NEW APPROACH FOR PRE-FORMULATION OF AN ORAL CYCLOSPORINE

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Development of new oral cyclosporine formulation using cyclodextrin tetrapolymer (P-αβγ-CD) in attempt to enhance its stability and dissolution rate. Two spray-dried dispersion formulations containing poorly water-soluble cyclosporine (CsA) were prepared with cyclodextrin tetrapolymer in water (F\textsubscript{H2O}) and ethanol (F\textsubscript{EOH}) then characterized by scanning electron microscopy, powder X-ray diffraction, particle size distribution, circular dichroism and nuclear magnetic resonance (NMR) along with the dissolution study which was compared to Neoral® and Sandimmune®. The physicochemical characterization studies showed an interaction between cyclosporine and P-αβγ-CD without secondary structure changes of cyclosporine. The order of cyclosporine release was as follows: (F\textsubscript{H2O}) = Neoral® > (F\textsubscript{EOH}) > Sandimmune®. The results could be explained by hydrophylisation and absence of crystallinity of cyclosporine. In conclusion, F\textsubscript{H2O} formulation revealed similar dissolution profile as Neoral® and better than Sandimmune®.

(Received November 14, 2011; Accepted February 3, 2012)

Key words: Cyclosporine, Spray-dried dispersion, cyclodextrin tetrapolymer, formulation.

1. Introduction

Cyclosporine (CsA) is an oligopeptide with an empirical formula of C\textsubscript{62}H\textsubscript{111}N\textsubscript{11}O\textsubscript{12} and molecular weight (MW) of 1202.64 Da. It was first isolated from crude extract of the fungus Tolypocladium inflatum gams by Sandoz in 1971[1]. In November 1983, the FDA approved cyclosporine for prevention of transplant rejection. Cyclosporine has been widely used for the prophylaxis and treatment of graft rejection in almost all types of organ transplantations. It has significantly enhanced the initial and long-term survival of transplant patients. In 1984, Wenger [2] reported its complete chemical synthesis which was categorized as a highly lipophilic neutral cyclic peptide composed of 11 amino acids, seven of which are N-methylated (Figure 1). Amino acid incorporates several methylated moieties including the unusual methylated butenyl-methyl-L4 threonine (MeBmt) in position 1 which is crucial for immunosuppressive activity. These hydrophobic amino-acids contribute to the poor aqueous solubility of CsA. It contains four intra-molecular hydrogen bonds, which impart high rigidity to its cyclic structure [3-5]. This unusual structural property confers a very low aqueous solubility to this drug. The low water solubility of the drug is a serious problem causing undesirable biopharmaceutical properties, such as erratic bioavailability from oral and topical routes and according to biopharmaceutical classification system, CsA has been classified as class 2 indicating that its bioavailability is dissolution dependent [6, 7].

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Cyclosporine is administered orally through self-emulsifying drug delivery systems such as self-emulsifying drug delivery system (SEDDS, Sandimmune®) and self-microemulsifying drug delivery system (SMEDDS, Neoral®). The main difference between those two concepts was in the particle size distribution of created dispersion. The droplet size of SEDDS is ranging from few nanometers to several micrometers while in SMEDDS indicates transparent microemulsions with oil droplet size ranging between 100 to 250 nm [8]. This difference in physico-chemical characteristic resulted from a change in composition, where new surfactants like cremophor RH-40 and cremophor-35 with hydrophilic-lipophilic balance (HLB) 14-16 and 12-14, respectively, were used as carrier drug and were shown to improve the bioavailability of cyclosporine [9, 10]. However, Neoral® contains a high concentration of a surfactant, polyoxethylated castor oil (Cremophor), which is known to exert some adverse effects, such as hypersensitivity, nephrotoxic and anaphylactoid reactions [11, 12]. Therefore, to overcome this problem, various strategies like micronization, complexation with cyclodextrins, and solid dispersion methods were reported in the literature [13] not only to improve the CsA solubility and bioavailability but also to reduce the side effects of the surfactants used. Another approach mediated by solid dispersion technique was applied to enhance water solubility, dissolution rate and bioavailability of poorly water soluble drugs and the most common polymers have been used were Eudragit® [14], hydroxypropylcellulose and hypromellose [15], chitosan and hydroxypropylmethylcellulose phthalate [16] which improved the solubility but delay the drug release. However, hydrophilic or amphiphilic polymers such as polyethylene glycol [17], poloxamer [18], hydroxyethyl cellulose, mannitol [19], polyvinylpyrrolidone [20], phospholipid [21] and natural cyclodextrins (α, β and γ) and their copolymers (poly-α-cyclodextrin, poly-β-cyclodextrin and poly-γ-cyclodextrin) [22, 23] improved the dissolution rate and oral absorption of lipophilic drugs. In regards to cyclosporine, literature stated the use of polyoxyethylene (40) stearate [24], inulin [25], dimyristoyl phosphatidylcholine [20], sodium lauryl sulfate and dextrin [26], and hydroxypropylmethylcellulose phthalate and polyoxyethylene hydrogenated castor oil [27] for the enhancement of its intrinsic solubility, dissolution rate, absorption rate, and hence its oral bioavailability. 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. Therefore, 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 only one excipient which is cyclodextrin tetrapolymer (P-αβγ-CD) mediated by spray-dryer technique. Such “spray-dried dispersions” (SDD) 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 (GI) 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; and (4) provide a solid drug form that can overcome the harsh
conditions of the gastrointestinal tract such as acidity and enzymatic degradation. Consequently, the present study aims at preparation of spray-dried dispersion of cyclosporine using P-αβγ-CD as excipient mediated by spray dryer technique. The physicochemical characterizations were investigated and in vitro studies on the efficacy of the tetrapolymer (P-αβγ-CD) on CsA release were carried out. Another main aspect of the study was to evaluate prepared spray-dried dispersion for their stability and for possible alterations of the secondary structure due to the interaction with the excipient. The latter was evaluated by circular dichroism.

2. Experimental detail

2.1. 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% in water (LDAO) was purchased from Molekula, United Kingdom. Ethanol, methanol, heptane and acetonitrile were obtained from VWR, France. Other reagents of analytical grade were used.

2.2. Preparation of tetrapolymer P-αβγ-CD

The tetrapolymer P-αβγ-CD was synthesized according to Skiba M by a fusion method [28]. Briefly, a mixture of known amount (w/w) of natural cyclodextrins (α, β, γ), citric acid and sodium phosphate dibasic was transferred into a reactor which was maintained at a temperature ranging between 140-150°C with fixed time. The obtained solid form was dissolved in water and then dialyzed using polyether sulfate membrane filter with molecular weight cut off of 10000 Da. The dialysis was controlled by measuring the conductivity of the purified water at T0 and after 4 hours 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)-(-50) mbar.

2.3. Phase Solubility Study

Solubility study was carried out by adding excess amount of CsA into 2 ml of increasing concentration solution of cyclodextrin tetrapolymers P-αβγ-CD ranging from 0 to 20% (w/v). The mixture was put on horizontal shaker at 600 rpm and 25°C for 48 hours. After shaking, the samples were filtered (0.45μm, low protein binding PVDF, thermofisher, France). The solubilized CsA was determined by HPLC and experiment was performed in triplicate.

2.4. Preparation of Spray-Solid Dispersion (SSD)

A Buchi 290 nozzle type mini spray dryer (Flawil, Switzerland) was used for the preparation of the cyclosporine-loaded spray-dried dispersions. Based on the obtained solubility data, two cyclosporine spray-dried dispersions were prepared with an amount of 0.3-1 g for cyclosporine and 3-10g for P-αβγ-CD in water and ethanol. In the organic method (F_EOH), cyclosporine was first dissolved in 300 ml of ethanol then P-αβγ-CD was dispersed into the solution. Conversely, in the aqueous method (F_H2O), P-αβγ-CD was first dissolved in 300 ml of water then cyclosporine was dispersed into the solution. They were then delivered to the nozzle with 1.4mm 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 was set at (-)40 mbar. The direction of air flow was the same as that of sprayed products.

2.5. Determination of CsA

The concentration of CsA in the resulting solution was analyzed using a USP method [29]. HPLC (Jasco PU-987) equipped with Nova-Pack® C18 (Waters, 5μm, 3.9 x 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.
2.6. Particle size and size distribution (PSD)
PSD was measured using Malvern Mastersizer (Malvern Hydro 2000S). Heptane was used as a dispersant (refractive index of 1.385-1389 and polarity index of zero). Each sample was dispersed in heptane and added to the sample dispersion unit containing stirrer and stirred at 2000 rpm in order to reduce the interparticle aggregation. The obscuration range was maintained between 10-20%. The average particle sizes were measured after performing the experiment for each batch in triplicate.

2.7. 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.

2.8. 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 (40kV, 40mA, Kα1 radiation: 1.5406Å, Kα2 radiation: 1.5444Å) and mounted with an angular detector-Lynx eye TM. The scan step was fixed at ~0.04° with a counting time of 0.5sec/step over an angular range 3°-30°.

2.9. Nuclear resonance magnetic (NRM)
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 12500 Hz (MAS), CP3lev with ramp up between 60 to 100% (contact time: tcp 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 H2O), (F EOH), cyclosporine, physical mixture: P-$\alpha\beta\gamma$-CD/CsA and P-$\alpha\beta\gamma$-CD were used for analysis.

2.10. Cicular dichroism (CD)
CD spectra were measured using a Jobin Yvon-Spex CD 6 at room temperature. Far-UV spectra (190–260 nm) were recorded in a 0.05 cm-path-length cell. The spectra were recorded with a response time of 4s, 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 spray-dried dispersion formulations (F H2O) 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.

2.11. In-vitro dissolution
Dissolution tests were carried out using apparatus II (Vankel, VK7000) for 90 min. The dissolution medium used was a simulated gastric fluid (SGF). The composition of SGF was 2g sodium chloride and 3.2g purified pepsin in 7 mL hydrochloric acid and sufficient water to make one liter including 0.4% LDAO (v/v). Sufficient volume of SGF medium was prepared to run the in-vitro study. Neoral®, Sandimmune® and CsA-loaded spray-dried dispersion formulations equivalent to 25 mg of CsA was added to a vessel containing 500 mL of SGF maintained at 37°C±0.5°C with a paddle speed of 100 rpm. At predetermined time intervals (10, 20, 30, 45, 60 and 90 min), 1 ml of the medium was sampled and filtered. The 1 mL sample was not replaced but it was taken into account during the calculation of the CsA percent release. The filtrate was analyzed by HPLC at the wavelength of 210 nm as described in the above method.

3. Results and discussion
3.1. Phase solubility study and percent yield of cyclosporine
Solubility study revealed a progressive increase in the solubility of CsA with P-$\alpha\beta\gamma$–CD concentration. According to the phase-solubility diagram classification introduced by Higuchi and Connors [30], the solubility diagrams of CsA in the presence of P-$\alpha\beta\gamma$-CD at 25°C correspond to Bs profile. The results obtained for the solubility of CsA in water and 20% w/v P-$\alpha\beta\gamma$-CD were 17.8 and 95.4 (µg/ml), respectively, and that correspond to 5.4-fold increased in its solubility by P-
αβγ-CD. Also, the percent yield of cyclosporine from both SDD (F\textsubscript{H2O}) and (F\textsubscript{EOH}) was determined and found out to be 82.1 and 86.7%, respectively.

### 3.2. Physicochemical characterisations

Dissolution profiles of SDD formulations, Neoral\textsuperscript{®} and Sandimmune\textsuperscript{®} are shown in Figure 2, A. A complete release (100%) of CsA from SDD (F\textsubscript{H2O}) and Neoral\textsuperscript{®} was observed after 10 minutes and was maintained steady up to 90 min. However, the percent release of cyclosporine was 76.6, 84.1, 92.4, 94.3, 93.7 and 99.7% for SDD (F\textsubscript{EOH}) and 75.8, 85.5, 85.4, 94.3, 93.4 and 99.5% for Sandimmune\textsuperscript{®} at 10, 20, 30, 45, 60 and 90 minutes, respectively. The depicted dissolution rank order in terms of percentage of CsA release was as follows: SDD formulation (FH2O) = Neoral\textsuperscript{®} > SDD formulation (FEOH) > Sandimmune\textsuperscript{®}.

Generally, solid dispersion 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 spray-dried dispersion formulations of cyclosporine-tetrapolymers in either aqueous (F\textsubscript{H2O}) or organic (F\textsubscript{EOH}) media was conducted and compared to Sandimmune\textsuperscript{®} and Neoral\textsuperscript{®}. These data were superior at the one obtained in solid dispersion of CsA with hydroxypropyl cellulose HPC (SSL) [31]. Additionally, this data is in-line with previous finding of research scientists that reported an increase in the dissolution of hydrophobic drug where it was molecularly dispersed as in the case of solid dispersion[32-35]. The increase in the dissolution rate of CsA in SDD formulations (FH2O) could be explained by P-αβγ-CD hydrophilicity (aqueous solubility greater than 1g/mL ) that causes wetting of drug particle, local enhancement of drug solubility at the diffusion layer surrounding the drug particles [32] and absence of crystallinity [36]. 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, \( \eta \) is the apparent viscosity, \( r \) is the radius of a spherical drug molecule and N is Avogadro’s number.

The particle size distribution of cyclosporine in F\textsubscript{H2O} and F\textsubscript{EOH} was analyzed by Malvern 2000S. A narrow distribution was observed as illustrated in Figure 2, B where D\textsubscript{10}, D\textsubscript{50} and D\textsubscript{80}...
were 2.5, 14.9, 26.5 μm and 1.60, 10.9 and 23.5 μm for \( F_{EOH} \) and \( F_{H2O} \), respectively. Therefore, diffusion coefficient was largely increased and the dissolution rate of drug became faster when the particle size is reduced as shown by the particle size distribution (PSD) data of \( F_{H2O} \) and \( F_{EOH} \) formulations (Figure 2, B).

Also, the sameness or equivalence between each two curves (i) \( F_{H2O} \) vs Neoral®; (ii) \( F_{EOH} \) vs Neoral®, (iii) \( F_{H2O} \) vs Sandimmune® and (iv) \( F_{EOH} \) vs Sandimmune® was established by calculating a parameter called \( f_2 \).

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

\( n \) : Number of Time Points

\( R_t \) : Dissolution value of the reference product (Neoral® or Sandimmune®) at Time t.

\( T_t \) : Dissolution value of the SSD (\( F_{EOH} \) or \( F_{H2O} \)) 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 for \( F_{H2O} \) and \( F_{EOH} \) was 92.4 and 42.6%, respectively when compared to Neoral®. However, the \( f_2 \) value obtained for \( F_{H2O} \) and \( F_{EOH} \) was 42.1 and 75.4%, respectively when compared to Sandimmune®. Consequently, on one hand, the SDD (\( F_{H2O} \)) was within specification (50–100%) when compared to Neoral® but was not with Sandimmune®. On the other hand, the SDD (\( F_{EOH} \)) was within specification (50–100%) when compared to Sandimmune® but was not with Neoral®.

Based on the similarity test data, Neoral® and \( F_{H2O} \) are likely to behave similarly upon administration and likely to exhibit similar absorption. The same statement could be applied to Sandimmune® and \( F_{EOH} \).

Also, these findings were confirmed by SEM micrographs of crystalline CsA and P-αβγ-CD based SDD formulation which revealed clear changes in the morphology of the powder particles after spray drying to the evident formation of solid dispersion as illustrated by Figure 3, A-E.

Fig. 3. SEM photomicrograph from cyclosporine samples, including (A) P-αβγ-CD, (B) crystalline CsA, (C) physical mixture of P-αβγ-CD: crystalline CsA, (D) spray-dried
Moreover, PXRD pattern of crystalline CsA alone showed several intense peaks which were indicative of a tetragonal crystal form [37] while in SDD formulations CsA exhibited a halo diffraction pattern indicating its transition from crystalline to amorphous form (Figure 4, A and B).

Fig. 4. PXRD patterns of cyclosporine samples: (A) Cyclosporine, P-αβγ-CD, physical mixture (P-αβγ-CD: CsA) and SDD (FH2O); (B) Cyclosporine, P-αβγ-CD, physical mixture (P-αβγ-CD: CsA) and SDD (FEOH).

Although amorphization has been used to improve the dissolution properties of poorly water-soluble drugs, the excess of free energy drives its nucleation and crystallization. However, the matrix polymers in the solid dispersion 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 [38]. This latter statement explained the behavior of SDD (FEOH) where the P-αβγ-CD had low solubility in ethanol and was unable to prevent the crystallization of CsA which is highly soluble in ethanol (10 mg/mL). This phenomena led to a slow release of CsA from SDD (FEOH) in which the drug was transferred into an amorphous state, as it was soluble in the organic solvent followed by re-crystallizing onto the carrier surface (P-αβγ-CD) by the elimination of solvent during spay drying processus [39]. However, in SDD (FH2O), the dissolved P-αβγ-CD was attached to the surface of dispersed CsA and prevented its crystallization and hence result an enhancement of its dissolution rate due to an increase in both the surface area and solubilization [40-41].

Also, the interaction between CsA and the tetrapolymer in SDD-CsA/P-αβγ-CD was assessed by 13C CPMAS NMR spectral analysis. The spectrum of cyclosporine alone showed intense alkyl C-C peaks with a 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 its crystalline form (Figure 5). However, in both spray-dried dispersion formulations (FH2O) and (FEOH), the intensity of alkyl C-C peaks along with the chemical shifts was dramatically changed. The cyclosporine amorphization was probably a consequence of its interaction with P-αβγ-CD as confirmed by 13C CPMAS NMR spectra where in both spray-dried dispersion formulations (FH2O) and (FEOH), the intensity of alkyl C-C peaks 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 cyclodextrin tetrapolymer P-αβγ-CD through hydrophobic interactions.
Fig. 5. $^{13}$C CPMAS NMR spectra from top to bottom: SDD formulation (F$_{H2O}$), SDD formulation (F$_{EOH}$), cyclodextrin tetrapolymer (P-αβγ-CD) and crystalline cyclosporine.

To clarify whether the secondary structure of CsA in SDD formulations had been changed or not by either cyclodextrin tetrapolymer or under various stress factors during spray-drying (e.g. thermal stress and/or shear stress at the outlet of the spray nozzle), circular dichroism 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 [42-44]. 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 [43-45]. Figure 6 illustrates a long wavelength minimum occurs at near 225 nm having an ellipticity of approximately -25000, accompanied by a maximum at near 194 nm having an ellipticity of 16 000 was depicted indicating that cyclosporine exist as β-turns. These results indicate that cyclosporine exists as β-turns which are on line with other cyclic peptide such as the CD spectrum for Cyclo (L-α-L-Pro-DPhe) [46].

![CD Spectrum](image)

Fig. 6. Far –UV-CD spectrum of cyclosporine in the diluent: 55% ACN:H$_2$O (bleu), cyclosporine released from SDD formulation (F$_{EOH}$) into the diluent (brown) and cyclosporine released from SDD (F$_{H2O}$) into the diluent (green).
Also, the CD spectra of different ratio of P-αβγ-CD: 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 polymer (data not shown). After entrapment of cyclosporine in SDD (F\text{H2O}) and (F\text{EOH}) formulations, only minor differences were observed in the cyclosporine CD spectrum. Thus, these systems seem to be stable to carry cyclosporine and release it, while preserving its structure and thus, potentially, also maintaining cyclosporine activity.

4. Conclusions

Spray-dried dispersion formulations of CsA using cyclodextrin tetrapolymer P-αβγ-CD as matrix were developed and characterized fully by novel methodologies. Developed formulations revealed great enhancement in dissolution rate of CsA especially with SDD (F\text{H2O}). The dissolution improvement was due to hydrophilisation and lost of crystallinity of cyclosporine. Developed SDD formulation (F\text{H2O}) revealed same profile as Neoral® and better than Sandimmune® which implied that CsA was embedded in a solid dispersion as confirmed by physicochemical characterization studies and without relevant changes in the peptide secondary structure as revealed by CD and hence might increase its bioavailability.

Acknowledgements

The author is grateful to Pr. H. Oulyadi Dr. L. Guihaldis from Rouen University, UMR 6014 & FR3038 CNRS; Pr. G. Coquerel and d. Martin from Rouen University, Laboratoire SMS UC2M2 UPRES EA3233 UFR of SciencesNone declare.

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