Improvement on solubility of fluticasone propionate with cyclodextrins by mechanochemical activation

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Abstract: To enhance the solubility and *in vitro* dissolution of fluticasone propionate (FP), a novel approach was developed with mechanochemical treatment. The order of solubilizing effect of β-CD derivatives was observed as HP-β-CD-SBE-β-CD-β-CD-HE-β-CD, consequently, HP-β-CD showed the highest stability constant. To further improve FP solubility, FP and HP-β-CD were grinded using a roll mill, the optimal conditions, determined through single factor experiments, were as follows: rotation frequency of 60 Hz; milling time of 6h. mass ratio of 1: 7. In comparison with pure FP, a 280-fold increase in solubility and a 2.15-fold higher dissolution rate of ground mixture was obtained. The characterization of FP and HP-β-CD complexes had been analyzed by scanning electron microscopy (SEM), differential scanning calorimetry (DSC), powder X-ray diffractometry (PXRD) and fourier transform infrared spectroscopy (FT-IR). The results suggested that the interaction between FP and HP-β-CD was strengthened and an amorphous ground mixture was gained. After stored for 60 days, the ground mixtures were stable both chemically and physically.

Keywords: Mechanochemistry, Fluticasone propionate, 2-Hydroxypropyl-β-cyclodextrin, Solubility, Physicochemical properties

INTRODUCTION

In pulmonary drug delivery, active pharmaceutical ingredients with poor solubility in water tend to be prepared as dry powder inhalation and suspension. The typical size of drug particles here is generally below 5 μm, which can penetrate into the lower lungs (Chrystyn, 2001). Due to small particle size and high relative surface area of those drugs, they can easily form agglomerates in dry powder inhalation (Kendall and Stainton, 2001). Meanwhile, the disadvantages of suspensions, such as their tendency to settle over time, require shaking before using, in the purpose to redisperse the drug. However, even with "optimal" shaking, formulation issues such as caking, particle size and agglomeration, may potentially lead to a lack of dose uniformity (Stringer W and Bryant R, 2010), and result in bioavailability reduction. To enhance solubility and dissolution rate of insoluble APIs, liquid inhalation formulation can be a useful choice to solve those aforementioned issues.

Various techniques have been applied to enhance the dissolution rate of insoluble APIs, containing preparation of solid dispersions (Vasconcelos *et al*., 2007; Tiwari *et al*., 2009), use of cocrystallization (Shiraki *et al*., 2008), hot-melt extrusion technology (Albers, 2008; Jagtap *et al*., 2012), spray-freezing into liquid particle engineering technology (Rogers *et al*., 2003; Hu *et al*., 2002), and the formation of molecular complexes (Sanofi-Aventis, 2009). It has been well documented that, owing to lipophilic inner cavities and hydrophilic outer surfaces, cyclodextrins (CDs), α-, β and γ-cyclodextrin, could be regarded as useful pharmaceutical excipients to form a stable inclusion complex with hydrophilic molecules of a suitable size to enhance the solubility of APIs (Challa *et al*., 2003; Lofts son and Duchêne, 2007). Generally, the resulting inclusion complexes offer various physicochemical advantages over the free drug, involving increasing solubility and dissolution rate, decreasing volatility, altering release rates, and improving stability, etc (Roger and Valentino, 1996). 2-hydroxypropyl-β-cyclodextrin, with enhanced aqueous solubility and may be more toxicologically benign, is an alternative excipient to α-, β and γ-cyclodextrin (Gould and Scot, 2005). Mechanochemical treatment is also seen as a popular technique in the pharmaceutical field. Basic reasons for this rely on the possibility of enhancing the bioavailability of drugs with poor water solubility and the possibility of producing pharmaceutical formulations without the usage of organic solvents (whose elimination can be expensive, hard and can alter the activated status of drug) (Colombo *et al*., 2009).

Fluticasone propionate (fig. 1), a locally active glucocorticoid, when delivered by oral or intranasal routes, has no noticeable systemic side effects (Harding, 2009). The market available products of FP were dry powder inhalation (DPI), nasal spray, oral inhaled aerosol and ointment. However, aqueous solubility of pure FP at 25°C was detected to be only 0.14μg/mL (Lip worth and Jackson, 2000), no liquid formulation had been developed so far.

The purpose of this study was to evaluate the enhancement effect of HP-β-CD on FP solubility and *in*

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vitro dissolution utilizing the milling technique. Possible interactions between HP-β-CD and FP were investigated by SEM, DSC, PXRD and FT-IR. The stability of the ground mixture was inspected by FT-IR and with dissolution tests.

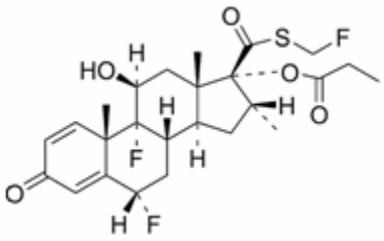

Fig. 1: Structure of fluticasone propionate

MATERIALS AND METHODS

Materials

FP (purity 99%) was kindly supplied by Zhejiang Taizhou Junye Pharmaceutical Co., Ltd. (Taizhou, Zhejiang). 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), 2-hydroxyethyl-β-cyclodextrin (HE-β-CD), sulfobutylether-β-cyclodextrin (SBE-β-CD) and β-cyclodextrin (β-CD) were purchased from Qianhui Fine Chemical Co., Ltd. (Zibo, Shan dong). Purified water was prepared by a Nano-purification system obtained from Thermo, USA. All commercially available solvents and reagents were used without further purification.

Methods

Preparation of ground mixtures and physical mixtures

Physical mixtures (PM) of FP were obtained with HP-β-CD at a ratio of 1:7 (w/w), using a spatula and a mortar until homogeneity of the mixtures was got. The mixtures were then sieved by a 200-mesh siever for further experiments.

1g FP and 7g HP-β-CD were loaded into a poly-urethane jar of 500mL with steel balls diameter of 22 mm in it, using a roll mill (QM-2, Sanxing instruments, Xiangtan) and grinded for 6h at 700 rpm to obtain ground mixtures (GM). All powder products were sieved by a 200-mesh sieve and stored in a vacuum desiccator over silica gel preserved from light at room temperature.

HPLC analysis of FP

The concentration of FP in the medium was tested using an Agilent HPLC system (series 1200, Agilent Technologies, Germany) equipped with Agilent ZORBAX SB-C18 reversed-phase column (250 mm × 4.6 mm, 5µm). A mobile phase of methanol, acetonitrile and buffer with 1.2g/L of monobasic ammonium phosphate, a pH of 3.5 adjusted with phosphoric acid, (50:15:35) was used at 1.5mL/min flow rate and 40°C column temperature. The UV detector was set at 239nm to analyze the column effluent. The presence of CD derivatives did not interfere with the analysis of FP. The solution was filtered by a 0.22µm membrane filter (Millipore Corp.).

Preliminary studies

The phase solubility studies (Higuchi and Connors, 1965) were performed as follow:

Approximately 50mg of FP was added into 10mL aqueous solution containing increasing concentrations of β-CD, HP-β-CD, HE-β-CD and SBE-β-CD (0.035, 0.007, 0.014, 0.028, 0.056mol/L). The suspensions were shaken at 25°C for 24h, then filtered by a 0.22µm membrane filter and analyzed by HPLC. The stability constant K_c was calculated by using the following equation Eq. 1, with the assumption of 1:1 stoichiometry from phase solubility diagrams:

$$Kc = slope/S(1 - slope) \quad (1)$$

Where, slope is gained from the linear relationship between the concentration of drug (y-axis) and the concentration of cyclodextrin (x-axis), Sis the intrinsic solubility (M, 25°C) in absence of CDs.

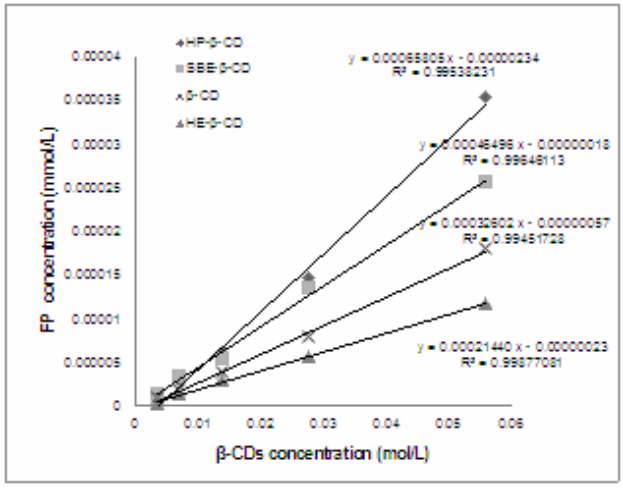

Fig. 2: Phase solubility diagram of FP/ β-CDs system at 25°C

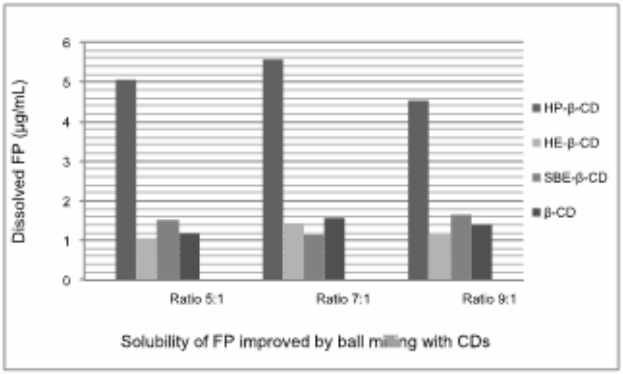

Fig. 3: Solubility of FP improved by ball milling with CDs for 4h at 45Hz

In addition to the stability constants, complexation efficiency (CE) of each cyclodextrin with FP was calculated (Lofts son *et al*., 2005). CE is defined asthe solubilizing efficiency of cyclodextrin and is equal to the ratio (complex to free cyclodextrin concentration), see Eq. 2:

$$CE = [D/CD]/[CD] = Slope/(1 - Slope) \quad (2)$$

Solubility test

Excess amount of the samples (50 mg physical mixtures or ground mixtures) was added into 10mL purified water. Then the suspensions were shaken using a Thermo Scientific MaxQ 4000 bench top orbital shaker at 37°C for 24h, after that 5mL sample was withdrawn and filtered by a 0.22µm filter membrane. Initial 2mL of each sample was discarded, while the rest 3mL was analyzed by HPLC.

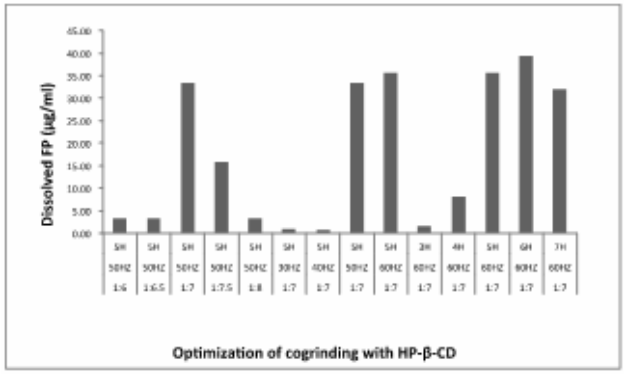

Fig. 4: Optimization of cogrinding with HP-β-CD

In vitro dissolution study

Dissolution tests of the physical/ground mixtures were conducted on an HTY-EU802 rotating paddle apparatus (Hangzhou Tailin Bioengineering Equipments Co., Ltd., China). In 250mL purified water with 16mg physical/ ground mixtures at 37±1°C, at 100rpm rotation speed. 5mL sample was withdrawn at 5, 10, 15, 20, 30, 45, 60, 90min and an equal volume of deionized water was added after each sample was withdrawn, respectively. After filtration by a 0.22µm filter membrane, initial 2mL of each sample was discarded, while the rest 3mL was analyzed by HPLC.

Stability study

A stability test was performed on the ground mixtures (1:7 w/w), which were stored in a desiccator at room temperature and preserved from light for 60 days. The samples were then further analyzed by dissolution tests and FT-IR.

Characterization of the co-ground products

SEM

The scanning electron micrographs of the samples were observed on a Hitachi S-4700 SEM (Hitachi Ltd., Japan). Samples were fixed by adhesive tape, sputtered with gold and then examined under high vacuum condition at an accelerating voltage of 15.0 kV (100, 500×magnification).

DSC

Thermal analysis was carried out on a Mettler Q100 instrument (TA Corporation, USA) equipped with a differential scanning calorimeter. The procedure was as follows: take about 4 mg sample and place it in an aluminum pan with sealed lid and crimp, then heat at 10°C/min rate from 50°C to 300°C, under a 10mL/min s nitrogen purge stream.

PXRD

The PXRD patterns were performed on an X' Pert PRO diffract meter with an X' Celerator (PANalytical Corporation, Holland). Samples were analyzed at a voltage of 40 kV, a current of 40mA and a scanning rate of 0.2° min^{-1} over a 2θ range of 5-45°.

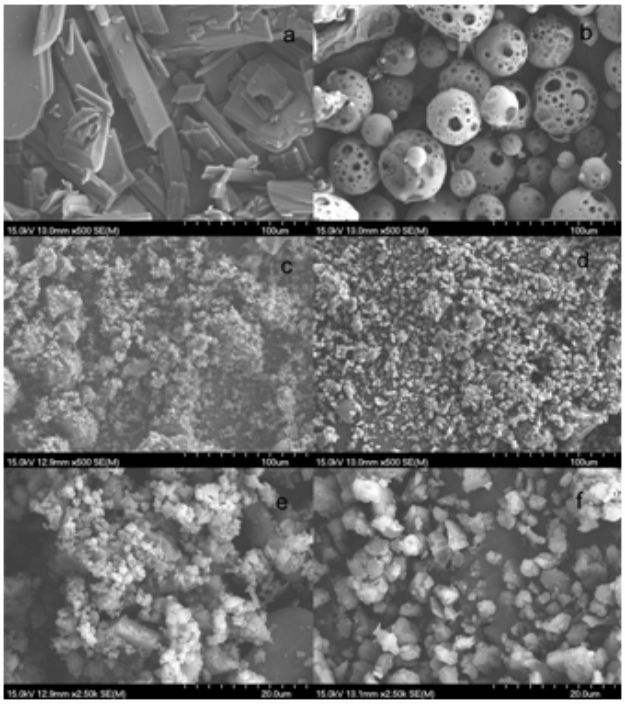

Fig. 5: Scanning electron micrographs (500× magnification) of (a) FP, (b) HP-β-CD, (c) physical mixture of FP and HP-β-CD (1:7 w/w), (d) ground mixture of FP and HP-β-CD (1:7 w/w), high magnification (2500× magnification) micrographs of a selected region (e) from c, (f) from d.

FT-IR Spectroscopy

Infrared spectra of the samples were obtained on a Niclolet Avatar 370 apparatus (Thermo Niclolet Corporation, USA). Each sample (about 1% w/w) was mixed with KBr powder and compressed to a 13 mm disc at 30MPa compression force for 60 sec. The FT-IR spectra were composed of 64 scans performed in range of 400-4000 cm^{-1} with 2cm^{-1} resolution.

RESULTS

Preliminary studies

To examine the compatibility of parent molecule β-CD and its substituted derivatives (HP-β-CD, HE-β-CD and SBE-β-CD) with FP, a phase solubility technique was introduced to investigate their effect on the solubility of FP (fig. 2). The phase solubility profile of the FP and β-CDs system illustrated that the solubility of FP increased linearly to β-CDs concentrations, According to Higuchi and Connors, as the slopes were less than 1, the curve of the FP and β-CDs should be classified as A_L-type, implying the formation of a 1:1 stoichiometric water-soluble molecular complex. The stability constants K_C (Table 1) for FP with HP-β-CD, SBE-β-CD, β-CD and HE-β-CD were determined to be 2351.7, 1661.3, 1164.7 and 765.9 M^{-1}, respectively. It has been argued that K_C must be greater than $10^5 M^{-1}$to have a positive effect on the drug pharmacokinetics after parenteral administration (Lofts son and Brewster, 2011). The small value of CE indicated that the interactions between β-CDs and FP were very weak, thereby β-CDs and FP systems were in need of new techniques to reinforce the molecular interaction.

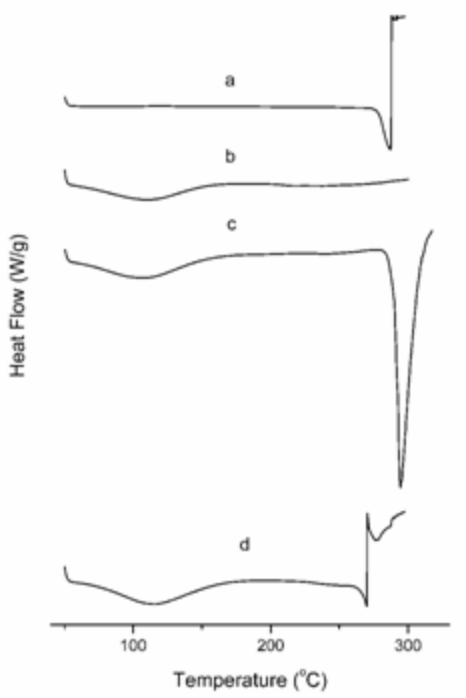

Fig. 6: Differential scanning calorimetrythermograms of (a) FP, (b) HP-β-CD, (c) physical mixture of FP and HP-β-CD (1:7 w/w), (d) ground mixture of FP and HP-β-CD (1:7 w/w)

Co grinding of FP with β-cyclodextrins in solid state

In an attempt to further improve FP solubility, the co-grinding technique was performed on FP and β-cyclodextrin and its derivatives in solid state. Based on the previous study, FP and the β-cyclodextrins should be weighed and mixed at an approximately 1:3 weight ratio corresponding to 1:1 molar ratio. As excess amounts of dextrin were required for solid state cogrinding (Fukami *et al*., 2006), drug/β-CDs weight ratios of 1:5, 1:7 and 1:9 were cog round for 4h at 45Hz (fig. 3). Weight ratio of 1:7 of HP-β-CD, HE-β-CD and β-CD had a slight superiority compared to 1:5 and 1:9, with an exception with SBE-β-CD. It was clearly evident that, with a propensity similar to the phase solubility diagram, HP-β-CD was more suitable for the enhancement of FP solubility. However, there was no notable difference between other β-CDs on the solubilizing effect. And considering the important role of powder properties in ball milling process, further investigation of preparation conditions of FP and HP-β-CD mixture was needed.

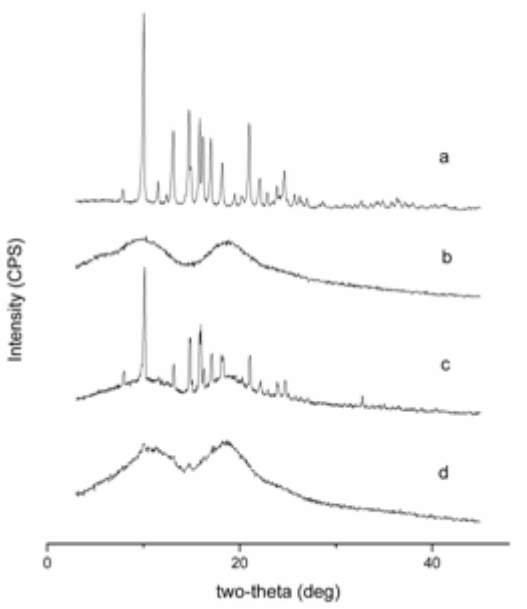

Fig. 7 Powder X-ray diffract grams of (a) FP, (b) HP-β-CD, (c) physical mixture of FP and HP-β-CD (1:7 w/w), (d) ground mixture of FP and HP-β-CD (1:7 w/w)

Optimization of co grinding with HP-β-CD

Through single factor experiments, several factors, mill time, and rotation frequency, as long as ratios of HP-β-CD, were explored to optimize preparation conditions (fig4). Interestingly, after grinded for 6 h at 60Hz with 1:7 w/w of FP/ HP-β-CD, the concentration of FP increased drastically up to about 40μg/mL. According to a previous study (Chen *et al*., 2008), FP solubility could be improved with HP-β-CD at the presence of ethanol or acetone; however, the concentration of FP was estimated to be 4.17 and 8.37μg/mL in our lab. In this case, mechanochemical activation showed its superiority in the enhancement of complexation between FP and HP-β-CD.

In all three factors of cogrinding, rotation frequency had a causative role. When it reached the maximum value (60 Hz) of the ball mill, the optimal value of solubilizing

effect was attained. The effect of mechanical energy remarkably occurred when the grinding accumulated to the certain degree, therefore rotation frequency of 50Hz and 40Hz had a marked impact on the enhancement of complexation. Extending milling time could not favor the transfer of complexation, on the contrary, the existed complexation could be destroyed. Weight ratio of 1:7 would be enough to gain a reasonable efficiency.

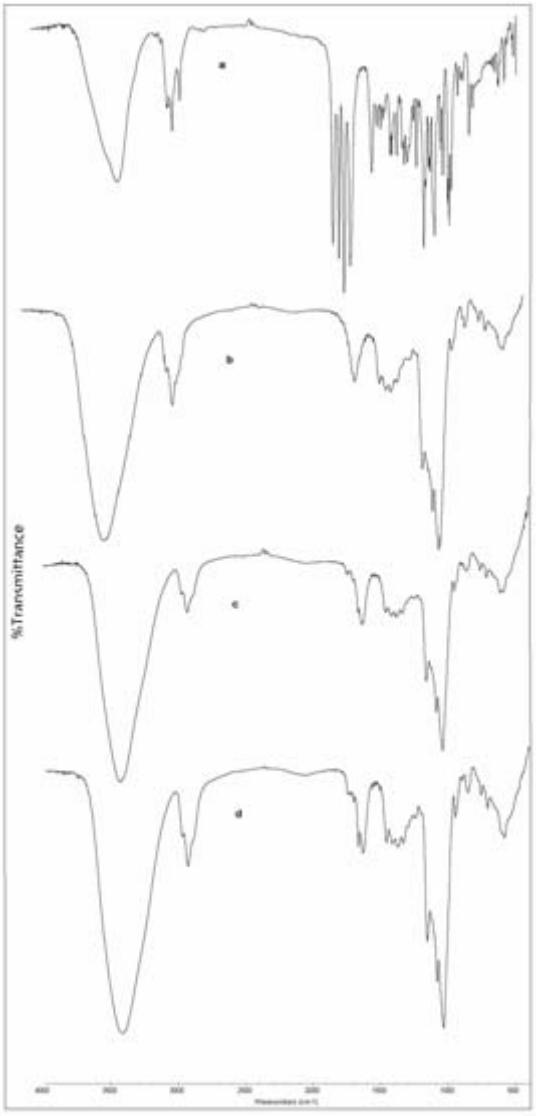

Fig. 8: Infrared spectra of (a) FP, (b) HP-β-CD, (c) physical mixture of FP and HP-β-CD (1:7 w/w), (d) ground mixture of FP and HP-β-CD (1:7 w/w)

SEM

The scanning electron micrographs of FP (fig. 5a), HP-β-CD (fig. 5b), the physical mixture (fig. 5c, e) and the ground mixture (fig. 5d, f) were present in fig. 5. Respectively, fig. 5e and f were the high magnification micrographs of a selected region from the physical mixture and the ground mixtures. FP particles appeared as irregular-shaped crystals meanwhile HP-β-CD existed as a sphere with numerous pores. Compared with fig. 5c and e, a relatively compact and homogeneous structure was obtained in 4d and f, whose size was smaller as well. Instead of the original morphology of both components, irregular particles were detected.

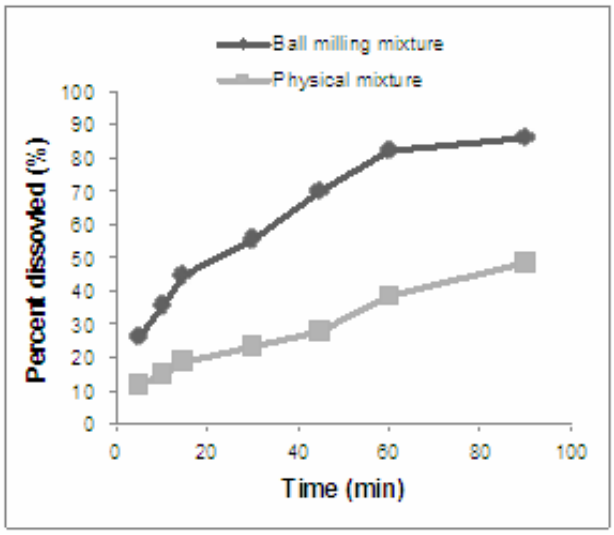

Fig. 9: Dissolution profiles of physical mixture of FP and HP-β-CD (1:7 w/w); ground mixture of FP and HP-β-CD (1:7 w/w)

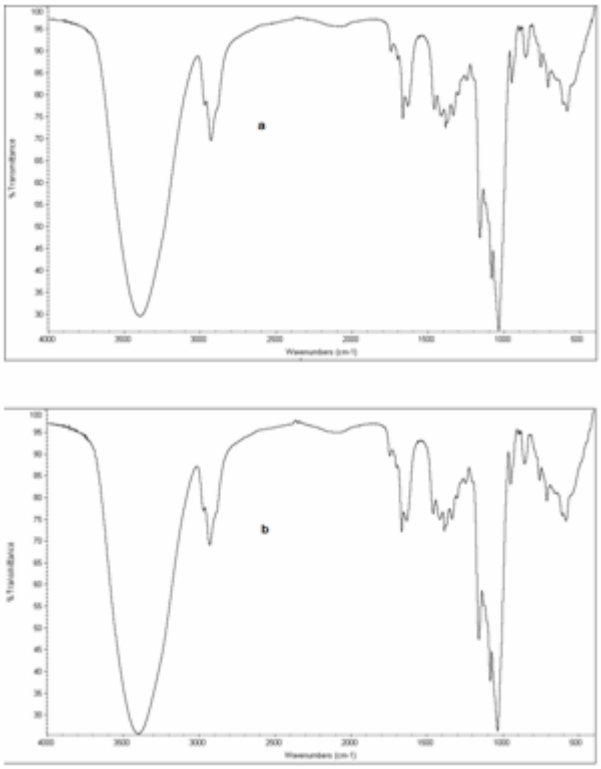

Fig. 10: Infrared spectra of (a) ground mixture of FP and HP-β-CD (1:7 w/w): day 0; (b) ground mixture of FP and HP-β-CD (1:7 w/w): day 60

DSC

DSC enabled quantitative detection of all processes, in which energy was demanded or generated. To identity probable interactions between FP and HP-β-CD in the solid state, DSC thermo grams of FP, HP-β-CD, the physical mixture and the ground mixture were recorded (fig. 6). The endothermic peak in the thermo gram of FP (fig. 6a) was assigned to the fusion of FP crystals. The curve of HP-β-CD (fig. 6b) only exhibited a very broad endothermal phenomenon due to the dehydration process of the cyclodextrin (Abd EL-Gawad *et al*., 2012), which emerged in the physical mixture (fig. 6c) and the ground mixture (fig. 6d) as well. The presence of sharp FP melting peak was still observed in the physical mixture (fig. 6c), on the contrary, in the thermo gram of the ground mixture (fig. 6d), the drug-melting endotherm had slightly moved from its original position 273.8-287.3^{o}C to 260.3-270.0^{o}C and did not appear as a sharp peak.

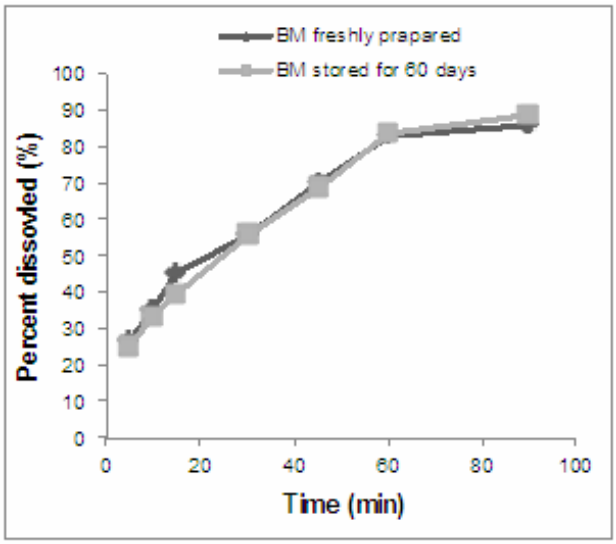

Fig. 11: Dissolution profiles of ground mixture of FP and HP-β-CD (1:7 w/w): day 0; ground mixture of FP and HP-β-CD (1:7 w/w): day 60

PXRD

The characteristic diffraction peaks of fig. a revealed that FP existed in a natural crystalline form and HP-β-CD in an amorphous state (fig. 7b). The physical mixture pattern was only superposition of FP and HP-β-CD pattern (fig. 7c). Otherwise, the ground mixture (fig. 7d) was similar to HP-β-CD without any crystallinity, meaning that an amorphous solid mixture was formed via ball milling.

FT-IR

Enhancement of IR absorption intensity of characteristic band might be related to possible drug-cyclodextrin interaction like the appearance of intermolecular hydrogen bonds. The infrared spectra of FP (fig. 8a) and HP-β-CD (fig. 8b) were in according with the previous reports (Ali *et al*., 2009; Zhu *et al*., 2012). The spectrum of physical mixture (fig. 8c) had no remarkable difference with the one of HP-β-CD, which suggested that no demonstrable interaction was existed between FP and HP-β-CD. In contrast, the presence of a characteristic band at 1666 cm^{-1} in IR spectrum of the ground mixture (fig. 8d) was assigned to C=O on the steroidal ring of FP. It showed that the carbonyl group of FP interacted with hydroxyl groups of HP-β-CD, resulting in the formation of intermolecular hydrogen bonds.

In vitro dissolution studies

In the dissolution profiles depicted in fig. 9, drug release of FP in the physical mixture reached 38.4% at 60min owing to the solubilizing property of HP-β-CD, while solubility of pure FP is too low to be detected by HPLC. A remarkable increase of the ground mixture was observed and its drug release amount was found to be 82.6% at 60min, 2.15-fold higher than the physical mixture. The improved dissolution could be attributed to a transformation of crystalline drug to amorphous, its complexation with the surface of the carrier and improved wet ability (Nicolescu *et al*., 2010).

Stability studies

No significant difference was founding the FT-IR spectra (fig. 10) and the dissolution profile (fig. 11) of ground mixtures between the samples stored for 60 day and freshly prepared. Thus, the ground mixtures exhibited good stability both chemically and physically.

DISCUSSION

It was speculated by SEM that mechanical shear stress forced FP to disperse homogeneously into HP-β-CD accompanied with size reduction. Meanwhile, the DSC demonstrated drug amorphization and enhancement of the capability of encapsuling the guest molecular FP of HP-β-CD with mechanochemical treatment. The result conformed to PXRD, which clearly showed the formation of amorphous solid mixtures. This result of DSC also agreed well with that of IR, which suggested that mechanochemical effect induced the interaction-probably

Table 1: Relationship between stability constant and complexation efficiency

Cyclodextrin	Slope	Intercept (M)	Corr.	K_c	CE
HP-β-CD	6.58E-04	-2.34E-06	0.995	2351.73	6.58E-04
SBE-β-CD	4.65E-04	-1.8E-07	0.996	1661.34	4.65E-04
β-CD	3.26E-04	-5.7E-07	0.995	1164.74	3.26E-04
HE-β-CD	2.14E-04	-2.3E-07	0.999	765.88	2.14E-04

intermolecular hydrogen bonds between FP and HP-β-CD molecules during the co grinding process.

CONCLUSIONS

To summarize, mechanochemical activation to the FP and HP-β-CD complex was a simple, efficient and environmentally friendly process. Both the solubility and dissolution rate were remarkably improved in contract with pure FP. During the process, beside with size reduction and homogeneous distribution owing to mechanical shear stress, the interaction between FP and HP-β-CD was strengthened and an amorphous ground mixture was gained. It exhibited good stability in 60 days. Mechanochemical activation would be effective for the solubilization of poorly water-soluble APIs.

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