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NOVEL ORAL FORMULATION OF CYCLOSPORINE-SPRAY-DRIED DISPERSION USING CYCLODEXTRIN COPOLYMERS

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The purpose of this study was to develop a new oral cyclosporine (CsA) formulation free from surfactant cremophor using only cyclodextrin copolymers (P-α-CD, P-β-CD and P-γ-CD) as excipients in attempt to enhance its stability, dissolution rate and reduce surfactant side effects. Two spray-dried dispersions (SDD) containing poorly water-soluble CsA were prepared with either P-α-CD, P-β-CD or P-γ-CD using water (F_{H2O}) and ethanol (F_{EOH}) via spray-drying technique and characterized by scanning electron microscopy, powder X-ray diffraction, particle size distribution, circular dichroism 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-α-CD, P-β-CD and P-γ-CD without secondary structure change of CsA. The order of the CsA release from the copolymers was ranked as follows: P-α-CD/CsA(F_{H2O})= P-β-CD/CsA(F_{H2O})=P-γ-CD/CsA(F_{H2O})= Neoral® > Sandimmune®> P-γ-CD/CsA (F_{EOH})> P-α-CD/CsA(F_{EOH})>P-β-CD/CsA (F_{EOH}). The results of (F_{H2O}) could be explained by reduction in particle size, the absence of crystallinity, and improved wettability of CsA while maintaining part of its cristallinity in the case of formulations (F_{EOH}). Developed SDD formulations (F_{H2O}) 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.

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Keywords: Cyclosporine, spray-dried dispersion, cyclodextrin copolymer, formulation, physico-chemical characterization.

1. 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 autoimmune disorders [1, 2].

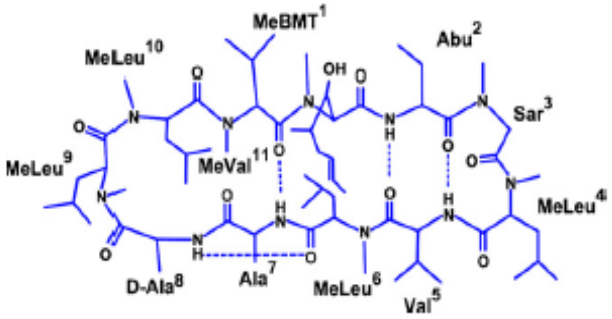

Fig. 1. Structure of cyclosporine A (CsA)

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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 of created dispersion. The droplet size in Sandimmune® and Neoral® is ranging from few nanometers to several micrometers and 100 to 250 nm, respectively [4]. This difference in physico-chemical characteristic resulted from a change in composition, where new surfactant like cremophor with higher hydrophilic-lipophilic balance (HLB) greater than12 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 was used to improve the solubility and bioavailability of lipophilic drugs. The most common polymers used were polyethylene glycol [8], poloxaamer [9], hydroxyethyl cellulose, mannitol [10], polyvinylpyrollidone [11], and phospholipid [12]. 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 solid dispersion (SD) with cyclodexrin copolymers. 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 development of a spray-dried solid amorphous dispersion technology using cyclodextrin copolymers. 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 severe conditions of the gastrointestinal tract such as acidity and enzymatic degradation.

The present study aims at preparation of spray-dried dispersion of cyclosporine using P-α-CD, P-β-CD and P-γ-CD mediated by spray dryer technique. The physicochemical characterizations were investigated and in vitro studies on efficacy of the copolymers on CsA release were carried out. Also, the peptide secondary structure was evaluated by circular dichroism upon 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 copolymers.

2. Experimental

2.1 Materials

Native cyclodextrins α, γ and β were ordered from Wacker, France. Citric acid, sodium chloride, pepsin (1:10.000, from porcine stomack 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.

2.2 Preparation of copolymer P-α-CD, P-β-CD and P-γ-CD

The copolymer P-α-CD was synthesized according to M. Skiba [19]. Briefly, a mixture of known amount (w/w) of cyclodextrin α, citric acid and sodium phosphate dibasic was transferred into a reactor which was maintained at temperature ranging between 140-150°C for 15 to 30 minutes. The obtained solid form was dissolved in water and 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 T_0 and after 4 hours of dialysis. After the dialysis, the resulted solution was spray dried using BÜCHI Mini Sprayer Dryer B-290. Spray-dryer parameters were validated by preliminary works and were as follow: inlet temperature: 150ºC; outlet temperature: 80-90 ºC; aspiration: 100%; pump%: 20% and pressure: (-40) mbar. The same procedure was used to synthesize the P-β-CD and P-γ-CD.

2.3 Phase Solubility Study

The solubility study was conducted in three different flasks containing either 2 ml of increasing concentrations of cyclodextrin copolymer P-α-CD, or P-β-CD or P-γ-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 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 Büchi 290 nozzle type mini spray dryer (Flawil, Switzerland) was used for the preparation of the cyclosporine-loaded spray-solid dispersions. Based on the solubility data, two cyclosporine-spray solid dispersions were prepared with 0.3-1 g cyclosporine and 3-10g of either cyclodextrin copolymers P-α-CD, P-β-CD or P-γ-CD. In the solvent method, cyclosporine was dissolved in 300 ml ethanol and cyclodextrin copolymer was dispersed (F_{EOH}). Conversely, in the aqueous method each copolymer was dissolved in 300 ml water and cyclosporine was dispersed ($_{FH2O}$). They were then delivered to the nozzle with 1.4mm diameter, flow rate of pump at 20% and spray-dried at 150ºC 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.

2.5 Determination of CsA

The concentration of CsA in the resulting solution was analyzed by 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 212 nm and HPLC colum temperature controller. The mobile phase consisted of acetonitrile: water: methanol: phosphoric acid (55: 45: 5: 0.5, v/v) with flow rate of 1.0 mL/min and the column temperature was maintained at 70ºC

2.6 Particle size and size distribution (PSD)

PSD was measured using Malvern Mastersizer (Malvern Hydro 2000S). Heptane was used as a dispersant with refractive index of 1.385-1389. 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, and laser 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 ™. 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 13C 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: 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_{H2O}), (F_{EOH}), cyclosporine, physical mixture of copolymers /CsA and P-α-CD, P-β-CD and P-γ-CD were used for analysis.

2.10 Circular 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 4 s, sensitivity of 10 mdeg and scan speed of 10 nm/min and converted into mean residue ellipticity in deg · cm2 · 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

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 and CsA-loaded spray-dried dispersion formulations (F_{H2O}) 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 (pH1.2) 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 212 nm as described in the above method.

3. Results

3.1 Phase solubility study and percent yield of cyclosporine

Solubility study revealed a progressive increase in the solubility of CsA with the copolymers (P-α –CD, P-β –CD and P-γ –CD) concentration. According to the phase-solubility diagram classification introduced by Higuchi and Connors [20], the solubility diagrams of CsA and copolymers at 25°C correspond to A_n profile for P-α –CD while P-β –CD and P-γ –CD followed B_s profiles. As shown in Fig. 2. Results obtained for the solubility of CsA in water and 20% w/v P-α –CD, P-β –CD and P-γ –CD were 17.8, 173.9, 42.1 and 38.1 (μg/ml), respectively, and that correspond to 9.8, 2.4 and 2.1-fold increase in its solubility by P-α –CD, P-β –CD and P-γ –CD, respectively. Also, the percent yield of cyclosporine from P-α-CD/CsA (F_{H2O}), P-β-CD/CsA (F_{H2O}), P-γ-CD/CsA (F_{H2O}), P-α-CD/CsA (F_{EOH}), P-β-CD/CsA (F_{EOH}), and P-γ-CD/CsA (F_{EOH}) was determined and found out to be 72.6, 79.2, 81.6, 88.1, 90.6 and 79.8%, respectively.

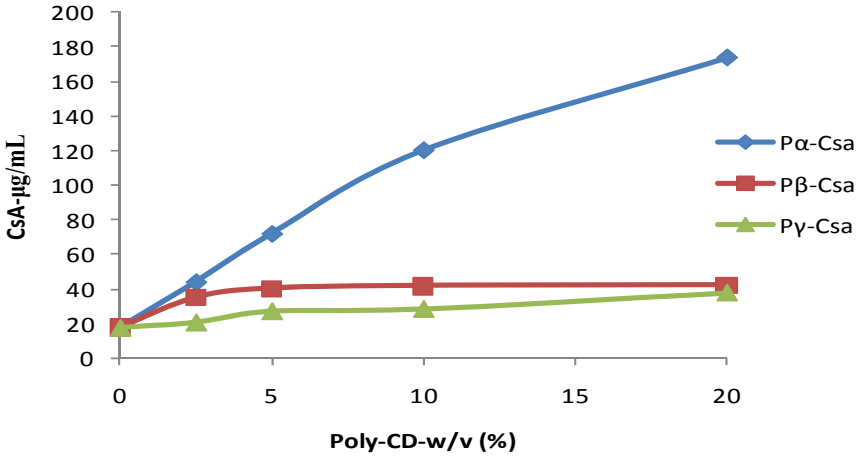

Fig. 2. Phase solubility of CyA in aqueous P-α-CD, P-β-CD and P-γ-CD at 25°C (n=3)

3.2 Dissolution study and particle size distribution

Dissolution profiles of SDD formulations, Neoral® and Sandimmune® are shown in Fig. 3. A complete release (100%) of CsA from P-α-CD/CsA (F_{H2O}), P-β-CD/CsA (F_{H2O}), P-γ-CD/CsA (F_{H2O}) SDD formulations and Neoral® was observed after 10 minutes while only 76, 49, 47 and 56% was released from Sandimmune®, P-α-CD/CsA (F_{EOH}), P-β-CD/CsA (F_{EOH}), and P-γ-CD/CsA (F_{EOH}), respectively. There was significant difference in the dissolution profile of Neoral®, Sandimmune® and SSD formulations followed by a progressive increase in the dissolution rate of CsA after 10 min for the spray-dried dispersion in ethanol and sandimmune® reaching up more than 95% release of CsA after 90 minutes of dissolution . The particle size distribution of cyclosporine in F_{H2O} and F_{EOH} was analyzed by Malvern 2000S. A narrow distribution was observed as illustrated in Fig. 4 and the data are tabulated in table 1 as the dissolution rate of drug became faster when the particle size is reduced.

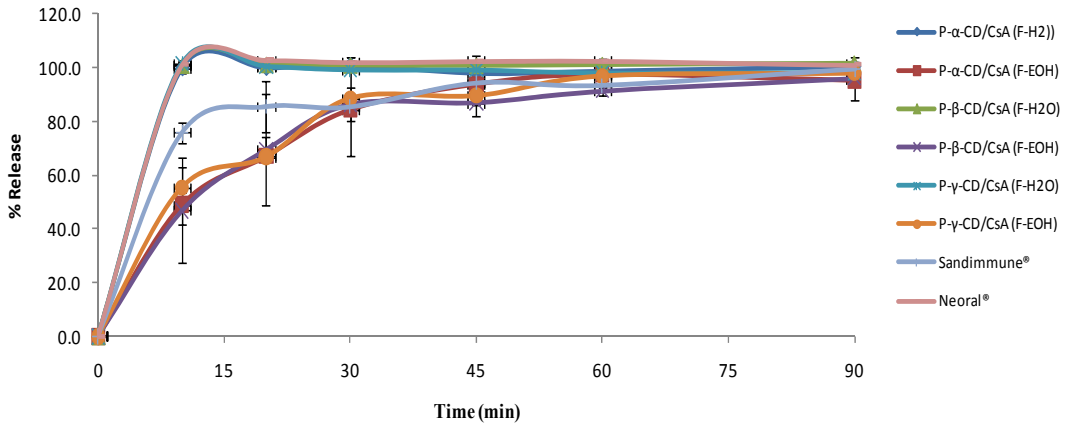

Fig. 3. Dissolution profile of cyclosporine in samples: SDD (F_{H2O}), SDD (F_{EOH}), Noeral® and Sandimmune® in a simulated gastric fluid.

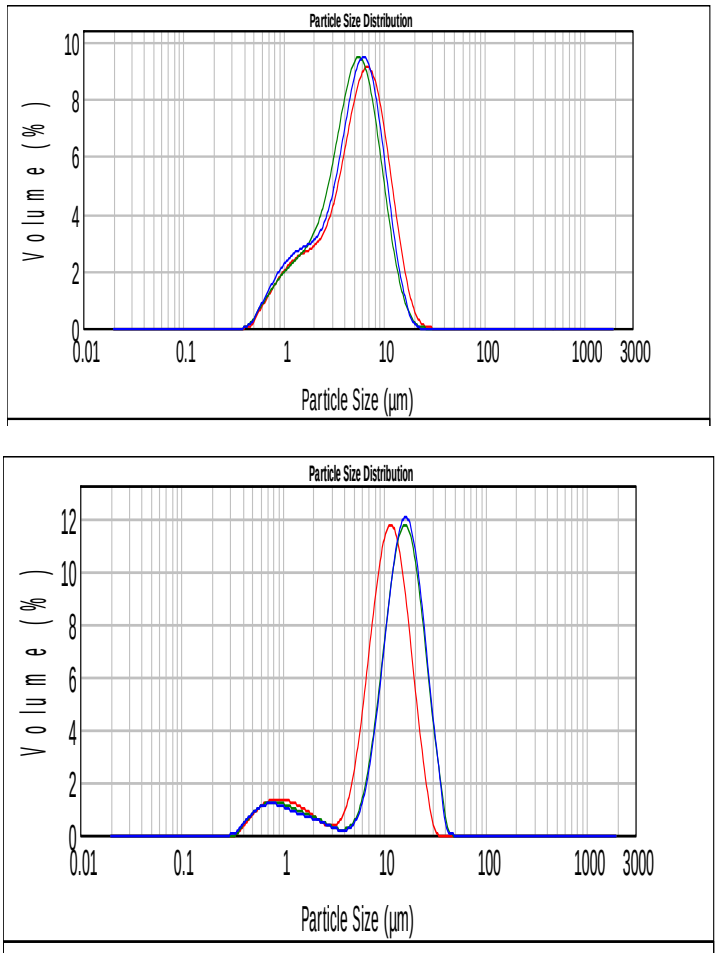

Fig. 4. Particle size distribution of CsA in samples (a): (Red) P-α-CD/CsA (F_{EOH}), (green) P-β-CD/CsA (F_{EOH}) and (bleu) P-γ-CD/CsA (F_{EOH}); (b) (Red) P-α-CD/CsA (F_{H2O}), (green) P-β-CD/CsA (F_{H2O}) and (bleu) P-γ-CD/CsA (F_{H2O}).

Table 1. Particle size distribution of cyclosporine in spray-dried dispersion formulations (F_{H2O}) and (F_{EOH})

	P-α-CD/CsA (F_{H2O})	**P-β-CD/CsA (F_{H2O})**	**P-γ-CD/CsA (F_{H2O})**	**P-α-CD/CsA (F_{EOH})**	**P-β-CD/CsA (F_{EOH})**	**P-γ-CD/CsA (F_{EOH})**
D10(µm)	1.6	1.8	2.0	1.4	1.4	1.3
D50(µm)	10.4	14.4	14.6	5.5	4.7	5.0
D90(µm)	18.8	26.0	26.1	11.6	9.8	10.3

3.3 Physico-chemical characterization

As baseline, Figure 5 a, *b* and *c* shows SEM micrographs of P-α-CD, crystalline CsA, physical mixture of P-α-CD: CsA where the cyclosporine preserves its crystalline form. In contrast, Figure 5d and e indicate a change in its morphology. These results are supported by PXRD spectra as shown by Figure 6*a* where several intense peaks were observed in the PXRD pattern of crystalline CsA while it exhibits a halo diffraction pattern in F_{H2O} indicating the amorphous form of CsA was kept during the spray-drying processes. The same results were obtained in F_{EOH} formulation as illustrated by Fig. 6b. The same data were observed with P-β-CD and P-γ-CD (data not shown)

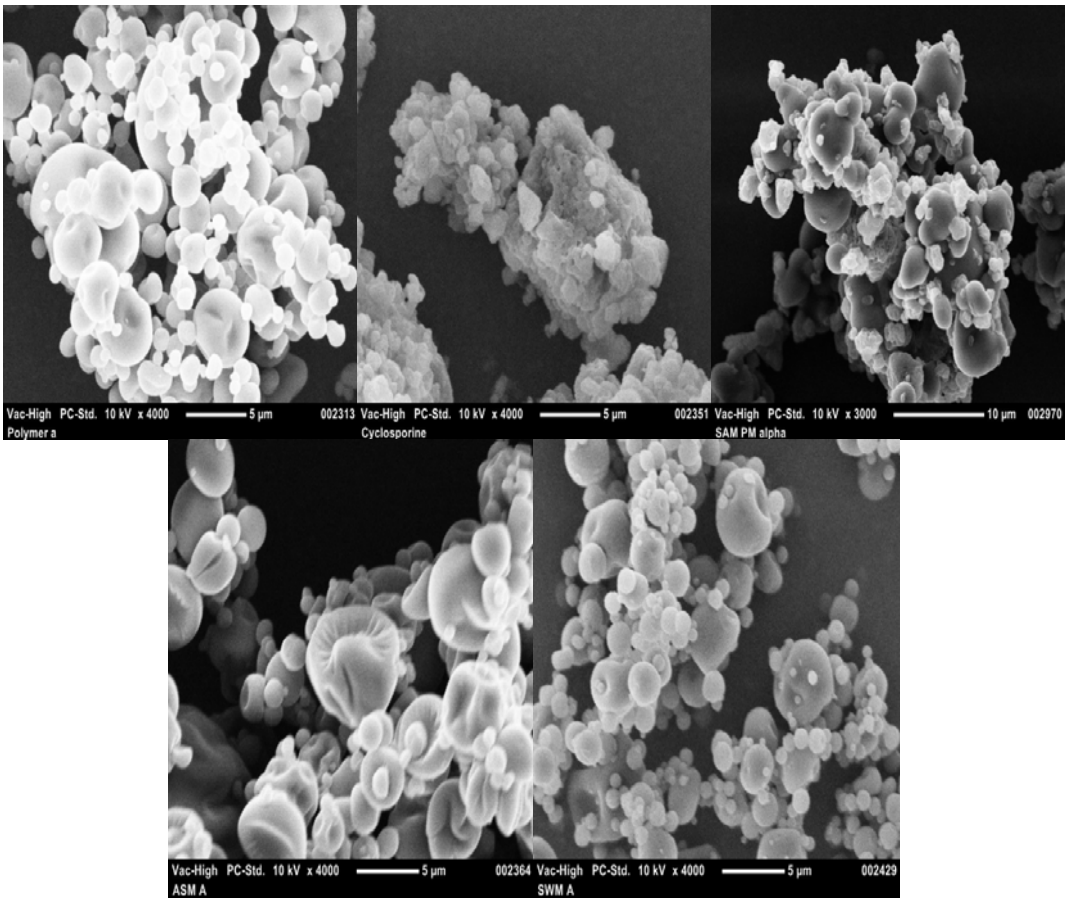

Fig. 5. SEM photomicrographs from cyclosporine samples, including (a) P-α-CD, (B) crystalline CsA, (c) physical mixture of P-α-CD: crystalline CsA, (d) spray-dried dispersion of CsA with P-α-CD using water (F_{H2O}) and (e) spray-dried dispersion of CsA with P-α-CD using ethanol (F_{EOH}).

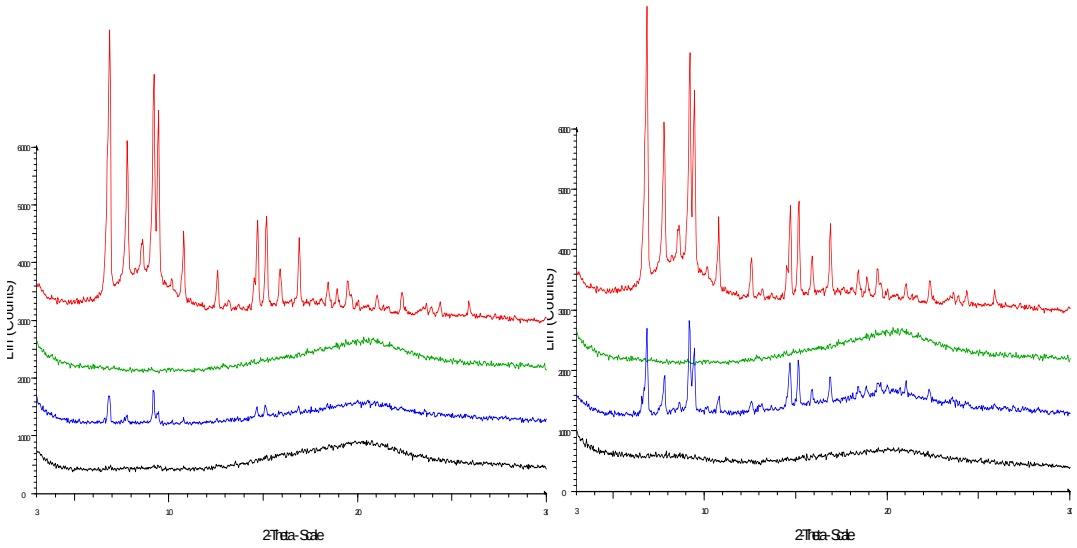

Fig. 6. PXRD patterns of cyclosporine samples: (a) Cyclosporine, P-α-CD, physical mixture (P-α-CD: CsA) and SDD (F_{H2O}); (b) Cyclosporine, P-α-CD, physical mixture (P-α-CD: CsA) and SDD (F_{EOH}).

Also, the interaction between cyclosporine and the copolymers was assessed by ^{13}C CPMAS NMR spectral analysis. The physical mixture P-α-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 (Fig. 7). However, in both spray-dried dispersion formulations (F_{H2O}) and (F_{EOH}), the intensity of alkyl C-C along with the chemical shifts was dramatically changed. The P-β-CD/CsA and P-γ-CD/CsA spray-dried dispersion formulations (F_{H2O}) and (F_{EOH}) were also analyzed by ^{13}C CPMAS NMR and showed the same pattern (data not shown).

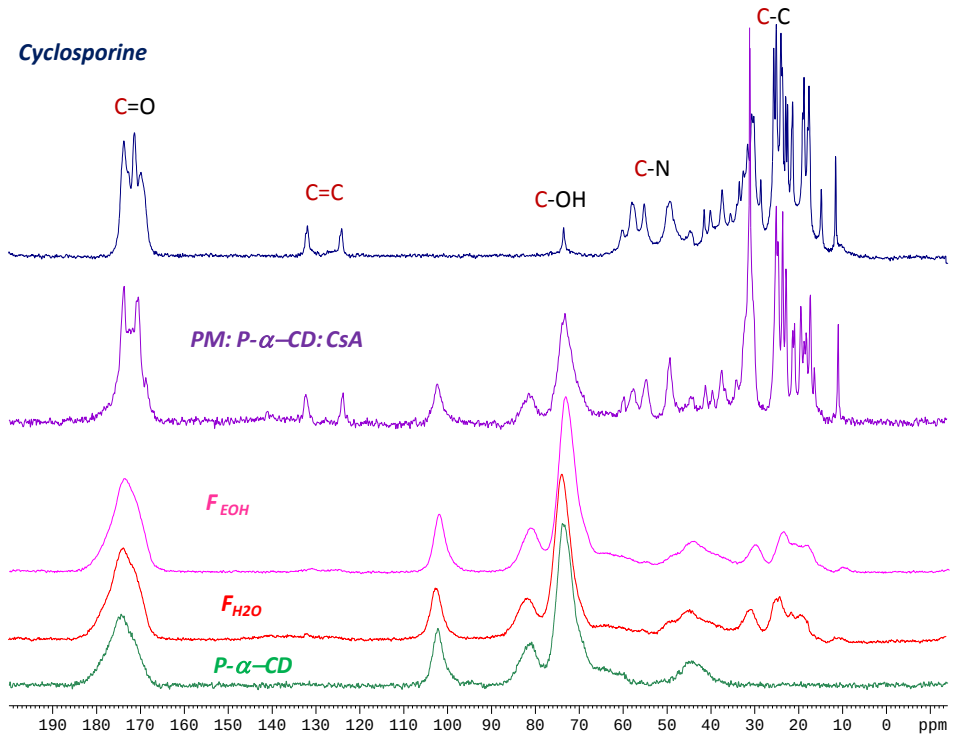

Fig. 7. ^{13}C CPMAS NMR spectra from top to bottom: crystalline cyclosporine, physical mixture (P-α-CD: CsA), SDD formulation (F_{EOH}), SDD formulation (F_{H2O}) and cyclodextrin copolymer (P- α-CD).

To clarify wither the secondary structure of CsA in SDD formulations had been changed or not by either cyclodextrin copolymers or 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. Fig. 8 illustrates a long-wavelength minimum occurs near 225 nm having an ellipticity of approximately -25000, accompanied by a maximum near 194 nm having an ellipticity of 16 000 was depicted indicating that cyclosporine exist as β-turns.

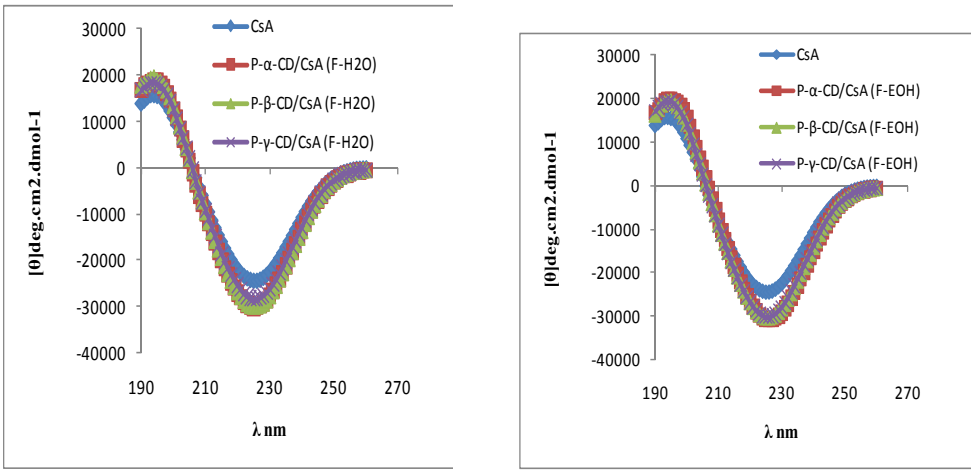

Fig. 8. Far –UV-CD spectrum of cyclosporine in samples (a) CsA in the diluent: 55% ACN:H2O (bleu), P-α-CD/CsA (F_{H2O}) (brown) and P-β-CD/CsA (F_{H2O}) (green) and P-γ-CD/CsA (F_{H2O}) (purple) ; (b) CsA in the diluent: 55% ACN:H2O (bleu), P-α-CD/CsA (F_{EOH}) (brown) and P-β-CD/CsA (F_{EOH}) (green) and P-γ-CD/CsA (F_{EOH}) (purple).

The CD spectra of different ratio of copolymers: 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 in SDD (F_{H2O}) and (F_{EOH}) formulations, only minor differences were observed in the cyclosporine CD spectrum.

4. Discussion

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_{H2O}) or organic (F_{EOH}) media was conducted and compared to Sandimmune® and Neoral® as shown in Fig. 3. These data were superior at the one obtained with solid dispersion of CsA with hydroxypropyl cellulose HPC (SSL) and CsA/ polyethylene glycol (PEG-6000) [21, 22]. The depicted dissolution rank order in terms of percentage of CsA released were ranked as follows: P-α-CD/CsA (F_{H2O})= P-β-CD/CsA (F_{H2O})=P-γ-CD/CsA (F_{H2O})= Neoral® > Sandimmune®> P-γ-CD/CsA (F_{EOH})> P-α-CD/CsA (F_{EOH})>P-β-CD/CsA (F_{EOH}). This data is in-line with previous finding of research scientists which reported an increase in the dissolution of hydrophobic drug where it is molecularly dispersed as in the case of solid dispersion [23-26]. 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.

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

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 particle size distribution (PSD) data of SDD formulations (F_{H2O}) and (F_{EOH}) which are tabulated in Table 1.

The increase in the dissolution rate of CsA in SDD formulations (F_{H2O}) could be explained by P-α –CD, P-β –CD and P-γ –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 [24] and absence of cristallinity [27]. These finding were confirmed

by SEM micrographs of crystalline CsA and copolymers based SDD formulations (F_{H2O}) and (F_{EOH}) which revealed clear changes in the morphology of the powder particles after spray drying to the evident formation of solid dispersion. As shown in Fig. 5.

Moreover, PXRD pattern of crystalline CsA showed several intense peaks which were indicative of a tetragonal crystal form [28] while in SDD formulations CsA exhibited a halo diffraction pattern indicating its armophous 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) [29, 30]. 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 [30]. Additionally, in the case of SDD formulations (F_{EOH}), the copolymers and due to their low solubility in ethanol they were unable to prevent the crystallization of CsA which is highly soluble in ethanol (10 mg/mL) leading to a slow release of CsA observed in SDD formulations (F_{EOH}) in which the drug was transferred in an amorphous state, as it was soluble in the organic solvent followed by re-crystallizing onto the carrier surface by the elimination of solvent [31]. On the other hand, in SDD formulations (F_{H2O}), the dissolved copolymer was 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 solubilization [32, 33].

The cyclosporine amorphization was probably a consequence of its interaction with copolymers in SDD-CsA/P-α-CD, P-β-CD and P-γ-CD as was confirmed by ^{13}C CPMAS NMR spectral analysis where in both spray-dried dispersion formulations (F_{H2O}) 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 copolymer through hydrophobic interactions.

To clarify whither the secondary structure of CsA in SDD formulations had been changed or not by either different cyclodextrin copolymers or various stress factors during spray-drying, 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 [34-36]. 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 [35, 37]. Like all spectroscopic techniques, the CD signal reflects an average of the entire molecular population. Fig. 8 illustrates a long-wavelength minimum occurs near 225 nm having an ellipticity of approximately -25000, 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) [38]. After entrapment in SDD (F_{H2O}) 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.

5. Conclusions

A spray-dried dispersion formulations of CsA using cyclodextrin copolymers (P-α-CD, P-β-CD, P-γ-CD) as matrices were developed and characterized fully by novel methodologies. Developed formulations revealed great enhancement in dissolution rate of CsA especially with SDD formulations (F_{H2O}). The increase dissolution rate is thought to be related to the reduction in

particle size, the absence of crystallinity, and improved wettability of CsA. Developed SDD formulation (F_{H2O}) showed same profile as Neoral® and better than Sandimmune® which thought to be 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.

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