Research Article

Formulation and Evaluation of a Protein-loaded Solid Dispersions by Non-destructive Methods

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Abstract. The purpose of this investigation was to develop solid dispersion (SD) formulation of cyclosporine (CyA) using polyethylene glycol (PEG-6000) to enhance its dissolution rate followed by nondestructive method for the prediction of both drug and carrier. SD formulations were prepared by varying the ratio of CyA and PEG-6000 by solvent evaporation technique and characterized by dissolution, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), Fourier transform infrared (FTIR), powder X-ray diffraction (PXRD), near infrared (NIR) and near infrared chemical imaging (NIR-CI). Dissolution data revealed enhanced dissolution of CyA when compared with pure CyA. DSC results showed that the crystallinity of PEG-6000 has decreased as indicated by decrease in the enthalpy of fusion and melting peak in the formulations. FTIR data demonstrated no chemical interaction between drug and carrier. The surface morphology of SD formulations was similar to PEG-6000 particle. NIR-CI disclosed homogeneity of SD matrix as indicated by symmetrical histograms with smaller values of skewness. Similar to NIR, a multivariate peak evaluation with principal component analysis and partial least square (PLS) were carried out with PXRD spectral data. PLS models with both techniques showed good correlation coefficient and smaller value of root mean square of errors. The accuracy of model for predicting CyA and PEG-6000 in NIR and PXRD data were 5.22%, 5.35%, 5.27%, and 2.10%, respectively. In summary, chemometric applications of non-destructive method sensors provided a valuable means of characterization and estimation of drug and carrier in the novel formulations.

KEY WORDS: cyclosporin; NIR; PEG-6000; PXRD; solid dispersion.

INTRODUCTION

Cyclosporin (CyA), a cyclic oligopeptide, is a potent immunosuppressant and has been used primarily to prevent xenograft rejection after organ transplantation. The oral bioavailability of cyclosporine is poor which is related to its high molecular weight (1202.61 Da), low water solubility (7.3 µg/mL at 37°C) (1) and high octanol-water partition coefficient (log *P*=2.92) (2). According to biopharmaceutical classification system, CyA has been classified as class 2 indicating that its bioavailability is dissolution dependent (3,4). Solubility enhancement is one of the way to increase dissolution rate and hence bioavailability. Various technique used to enhance solubility are use of surfactant (5), complexation with cyclodextrin (6) and solid dispersion (7).

Solid dispersion technique is used to enhance water solubility, dissolution rate and bioavailability of lipophilic molecule and the most commonly used for solid dispersions

carriers are polymers. Solid dispersion system of Eudragit® (8), hydroxypropylcellulose and hypromellose (9), chitosan, and hydroxypropylmethylcellulose phthalate (10) improve the solubility but retard the drug release. While solid dispersion with hydrophilic or amphiphilic polymer such polyethylene glycol (11), poloxamer (12), ammonium glycyrrhizinate pentahydrate (13), hydroxyethyl cellulose, mannitol (14), polyvinyl pyrrolidone (15), and phospholipid (7) improve the dissolution rate and oral absorption of lipophilic drugs. Investigators reported the solid dispersion of CyA with polyoxyethylene (40) stearate (5), inulin (16), dimyristoyl phosphatidylcholine (7), sodium lauryl sulfate and dextrin (17), and hydroxypropylmethylcellulose phthalate and polyoxyethylene hydrogenated castor oil (18) for the enhancement of intrinsic solubility, dissolution rate, absorption rate, and hence bioavailability. No information was found in literature for the improvement of CyA dissolution and bioavailability by its solid dispersion (SD) with polyethylene glycol-6000 (PEG-6000).

Process analytical technology (PAT) is a system to design, analyze, and control raw material, intermediate, and pharmaceutical manufacturing processes through the measurement of critical parameters which affect critical quality attributes of finished product (19). The concept aimed at defining critical parameters, measuring and monitoring by in-line or the on-line sensors thus results in more efficient testing which at the same time results in more consistency and reduces rejection of final products. Most widely investigated

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process analytical sensors are near infrared spectroscopy (NIR) and Raman spectroscopy (7) and less commonly used are mid-infrared (IR) (20) for reaction monitoring, light-induced fluorescence for blend homogeneity determination (21), in-line nuclear magnetic resonance (and gas chromatography) in the petrochemical industry (22) and near infrared chemical imaging (NIR-CI) (23). In all the technology, NIR technology emerged as the successful PAT sensor in in-line, on-line, and at-line monitoring process because of its versatility, speed, simplicity, non-destructiveness, and requires no sample preparation (7,24).

Powder X-ray diffraction (PXRD) is the common technique for the identification of crystalline substance. Beside its qualitative application, it can also be used quantitatively (25). Investigators reported its application in monitoring pharmaceutical process. Davis *et al.* (26) successfully demonstrated the on-line application of PXRD in monitoring the transformation of metastable polymorphs to stable polymorphs during the wet granulation of fluphenic acid. PXRD has also been reported for the quantification of crystalline tolnafate from ethylcellulose microspheres (27) and acetoaminophen from suppositories containing hydrogenated vegetable oil, polyoxyethylene stearate, glycol monostearate, and preservatives (25). Different methods of data analysis, such as spectral subtraction (25), Rietveld analysis (28), whole pattern matching software (29) and partial least square (PLS) methods (30), can also be used to obtain lower detection levels or quantitate materials with significant overlap of diffractogram.

The objective of present investigation is to prepare solid dispersion of CyA with polyethylene glycol as a carrier by solvent evaporation technique and evaluate it for drug and polymer content by nondestructive method such as NIR, PXRD, and NIR-CI and construct model for that using chemometrics.

MATERIALS AND METHODS

Materials

Cyclosporine (purity 99%) was purchased from Poli Industria Chimica S.P.A (Rozzano, Milano, Italy). Polyethylene glycol-6000 (PEG-6000) and hard capsule gelatin shell were obtained from Alfa Aesar, MA, USA) and Parke Davis, Detroit, MI, USA, respectively. Methanol and acetonitrile (high performance liquid chromatography (HPLC) grade) was obtained from Fisher Scientific Co. (Norcross, GA, USA). All other chemicals and solvents used were of analytical or HPLC grade.

Phase Solubility Study

Solubility study was carried out by adding excess amount of CyA in 5 ml of different concentrations of PEG-6000 (0-10% w/v) solution. The mixture was vortexed for 10 s and put on horizontal shaker at 120 rpm and 25°C for 48 h. Samples were filtered

through 0.2 µm filter (Milipore, Nylon). The solubilized CyA was determined by HPLC and experiment was performed in duplicate.

Preparation of Solid Dispersion

SD was prepared by solvent evaporation method (31). Six formulations were prepared by varying amount of CyA and PEG-6000 as shown in Table I. Briefly, CyA and PEG-6000 were dissolved in 20 ml of methanol. Subsequently, methanol was removed by evaporation at room temperature (21-25°C) and SD formulations were further dried in vacuum drying at 30°C for 48 h. The formulation was grounded by a pestle mortar and passed through a set of sieve 70/120 (ASTM). The resultant powder which was passed through sieve 70 and retained on sieve 120 was used in dissolution and characterization studies. Dry SD was kept in desiccator at room temperature until further evaluation.

Dissolution Study

The SD formulation equivalent to 25 mg CyA was filled in hard gelatin capsule shell size '0'. The dissolution study was carried out by USP 23 paddle method. The dissolution experiment was performed in 500 ml of 0.1 N HCl at paddle speed of 50 rpm and 37°C for 2 h. The capsules were fitted in helical sinker before putting in the dissolution vessel to prevent their floating during the study. 1 ml samples were withdrawn at 10, 20, 30, 45, 60, 90, and 120 min, centrifuge for 15 min at 14,000 rpm. CyA release from SD formulation was quantitated using in-house developed and validated HPLC method with a Hewlett Packard (HP) HPLC instrument (Agilent Technologies, CA, USA) that consist of a quaternary HP 1050 pump, HP 1050 autosampler, and 1050 HP UV detector set at a wavelength of 203 nm and column compartment thermostatted at 70°C. The HPLC stationary phase was composed of a C8, 4.6×250 mm (3.5µm packing) reverse phase chromatography Zorbax SB-C8 column and a C8, 4.6×12.5 mm (5µm packing) Zorbax SB-C8 reliance guard column (Agilent Technologies, CA, USA). The mobile phase consisted of acetonitrile/methanol/water/phosphoric acid (8:4:3:0.05) and was pumped isocratically at a flow rate of 1.25 mL/min. The experiment was performed in duplicate.

Scanning Electron Microscopy

Shape and surface morphology of drug, the powdered PEG-6000 and SD formulations were performed by scanning electron microscopy (SEM, JSM-6390 LV, JEOL, Tokyo, Japan) measurements at the working distance of 15 mm and an accelerated voltage of 20 KV. Samples were gold coated with sputter coater (Desk V, Denton Vacuum, NJ, USA) before SEM observation under high vacuum and high voltage of 10 mV to achieve film thickness of 30 nm.

Table I. Solid Dispersion Formulation Composition and Dissolution Efficiency Results

Formulation	SD-1	SD-2	SD-3	SD-4	SD-5	SD-6	PM
CyA (g)	1	0.67	0.50	0.33	0.25	0.18	0.18
PEG-6000 (g)	1	1.33	1.50	1.67	1.75	1.81	1.81
DE	4.39±0.42	6.79±0.69	9.44±2.63	10.61±0.78	15.61±3.20	21.05±1.12	1.51±0.10

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) of CyA, PEG-6000, their physical mixture (composition equivalent to SD-6 formulation) and SD formulations was performed by SDT 2960 Simultaneous DSC/Thermogravimetric Analyzer (TA Instruments Co., New Castle, DE, USA). Nitrogen was used for purging the environment of sample holder at the rate of 20 ml/min. The temperature of equipment was calibrated by running standard indium metal. Sample equivalent to 1-2 mg was hermetically sealed in aluminum cap and empty pan was used as a reference and run from 20-250°C at scanning rate 10°C/min.

Fourier Transform Infrared Spectroscopy

Fourier transform infrared spectroscopy (FTIR) spectra of drug, polymer, their physical mixture and SD formulations were performed by FTIR instrument (Thermo Nicolet Nexus 670 FTIR, GMI Inc., Ramsey, MN, USA). The instrument was equipped with attenuated total reflectance accessory for fast and direct measurement of FTIR spectrum. OMNIC ESP software (version 5.1) was used to capture and analyze the spectra.

Powder X-ray Diffraction

PXRD experiments were performed on X-ray diffractometer (MD-10 mini diffractometer, MTI Corporation, Richmond, CA, USA) using Cu K 2α rays ($\lambda=1.54056\text{\AA}$) with a voltage of 25 KV and a current of 30 mA, in flat plate $\theta/2\theta$ geometry, over the 2θ ranges 14-70°, with a step width 0.05° and a scan time of 2.0 s per step. A 100 mg tightly packed sample was prepared by pressing against a flat glass plat on the glass holder. Diffraction patterns for CyA, PEG-6000, their physical mixture, and SD formulations were obtained. Six diffractogram were collected for each sample. Diffractogram processing and chemometric analysis were performed using Unscrambler v9.2 software (CAMO Software Inc., Woodbridge, NJ, USA).

Near Infrared Spectroscopy

NIR spectra for each SD formulation were collected using a Foss NIR spectrometer (Rapid Content TM Analyzer, AP-2020, Model 5000, Foss NIR Systems Co., Laurel, MD, USA) equipped with a diffuse reflectance apparatus over the range 1,100-2,500 nm. Sample was filled in 2 ml borosilicate glass vials as it is transparent to NIR beam (32). Spectrum was obtained directly scanning through the base of vials (4 mm³). Shift in spectral baseline was dependent upon sample composition and positioning during measurement. To minimize baseline shifting due to sample positioning, all spectra were collected in sextet with rotation of the sample vials to ensure representative spectra. Processing of spectra and chemometric analysis were performed using Unscrambler v9.2 software (CAMO Software Inc., Woodbridge, NJ, USA).

Near Infrared Chemical Imaging Spectroscopy

The image data sets of the SD formulations were collected by the SapphireIM NIR Spectral Imaging System (Spectral Dimensions, Inc., Olney, MD, USA). The imaging system consists of a liquid crystal tunable filter (LCTF) coupled with a

NIR sensitive focal plane array (FPA) detector. The diffuse reflectance image of the sample is passed through LCTF. The tunable filter element rapidly selects wavelengths over a spectral range of 1,400-2,450 nm. A series of images are then captured by the indium-gallium-arsenide NIR FPA detector with a total acquisition time of ~2 min. Each pixel in the detector array corresponds to ~1,600 μm^2 (40×40 μm) area of the sample surface and the resulting data set contains 125 wavelength increment scans per spectrum. The data sets are generally referred to as image cubes or hyperspectral image cubes. Data were analyzed using ISysIM software (Spectral Dimensions, Inc., Olney, MD, USA), a graphical user interface with an integrated software package designed specifically for the acquisition, visualization, and analysis of hyperspectral image cubes. Localized NIR spectra associated with each pixel and images associated with each NIR wavelength are readily displayed. Based on processing, all images were PLS score images. Spectralon background image cube was used to correct both the spatial and spectral response of the system. All images were preprocessed by taking the inverse common logarithm to convert to log (1/R). PLS score images were generated using PLS analysis type 2. A library was built from the pure component spectra representing this binary system (CyA and PEG-6000). Spectral absorbance for each pixel was decomposed into score values associated with each component. The intensity values for the PLS score images shown represent the score values for CyA PEG-6000, respectively. The CyA and PEG-6000 amounts in the SD formulation could be determined qualitatively from the NIR-CI by visual inspection. A quantitative measure of CyA and PEG-6000 in the SD formulations were established by calculating the mean representing CyA and PEG-6000 in the SD formulations from the histograms of their PLS score images.

RESULTS AND DISCUSSION

Physicochemical Characterization

Phase Solubility Study

Solubility study revealed the progressive increase in the solubility of CyA with PEG-6000 concentration. According to the phase-solubility diagram classification introduced by Higuchi and Connors (33), the solubility diagrams of CyA and PEG-6000 at 25°C correspond to A_p-type profiles as indicated by R^2 value 0.9601 (Fig. 1). Results obtained for the solubility of CyA in water and 10% w/v PEG-6000 were 5.32 ± 0.10 and 25.51 ± 0.45 ($\mu\text{g/ml}$), respectively, and that correspond to 4.8-fold increased in its solubility by PEG-6000.

Dissolution Rate

Dissolution profiles of SD formulations, physical mixture and raw CyA are shown in Fig. 2a. The percentage of CyA dissolved after 2 h varied from 16.69% (SD-1) to 78.25% (SD-6). There was significant difference in the dissolution profile of raw CyA, physical mixture, and SD formulations. The depicted dissolution of raw CyA, physical mixture, and SD formulations rank order in terms of percentage of CyA dissolved in 2 h were SD formulations>physical mixture>raw CyA. There was progressive increase in the dissolution rate of

Fig. 1. Phase solubility of CyA in aqueous PEG-6000 at 25°C. Data are expressed as mean $\pm$ SD ($n=2$)

CyA by increasing PEG-6000 content as shown in the Fig. 2a. This data is in-line with previous finding of investigators that reported increase in the dissolution of hydrophobic drug irrespective whether drug is present as a physical mixture or molecularly dispersed as in the case of solid dispersion (34). The increase in the dissolution by increasing PEG-6000 loading in SD formulations could be explained by its hydrophilicity that causes wetting of drug particle and local enhancement of drug solubility at the diffusion layer surrounding the drug particles (34). The dissolution rate of SD formulation was superior compared to physical mixture that was prepared with the same weight ratio. This could be explained by the fact that in the solid dispersion, drug is present at the absolute minimum particle size that is molecular level. The fact that the intimate mixing of drug and carrier is at molecular degree during manufacturing step, provides more wetting of drug molecules with the dissolution medium and improved dissolution (34).

To further explain the effect of polymer/drug ratio on the dissolution characteristic, the extent of dissolution was expressed as dissolution efficiency (DE) which was described by Khan (35) in the following equation

$$DE = \frac{\int_0^t Y \times dt}{Y_{100} \times t} \times 100\%$$

Y is the drug release at time t .

Fig. 2. **a** Dissolution profile of CyA from SD formulation in 0.1 N HCl. **b** Linear relation among PEG-6000 loading in SD formulations, dissolution efficiency and percent CyA dissolved in 2 h

The value of DE varied from 4.39 (SD-1) to 21.05 (SD-6). Significance ($p < 0.05$) difference was observed among DE of SD-2, SD-3, SD-4, SD-5, and SD-6 when compared with raw CyA and PM ($p < 0.05$). Linear correlation was obtained between percentages of PEG-6000 or CyA in the SD formulations against cumulative percentage of CyA dissolved in 2 h and the DE values (Fig. 2b) as indicated by the value of correlation coefficient of 0.925 and 0.887, respectively.

The dissolution data were fitted into zero, first order, Higuchi model (36) to understand the mechanism of drug release from SD formulations. It was found that dissolution of CyA from SD formulations was best fitted in zero order release model as indicated by highest value of determination coefficient ' R^2 ' (0.957-0.995) followed by first order (0.916-0.972), and then Higuchi (0.874-0.964).

Scanning Electron Microscopy

Figure 3 showed the photomicrographs of pure components and SD formulations. CyA exist as spherical shape granules with greasy texture indicating its hydrophobicity while PEG-6000 exists as crystalline particles with smooth surface. SD formulations showed crystalline particle with smooth surface similar to PEG-6000. It was not possible to detect the presence of dissolved CyA particles in PEG-6000 matrix in the SD formulations. These photomicrographs suggest homogeneity of

Fig. 3. Scanning electron microscope photomicrograph of CyA, PEG-6000, SD-1 and SD-6 formulations

SD formulations and the formation of solid solution that might be responsible for the enhanced dissolution rate of CyA.

Differential Scanning Calorimetry

DSC experiment was performed to reveal physical state of drug and polymers in the SD formulation and to determine

possible drug-polymer interaction during the manufacturing step. It is known that CyA shows characteristic melting peak at 190°C for an orthorhombic crystal form and around 110°C for a tetragonal form (37). Thermogram of raw CyA did not show any peak indicating that drug used in this study was amorphous in nature while raw PEG-6000 (Fig. 4a) showed melting peak at 61.80°C with enthalpy of fusion (ΔH) of 194.1 J/g. The physical

Fig. 4. **a** Differential scanning calorimetry thermogram of CyA, PEG-6000, physical mixture, and SD formulations. **b** Linear relationship among PEG-6000 loading, melting peak temperature and enthalpy of fusion

Fig. 5. Fourier transform infrared spectra of CyA, PEG-6000, physical mixture and SD formulations

mixture and SD formulations showed broad melting peak of PEG-6000 and the value ranged from 57.52 to 61.79°C for SD-1 to SD-6, respectively. The reduction in the onset temperatures of melting, melting peak and enthalpy of fusion were found with increase of CyA content from 9.09% (SD-6) to 50% (SD-1) and linear correlation was obtained between percentage of PEG-6000, enthalpy of fusion and melting peak temperature as shown by *R* value of '0.990' and 0.951, respectively (Fig. 4b). These

results suggest that CyA causes distortion of crystal lattice of PEG-6000 and hence is responsible for decrease in onset temperature, melting peak, and enthalpy of fusion. Moreover, the thermogram of physical mixture and SD formulations did not show any extra endo/exothermic peak suggesting no chemical interaction between CyA and PEG-6000 and retaining physical states of drug and polymer, i.e., amorphous and crystalline, respectively.

Fig. 6. Powder X-ray diffractogram of CyA, PEG-6000, physical mixture and SD formulations

Fourier Transform Infrared Spectroscopy

FTIR study results are shown in the Fig. 5 spectra of pure CyA showed absorption band of N-H stretching vibration at $3,320\text{ cm}^{-1}$, C = O stretching vibration at $1,624\text{ cm}^{-1}$ and C-H stretching vibration at $2,954\text{ cm}^{-1}$. PEG 6000 showed a C-H stretching at $2,890\text{ cm}^{-1}$ of OC_2H_5 and a C-O stretching at $1,110\text{ cm}^{-1}$. Spectra of their physical mixture were almost addition spectra encompassing absorption band of CyA and PEG-6000. A decrease in the intensity of CyA peaks and increase in the intensity of PEG-6000 in the formulations from SD-1 to SD-6 were observed which correlate with increased loading of PEG-6000. However, there was no appearance of extra absorption band, absence of major shift in the peak positions, retention of the drug and polymer peaks, and the almost equivalent addition spectra (of CyA PEG 6000, respectively) for SD formulations and their physical mixtures

indicated the absence of interactions in the solid state between PEG 6000 and the CyA and there was no change in the physical state of drug and polymer.

Powder X-ray Diffraction

XRD diffractogram is shown in Fig. 6 CyA spectra revealed halo diffractogram indicating its amorphous nature and spectra of PEG-6000 showed sharp peak at 18.50 , 22.15 , and $24.86^\circ 2\theta$, characteristics of its crystallinity. The physical mixture showed the peak of PEG-6000. The SD formulations showed peaks corresponding to pure PEG-6000. The intensity of these peaks decreased with decrease in the PEG-6000 loading in the SD formulations from 90.09% (SD-6) to 50% (SD-1) possibly because of dilution with CyA or distortion of crystal lattice of PEG-6000. Furthermore, SD formulations diffractogram did not show

Fig. 7. **a** Original NIR spectra of SD formulations; **b** multiple scattering corrected NIR spectra of SD formulations

Fig. 8. PC1 and PC2 of **a** MSC-NIR spectra and **b** PXRD diffractogram of SD formulation computed by principal component analysis

any new peak or disappearance of peak suggesting no new polymorphs formation and no change in the physical states of drug and carrier that may have occurred during manufacturing step. In addition, the diffractogram pattern of PEG-6000 and SD systems were the same and superimposable, which ruled out the possibility of chemical interaction between CyA and PEG 6000. These results suggest that CyA is dispersed homogeneously in an amorphous state or dissolved into PEG-6000 (38).

Chemometric Evaluation

Near Infrared and Powder X-ray Spectroscopy

Scarce data is found in the literature for estimating drug and carrier in the solid dispersion using NIR and PXRD technique. Zidan *et al.* (7) successfully demonstrated application of non-destructive NIR technology in estimating dimyristyl phosphotyl choline from solid dispersion dosage form of CyA

Table II. Results of PLS Regression of MSC-NIR and PXRD Data for Calibration and Prediction of CyA and PEG-6000 from SD Formulations

Parameters	MSC-NIR				PXRD			
	CyA		PEG-6000		CyA		PEG-6000	
	Calibration	Prediction	Calibration	Prediction	Calibration	Prediction	Calibration	Prediction
Correlation	0.9946	0.9941	0.9946	0.9941	0.9977	0.9974	0.9977	0.9974
Offset	0.2114	0.1922	0.6544	0.7041	0.0887	0.3064	0.2806	0.9314
Slope	0.9891	0.9887	0.9891	0.9887	0.9953	0.9845	0.9953	0.9845
Root mean square of error	1.1669	1.2207	1.1669	1.2207	0.7802	0.8268	0.7802	0.8268
Standard error	1.1835	1.2377	1.1835	1.2377	0.7913	0.8384	0.7913	0.8384

and Shahroodi *et al.* (39) used PXRD to estimate new crystalline form of meloxicam in the solid dispersion formulation of meloxicam and PEG-4000. In this aspect, we attempted to investigate the non-destructive PAT sensors such as NIR and PXRD to estimate both CyA and PEG-6000 in the solid dispersion dosage forms. The major problem of these techniques is the variation in data during collection of spectrum. Dissimilar packing density of particles cause multiplicative variation in the recorded spectra which result from scattering effect causing difference in light path length (40). Other source of variation in NIR data is base line shift (41). Similarly, sources of variation in

PXRD data are insufficient homogeneity in the crystalline sample and preferential orientation of crystal in a sample holder (42).

Many data pretreatment methods have been proposed in the literature such as multiplicative scatter correction (MSC; 43), standard normal variate (SNV) transformation (44), first derivative, second derivative, second-derivative/logarithm (SDL; 45). The derivative and SDL methods can remove both parallel shifts and slope changes in the baseline, but at the same time enhance the noise in the spectra. MSC and SNV are proposed as methods for correcting multiplicative scatter effect in NIR spectra, but they can also be used to correct sloping background.

Fig. 9. **a** Loading vectors of the two PLS factors and MSC-NIR spectra of the individual components; **b** loading vectors of the two PLS factors and PXRD diffractogram of the individual components

Original NIR data of solid dispersion formulation is depicted in Fig. 7a showing the effect of changing CyA and PEG-6000 content. In order to build a robust calibration model, original NIR spectra was pretreated for MSC (MSC-NIR) to correct for baseline shift and multiplicative effect (Fig. 7b). The data was split into homogeneous calibration and prediction subsets, each consisting of 18 spectra. The principal component analysis was performed using two components on MSC-NIR and original PXRD data to study the data distribution. Figure 8a and b shows the scores of first principal component (PC1) versus second principal component (PC2) of calibration and validation data set of MSC-NIR and PXRD that represent approximately 100% and 76% of data variance. The model allow satisfactory clustering of SD formulation according to CyA and PEG-6000 loading and approximate value of PC1 of SD-1, SD-2, SD-3, SD-4, SD-5, and SD-6 of MSC-NIR and PXRD data were -0.45 , -0.25 , -0.05 , 0.10 , 0.25 , 0.40 , -0.45 , -1800 , -700 , -50 , 600 , $1,200$, and $1,000$, respectively. SD formulations could be arranged in increasing order of PC1 as $SD-1 < SD-2 < SD-3 < SD-4 < SD-5 < SD-6$. This arrangement of PC1 scores were in accordance with increased loading of PEG-6000 or conversely, deceased loading of CyA. This suggests that PC1 could represent either CyA or PEG-6000.

The partial least square (PLS) regression is one of the most commonly used method for modeling between multi-dependent variables and multi-independent variables in the

field of chemometrics and spectroscopy because of its simplicity to use, speed, relative good performance, and easy accessibility. PLS when applied to spectra, may provide identification and quantification of compounds in a second or higher order mixture, with little or no sample preparation. This may be achieved by developing a calibration model and correlating the instrumental responses with the property of interest (46). PLS regression was applied to MSC-NIR and original PXRD data with two PLS factor. PLS1 and PLS2 factor represent 97%, 2%, 74%, and 2% variance in MSC-NIR and PXRD data, respectively. The results of calibration and prediction of CyA and PEG-6000 are given in Table II. Linear correlation were obtained between actual and predicted value of CyA and PEG-6000 in both MSC-NIR and PXRD data as indicated by the value of correlation coefficient >0.9941 in all the cases. The model was equivalent in simultaneously predicting CyA and PEG-6000. The prediction ability of model is assessed through root mean square error of calibration and prediction (RMSEC and RMSEP). RMSEC and RMSEP of CyA and PEG-6000 from PXRD data were smaller than MSC-NIR data indicating PXRD is more accurate in assessing CyA and PEG-6000 in SD formulations.

Accuracy measure the closeness between reported and true value and accuracy of the calibration curve was evaluated based on validation data set using mean bias and mean accuracy and determined by following equation (47)

Fig. 10. PLS images and associated histograms of SD formulations

Table III. Results of Histogram Distributions from PLS Score Images of SD Formulations

Formulation	CyA			PEG-6000		
	Number of pixel	Mean±SD	Skewness	Number of pixel	Mean±SD	Skewness
SD-1	2,482	0.752±0.098	0.582	2479	0.328±0.041	0.469
SD-2	2,470	0.539±0.069	0.637	2482	0.419±0.043	0.305
SD-3	2,456	0.378±0.059	0.548	2484	0.571±0.052	0.428
SD-4	2,475	0.236±0.039	0.344	2482	0.607±0.048	0.292
SD-5	2,477	0.185±0.035	0.239	2484	0.651±0.044	0.356
SD-6	2,487	0.069±0.030	0.006	2492	0.653±0.041	0.233

$$B_m = \frac{\sum_{i=1}^n X_c - X_t}{n} \times 100$$

$$A_m = \frac{\sum_{i=1}^n \frac{|X_c - X_t|}{X_t}}{n} \times 100$$

where, B_m is the percentage mean bias, A_m is the percentage mean accuracy, X_c is the predicted drug/polymer loading value, X_t is actual drug/polymer loading and n is number of experiments. The mean accuracy for CyA and PEG-6000 prediction from MSC-NIR data were 5.22% and 5.27%, respectively, and mean bias is zero indicating that model is equally accurate in predicting the CyA and PEG-6000 loading in the SD formulations. Similarly, the mean bias and accuracy for CyA and PEG-6000 prediction from PXRD data were -0.27%, 0.28%, 5.35%, and 2.10%, respectively, suggesting that model is more accurate in predicting the loading of PEG-6000 than CyA. PXRD data is more powerful in predicting PEG-6000 loading than MSC-NIR as it indicated lower value of mean accuracy.

Each PLS factor describe certain portion of overall spectra and explain physical and chemical information in the model. This information is given as percentage explained variance for the physical and chemical part of the model. In order to understand these PLS factor, loading vectors for these two PLS factors of MSC-NIR and PXRD data were compared with respective spectra of MSC-NIR and PXRD of CyA and PEG-6000 (Fig. 9a, b). In case of MSC-NIR,

PLS1 loading vector showed positive peaks at 1,260, 1,758, 2,316, and 2,410 nm, and negative peaks at 1,372, 1,696, 1,944, 2,058, 2,180, 2,272, and 2,436 nm which were attributed to PEG-6000. PLS2 loading vectors showed positive peak at 1,768, 1,894, 2,064, and 2,174 nm and negative peaks at 1,328, 1,620, 1,768, 2,310, and 2,468 nm which were attributed to CyA. Likewise, PLS 1 loading vector of PXRD was superimposable to diffractogram of PEG-6000 and showed characteristics peak at 18.50, 22.15 and 24.86° 2 θ indicating PLS1 represent PEG-6000. PLS2 of PXRD did not show any peak similar to halo diffractogram of CyA which could be attributed to it

Near Infrared Chemical Imaging

NIR-CI differs from classical NIR spectroscopy in that it record spatial feature beside recording spectral character of a sample. The measurement of spatial feature helps to identify chemical species inside the sample and map their distribution. This technique was applied to SD formulations to probe the distribution of CyA and PEG-6000 by generating image contrast based on the absorbance at a particular wavelength for all pixels in the image. PLS images score of SD formulations concatenated according to CyA and PEG-6000 concentration from prebuilt PLS library (Fig. 10). The histogram representing the distribution of CyA and PEG-6000 in the SD formulations can correspond to their spatial distribution. PLS images and showed the homogenous distribution of CyA and PEG-6000 in SD formulations. The width of histogram of SD formulation can be used to quantitate CyA and PEG-6000. The statistics of histogram is shown in Table III and smaller value of skew indicates symmetrical

Fig. 11. Quantile-quantile plots for the prediction of **a** CyA loading and **b** PEG-6000 loading using PLS score images' pixels intensities

distribution. The formulation could be arranged according to mean representing CyA and PEG-6000 loading from their PLS images as well as according to intensity of pixels representing CyA; SD-1>SD-2>SD-3>SD-4.SD-5>SD-6 and order would be reversed if arranged according to PEG-6000. This order of arrangement is in agreement with actual CyA and PEG-6000 loading in SD formulations. The quantile-quantile plot (Fig. 11) between mean representing CyA and PEG-6000 from PLS images and their actual loading produced correlation coefficient of 0.9883 and 0.9837 for CyA and PEG-6000, respectively, revealing the usefulness of nondestructive method of estimating of component of SD formulations.

CONCLUSION

A solid dispersion formulation of CyA using PEG-6000 as matrix was developed and characterized fully by novel methodologies. Developed formulation revealed greatly enhanced dissolution rate of CyA. The increase dissolution rate is thought to be the formation of solid solution as revealed by SEM and DSC studies. The improved dissolution of CyA in solid dosage form might increase its bioavailability and may be applicable to other hydrophobic drug. NIR-CI indicates uniform distribution of CyA inside the matrix. We successfully developed robust and reproducible models for the estimation of CyA and PEG-6000 using PLS regression technique to NIR and PXRD data. The model offer advantage of instantaneous and simultaneous quantization of both component of SD formulation.

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