2O}) (*brown*) and P-$\alpha\gamma$-CD/CsA (F_{H_2O}) (*green*) and P-$\beta\gamma$-CD/CsA (F_{H_2O}) (*purple*); **b** CsA in the diluent: 55 % ACN:H_2O (*blue*), P-$\alpha\beta$-CD/CsA (F_{EOH}) (*brown*) and P-$\alpha\gamma$-CD/CsA (F_{EOH}) (*green*) and P-$\beta\gamma$-CD/CsA (F_{EOH}) (*purple*). (Color figure online)

reflects an average of the entire molecular population. Figure 8 illustrates a long-wavelength minimum occurs near 225 nm having an ellipticity of approximately −25,000, accompanied by a maximum near 194 nm having an ellipticity of 16,000 was depicted indicating that cyclosporine exist as β-turns and this data is on line with other cyclic peptide such as the CD spectrum for Cyclo (L-Om-L-Pro-DPhe) [39].

The CD spectra of different ratio of terpolymers: CsA (e.g., 3:1, 6:1 and 10:1) were found to be coincident with CsA alone suggesting minor, if any, changes of cyclosporine secondary structure in the presence of the aforementioned polymers (data not shown). After entrapment in SDD (F_{H_2O}) and (F_{EOH}) formulations, only minor differences were observed in the cyclosporine CD spectrum. No substantial alterations were noted in the β-sheet minima. Thus, these systems seem to be stable to carry cyclosporine and release it, while preserving structure and thus, potentially, also maintaining cyclosporine activity.

Conclusion

Developed formulations revealed an improvement in dissolution rate of CsA especially with SDD formulations (F_{H_2O}). The increase dissolution rate is thought to

be related to hydrophilisation and absence of crystallinity of CsA. Developed SDD formulation (F_{H_2O}) showed same profile as Neoral® and better than Sandimmune® which thought to be that CsA was molecularly dispersed in cyclodextrin terpolymers as confirmed by physico-chemical characterization studies and without relevant changes in the peptide secondary structure as revealed by CD and hence might increase its bioavailability.

Acknowledgments The author is grateful to Pr. H. Oulyadi; Dr. L. Guilhaudis from Rouen University, UMR 6014 and FR3038 CNRS; and Pr. G. Coquerel from Rouen University, Laboratoire SMS UC2M2 UPRES EA3233 UFR of Sciences.

References

1. Dunn, C.J., Wagstaff, A.J., Perry, C.M., Posker, G.L., Goa, K.L.: Cyclosporine. An update review of the pharmacokinetic properties, clinical efficacy and tolerability of a microemulsion-based formulation (Neoral) in organ transplantation. Drugs **61**, 1957–2016 (2001)
2. Zijlstra, G.S., Rijkeboer, M., Jan van Drooge, D., Sutter, M., Jiskoot, W., Van de Weert, M., Hinrichs, W.L., Frijlink, H.W.: Characterization of a cyclosporine solid dispersion for inhalation. AAPS J. **9**, E190–E199 (2007)
3. Tarr, B.D., Yalkowsky, S.H.: Enhanced intestinal absorption of cyclosporine in rats through the reduction of emulsion droplet size. Pharm. Res. **6**, 40–43 (1989)
4. Gursoy, R.N., Benita, S.: Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed. Pharmacother. **58**(3), 173–182 (2004)
5. Klauser, R.M., Irschik, H., Kletzmayr, J., Sturm, I., Brunner, W., Woloszczuk, W., Kovarik, J.: Pharmacokinetic cyclosporine A profiles under longterm Neoral treatment in renal transplant recipients: Does fat intake still matter? Transplant. Proc. **29**, 3137–3140 (1997)
6. Luke, D.R., Kasiske, B.L., Matzke, G.R., Awni, W.M., Keane, W.F.: Effect of cyclosporin on the isolated perfused rat kidney. Transplantation **43**, 795–799 (1987)
7. Cavanak, T., Sucker, H.: Formulation of dosage forms. Prog. Allergy **38**, 65–72 (1986)
8. Chen, H., Jiang, G., Ding, F.: Monolithic osmotic tablet containing solid dispersion of 10-hydroxycamptothecin. Drug Dev. Ind. Pharm. **35**, 131–137 (2009)
9. Kanagale, P., Patel, V., Venkatesan, N., Jain, M., Patel, P., Misra, A.: Pharmaceutical development of solid dispersion based osmotic drug delivery system for nifedipine. Curr. Drug Deliv. **5**, 306–311 (2008)
10. Pathak, D., Dahiya, S., Pathak, K.: Solid dispersion of meloxicam: factorially designed dosage form for geriatric population. Acta Pharm. **58**, 99–110 (2008)
11. Rahamathulla, M., Hv, G., Rathod, N.: Solubility and dissolution improvement of Rofecoxib using solid dispersion technique. Pak. J. Pharm. Sci. **21**, 350–355 (2008)
12. Zidan, A.S., Habib, M.J., Khan, M.A.: Process analytical technology: nondestructive evaluation of cyclosporine A and phospholipid solid dispersions by near infrared spectroscopy and imaging. J. Pharm. Sci. **97**, 3388–3399 (2008)
13. Liu, C., Zhu, S.J., Zhou, Y., Wei, Y.P., Pei, Y.Y.: Enhancement of dissolution of cyclosporine A using solid dispersions with polyoxyethylene (40) stearate. Pharmazie. **61**, 681–684 (2006)
14. Lee, E.J., Lee, S.W., Choi, H.G., Kim, C.K.: Bioavailability of cyclosporine A dispersed in sodium lauryl sulfate-dextrin based solid microspheres. Int. J. Pharm. **218**, 125–131 (2001)
15. Takada, K., Oh-hashi, M., Furuya, Y., Yoshikawa, H., Muranishi, S.: Enteric solid dispersion of cyclosporin A (CyA) having potential to improve availability of CyA in rabbit. Chem. Pharm. Bull. **37**, 2542–2544 (1989)
16. Miyake, K., Arima, H., Irie, T., Hiraayama, F., Uekama, K.: Enhanced absorption of cyclosporin A by complexation with dimethyl-β-cyclodextrin in bile duct-cannulated and noncannulated rats. Biol. Pharm. Bull. **22**(1), 66–72 (1999)
17. Joudieh, S., Lahiani-Skiba, M., Bon, P., Ba, O., Le Breton, J.M., Skiba, M.: Nimesulide apparent solubility enhancement with natural cyclodextrins and their polymers. Lett. Drug Des. Discov. **5**, 406–415 (2008)
18. Joudieh, S., Bon, P., Martel, B., Skiba, M., Lahiani-Skiba, M.: Cyclodextrin polymers as efficient solubilizers of albendazole: complexation and physic-chemical characterization. Nanosci. Nanotechnol. **8**, 1–9 (2008)
19. Skiba, M.: Method for synthesizing calixarene and/or cyclodextrin coploymers, terpolumers and tetrapolymers, and uses thereof: PCT/FR/10/00875 (2010)
20. USP 30: Cyclosporine capsule. The United States Pharmacopeia and the National Formulary (USP 30/NF 25). The United States Pharmacopeial Convention; Rockville (MD); 1852–1854 (2007)
21. Higuchi, T., Connors, K.A.: Phase solubility techniques. Adv. Anal. Chem. Instrum. **4**, 117–212 (1965)
22. Onoue, S.H., Sato, H., Ogawa, K., Kawabata, Y., Mizumoto, T., Yuminokid, K., Hashimoto, N., Yamada, S.: Improved dissolution and pharmacokinetic behavior of cyclosporine A using high-energy amorphous solid dispersion approach. Int. J. Pharm. **399**, 94–101 (2010)
23. Rahman, Z., Zidan, A.S., Khan, M.A.: Formulation and evaluation of a protein-loaded solid dispersions by non-destructive methods. AAPS J. **12**(2), 158–170 (2010)
24. Zerrouk, N., Chemtob, C., Arnaud, P., Toscani, S., Dugue, J.: In vitro and in vivo evaluation of carbamazepine-PEG 6000 solid dispersions. Int. J. Pharm. **225**, 49–62 (2001)
25. Dutet, J., Lahiani-Skiba, M., Didier, L., Jezequel, S., Bounoure, F., Barbot, C., Arnaud, P., Skiba, M.: Nimesulide/cyclodextrin/PEG 6000 ternary complexes: physico-chemical characterization, dissolution studies and bioavailability in rats. J. Incl. Phenom. Macrocycl. Chem. **57**, 203–209 (2007)
26. Lahiani-Skiba, M., Barbot, C., Bounoure, F., Joudieh, S., Skiba, M.: Solubility and dissolution rate of progesterone–cyclodextrin–polymer systems. Drug Dev. Ind. Pharm. **32**(9), 1043–1058 (2006)
27. Ren, F., Jing, Q., Tang, Y., Shen, Y., Chen, J., Gao, F., Cui, J.: Characteristics of bicalutamide solid dispersions and improvement of the dissolution. Drug Dev. Ind. Pharm. **32**, 967–972 (2006)
28. Leuner, C., Dressman, J.: Improving drug solubility for oral delivery using solid dispersions. Eur. J. Pharm. Biopharm. **50**, 47–60 (2000)
29. Bertacche, V., Pini, E., Stradi, R., Stratta, F.: Quantitative determination of amorphous cyclosporine in crystalline cyclosporine samples by Fourier transform infrared spectroscopy. J. Pharm. Sci. **95**, 159–166 (2006)
30. Zanotto, E.D.: Do cathedral glasses flow? Am. J. Phys. **66**(5), 392–395 (1998)
31. Serajuddin, A.T.: Solid dispersion of poorly water-soluble drugs: early promises, subsequent problems, and recent breakthroughs. J. Pharm. Sci. **88**, 1058–1066 (1999)
32. Yamashita, K., Nakate, T., Okimoto, K., Ohike, A., Tokunaga, Y., Ibuki, R., Higaki, K., Kimura, T.: Establishment of new preparation method for solid dispersion formulation of tacrolimus. Int. J. Pharm. **267**, 79–91 (2003)

33. Corrigan, O.I.: Mechanism of dissolution of fast release solid dispersion. Drug Dev. Ind. Pharm. **11**(2–3), 697–698 (1985)
34. Li, D.X., Jang, K.Y., Kang, W.K., Bae, K.J., Lee, M.H., Oh, Y.K., Jee, J.P., Park, Y.J., Oh, D.H., Seo, Y.G., Kim, Y.R., Kim, J.O., Woo, J.S., Yong, C.S., Choi, H.G.: Enhanced solubility and bioavailability of sibutramine base by solid dispersion system with aqueous medium. Biol. Pharm. Bull. **33**, 279–284 (2010)
35. Greenfield, N.J.: Methods to estimate the conformation of proteins and polypeptides from circular dichroism data. Anal. Biochem. **235**, 1–10 (1996)
36. Jiskoot, W., Crommelin, D.J.: Methods for Structural Analysis of Protein Pharmaceuticals. AAPS Press, Arlington (2005)
37. Ng, K.Y., Zhao, L., Meyer, J.D., Rittmann-Grauer, L., Manning, M.C.: Use of circular dichroism spectroscopy in determining the conformation of a monoclonal antibody prior to its incorporation in an immunoliposome. J. Pharm. Biomed. Anal. **16**, 507–513 (1997)
38. Righetti, P.G., Verzola, B.: Folding/unfolding/refolding of proteins: present methodologies in comparison with capillary zone electrophoresis. Electrophoresis **22**, 2359–2374 (2001)
39. Bush, C.A., Sarkar, S.K., Kopple, K.D.: Circular dichroism of beta turns in peptides and proteins. Biochemistry **17**(23), 4951–4954 (1978)