Physico-chemical Characterization and *In Vitro* Evaluation of Biopharmaceutics Classification System Class II Drug Febuxostat

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Abstract

Aim: Febuxostat (FBX) is a non-purine selective inhibitor of xanthine oxidase/xanthine reductase. It belongs to Biopharmaceutics Classification System Class II with low solubility and high permeability. Because of low solubility, the bioavailability of the drug is hampered, food also interferes with the absorption of the drug and decreases the C_{max} by 38-49%. The bioavailability of a drug is a function of dissolution rate of the drug which is controlled by the surface area of the drug. In the category of poorly soluble drugs, the change in surface area of the drug will show considerable changes in the solubility and dissolution of the drug. **Materials and Methods:** In the present study, the attempts were made to improve the bioavailability of FBX by solid dispersions technique by employing poly vinyl pyrrolidone K30 (PVP K30) and hydroxy propyl methyl cellulose E15 (HPMC E15) as carrier molecules. Different ratios on weight basis were prepared, *viz*., 1:1,1:2,1:3,2:1 with PVP and 1:1, 1:2, 1:3,1:4 with HPMC were prepared. **Results and Discussion:** These preparations were characterized in liquid state by phase solubility studies and in solid state by differential scanning calorimetry, Fourier transform infrared spectroscopy, Powdered X-ray diffraction studies, and scanning electron microscopy. The aqueous solubility of FBX is favored by the presence of both the carriers. Solid state characterization indicated that FBX was present as fine amorphous form in the carrier polymeric molecules. **Conclusion:** In contrast to the solution rate of pure FBX, the dissolution of drug in carriers considerably improved the dissolution rate, this can be attributed to the increased wettability and dispersibility as well as decreased crystallinity and increased amorphous fraction of the drug.

Key words: Drug release studies, febuxostat, hydroxy propyl methyl cellulose E15, phase solubility, poly vinyl pyrrolidone K30, solid dispersions

INTRODUCTION

Febuxostat (FBX) is a non-purine selective inhibitor of xanthine oxidase/xanthine reductase. The chemical name of FEX is 2-[3-cyano-4-(2-methyl propoxy) phenyl]-4-methylthiazole-5-carboxylic acid. The molecular structure was given in Figure 1

It is indicated for the long-term management of hyperuricemia in patients with gout. It belongs to Biopharmaceutics Classification System (BCS) Class II with low solubility and high permeability. Because of low solubility, the bioavailability of the drug is hampered and it also undergoes enzymatic degradation in the intestine as well as in liver. Food interferes with the absorption of the drug and decreases the C_{max} by 38-49%. Thus, it has undesirable dissolution profile and poor bioavailability following oral administration. Poorly water-soluble drugs present significant challenges during dosage form designing due to their inadequate solubilization in digestive fluids.

Most of the newly discovered drugs receive little or no aqueous solubility as a challenge for the successful formulation development[1] and commercialization of new drugs in the pharmaceutical industry. The bioavailability of a drug is a function of dissolution rate of the drug which is controlled by the surface area of the drug. In the category of poorly soluble drugs, the change in surface area of the drug will show considerable changes in the solubility and dissolution of the drug. Micronization, nanosuspensions, polymorphs, complexation, solid dispersions, prodrugs, and salt formation can be employed to increase dissolution rate.[2] Among the various techniques of improving the surface area thus enhancing the solubility of drug substances, solid dispersion technique stands in the first row. Chiou and Riegelman define solid dispersions as "the dispersion of one or more active ingredients in an inert carrier matrix at solid state." Solid dispersions can be prepared by different methods using different water-soluble carriers. These solid systems[3] exhibit enhanced solubility and dissolution rate compared to the plain drug that may be attributed to the molecular/colloidal dispersion of the drug in mixture, the absence of aggregation of drug particles, particle size reduction, improved wettability and dispersibility, and polymeric transformation of drug crystals. Enhancement of solubility[4] may contribute directly to the improved bioavailability of poorly water-soluble drugs.

In the current research investigation,[5] trials were made to improve the dissolution rate of FBX by employing the solid dispersion technique. An attempt was made to improve the dissolution properties of FBX by preparing free-flowing solid dispersions[6] using poly vinyl pyrrolidone K30 (PVP K30) and hydroxy propyl methyl cellulose E15 (HPMC E15) as carrier systems. The prepared solid dispersions were characterized by Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), and X-ray diffraction study (XRD).

MATERIALS AND METHODS

The solid dispersions preparation required the following chemicals: FBX was generously donated by Sun Pharma Mumbai; PVP K30 and HPMC E15 are procured from Loba Chemicals; all other chemicals used in the study are of pharmacopeial grade.

Phase solubility studies

The phase solubility studies were conducted using a simple technique, which involves the addition of excess amount of FBX, i.e., 100 mg in 25 ml of water containing different weights of solubilizing agents, i.e. PVP K30 and HPMC E15. The solutions were[7] sonicated for 1 h at room temperature and maintained at 25°C for 48 h on an orbital shaker Orchid, Mumbai. The dispersions were filtered[1] through a 0.22 μm nylon membrane filter. The filtrates were suitably diluted

and analyzed spectrophotometrically (ultraviolet-visible [UV-Vis] spectrophotometer, Perkin-Elmer), for the dissolved drug at 318 nm. All trials were performed in triplicate.

Preparation of solid dispersions

The solid dispersions of FBX employing PVP K30 and HPMC E15 were prepared[8] using a simple method of solvent evaporation technique. The prepared solid dispersions were compared with pure FBX and the physical mixtures of drug and polymer.

Solvent evaporation method

Solid dispersions of the drug FBX in PVP K30 and HPMC E15 in different weight ratios (1:1, 2:1, 1:2, 3:1 of PVP denoted as FBPVP 1:1, 2:1, 1:2, 3:1 and 1:1, 1:2, 1:3, 1:4 of HPMC E15 denoted as FBMC 1:1, 1:2, 1:3, 1:4) were prepared by employing solvent evaporation method.[9-12] The required amount of polymer (PVP K30 or HPMC E15) was weighed and mixed with sufficient quantity[13-15] of the solvent methanol to obtain a clear solution. In this solution, the weighed quantity of drug was dispersed, and the solution was triturated continuously till the entire solvent was evaporated. Then, the mixture was further air dried for 24 h to completely remove the solvent and pulverized and sifted through sieve No. 60 to obtain the solid dispersions. Thus, prepared solid dispersions were stored in a desiccator until further evaluation.

Characterization of solid dispersions

FTIR spectroscopy

A Perkin-Elmer Spectrum Rx 1 FTIR spectrometer was used for infrared analysis. Samples were prepared by KBr disc method (2 mg sample in 100 mg KBr) and examined in the transmission mode. A resolution of 4/cm was used and 64 scans were co-added for each spectrum over a frequency range of 4000-450/cm. The software used for the data analysis was Perkin-Elmer Spectra MAX.

DSC analysis

Thermal analyses of prepared solid dispersions were performed in a Mettler Toledo, Switzerland, differential scanning calorimeter with a thermal analysis controller. Samples were accurately weighed (5-8 mg) into aluminum pans and thermograms obtained at a heating rate of 10°C/min over a temperature range of 25-210°C.

Powder X-ray diffraction (PXRD)

Diffraction patterns were obtained on a Bruker AXS D8 Discover Instrument (USA) modified for step-scan operations. Ni-filtered CuKa radiation was produced with a Philips PW 1130/00 X-ray generator. Powder samples of solid dispersions

were top loaded in a Philips PW 1066 (15/20 mm) flat sample holder. The patterns were collected with a voltage of 45 kV and a current of 32 mA in the angular range of 48B/2uB/758 in a step scan mode (step width 0.028, counting time 2 s/step) using the Philips PW 1710 microprocessor based control and measuring system.

Scanning electron microscopy (SEM)

The SEM analysis was carried out using an SEM (make). Before examination, samples were mounted on an aluminum stub using a double-sided adhesive tape and then making it electrically conductive by coating with a thin layer of gold (approximately 20 nm) in vacuum. The SEM operated at an acceleration voltage of 15 kV.

Assay of solid dispersions

The content of FBX in the prepared solid dispersions was determined using UV-Vis spectrophotometer. Solid dispersions equivalent to 10 mg drug were dissolved in methanol. 1 ml of the stock solution was diluted to 10 ml with methanol which was further diluted with pH 6.0 buffer to give a final concentration of 10 µg/ml (10 ppm) solution. Percent drug content was calculated spectrophotometrically from the absorbance obtained at 318 nm.

In vitro dissolution studies

In vitro dissolution studies were carried out for the pure drug, physical mixture, and all the different solid dispersions prepared in USP Type II dissolution test apparatus (Campbell Electronics, India) at 75 rpm in 900 ml of pH 6.0 phosphate buffer. 40 mg of pure drug and an equivalent amount of solid dispersions and physical mixture were used for the dissolution studies. 10 ml of the aliquot was withdrawn at predetermined intervals and filtered using 0.45 mm nylon membrane (Pall Life Sciences, India). The required dilutions were made with pH 6.0 phosphate buffer, and the solution was analyzed for the drug content UV spectrophotometrically at 318 nm against pH 6.0 phosphate buffer. An equal volume of the dissolution medium was replaced in the vessel after each withdrawal to maintain the sink condition. Three determinations were carried out for each formulation. From this, cumulative percentage of drug dissolved was calculated and plotted against the function of time to study the pattern of drug release. Each test was performed in triplicate ($n = 3$), and calculated mean values of cumulative drug release were used while plotting the release curves.

Tablet dosage form preparation

Based on the results obtained from the drug release studies, the solid dispersions with better release profile were selected and prepared in the form of tablet dosage forms employing wet granulation technique.

Stability studies

Stability study was performed according to ICH guidelines for 3 months. Dissolution studies were carried out at the end of 3 months to check inhibition of reversal of FBX to crystalline form.

RESULTS AND DISCUSSION

Phase solubility studies

Figure 2 shows the solubility phase diagram representing the effect of increasing the concentrations of PVP K30 and HPMC E15 on the apparent solubility of FBX in water at 25°C. The aqueous solutions of PVP K30 and HPMC E15 increased the solubility of FBX more when compared to pure drug. The polymers were selected for formulation of solid dispersions because of their higher molecular weight and better solubility of FBX in their aqueous solution.

FTIR studies

FTIR spectra of solid dispersions of FBX with PVP K30 and HPMC E15 are shown in Figures 3-7. The spectra of pure FBX presented characteristic peaks at 3450.40, 3533.40/cm (O,H stretching of free hydroxyl group); 2957.68/cm (C, H stretching
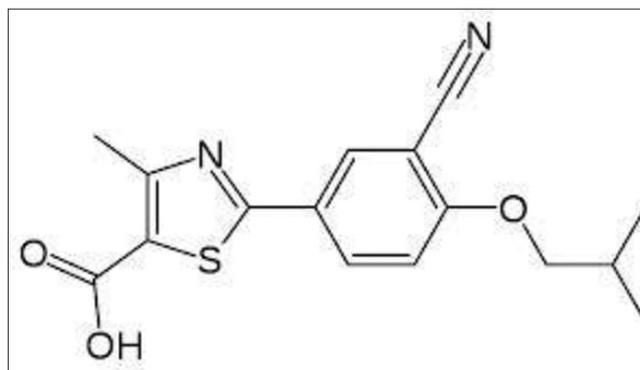

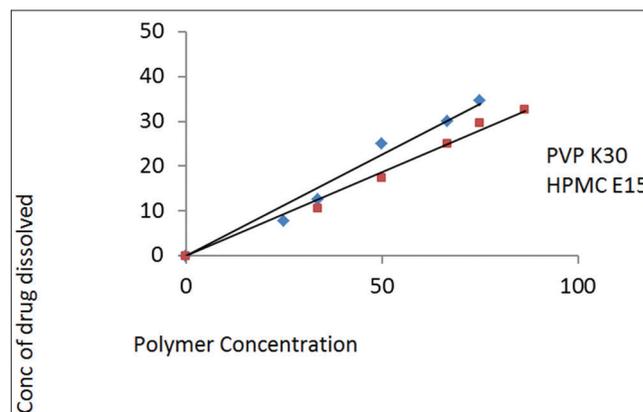**Figure 1:** Molecular structure of febuxostat

Figure 2: Phase solubility studies of drug in polymers

of alkanes); 1670/cm (C,O stretching of carboxylic acid); 1592, 1580, 1455/cm (C, C stretching of aromatic ring); 1498.71, 1457.44, 1413.90/cm (C-H stretching of alkanes), respectively. The spectrum of PVP K30 showed among others important bands at 2955/cm (C-H stretch) and 1654/cm (C=O). A very broadband was also visible at 3435/cm that was attributed to the presence of water (Van den Mooter *et al*., 1998) confirming the broad endotherm detected in the DSC experiments.

The characteristic peaks of FBX at 3450.40, 3533.4/cm (O, H stretching of acid), 2957.68/cm (C, H stretching of alkanes), and 1592, 1580, 1455/cm (C, C stretching of aromatic ring) are disappeared in spectra of solid dispersions with PVP K30 1:1 ratio, which indicates the trapping of FBX inside the matrix of PVP. In case of other ratios, 3450.40, 3533.40/cm (O, H stretching of free hydroxyl group), 2957.68/cm (C, H

stretching of alkanes), 1670.23/cm (C, O stretching of carboxylic group), 1592, 1580, 1455/cm (C, C stretching of aromatic ring), and 1498.71, 1457.44, 1413.90/cm (C, H stretching of alkanes) are retained which shows that the trapping of the drug with the polymer matrix is incomplete. The IR spectra of HPMC E15 showed characteristic sharp bands at 2920.62 (C, H stretching for alkanes), 2532.74 (H-C=O: C-H stretch aldehydes), broadband at 1036.23(C-N stretch aliphatic amines). The spectra of FBX with HPMC E15 1:3 ratio solid dispersion show bands of FBX at 2532 and 1284/cm, and the other peaks were disappeared indicating the trapping of the drug in the polymer matrix.

PXRD studies

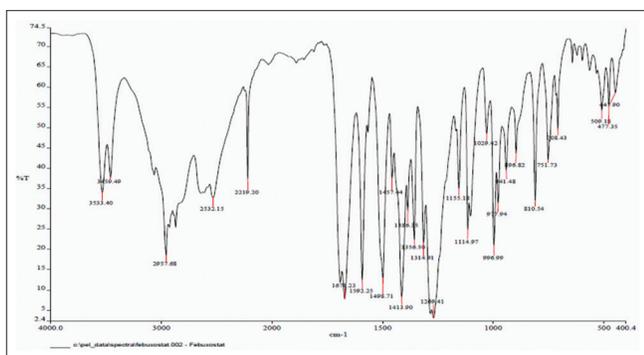The pure FBX, polymers PVP K30 and HPMC E15 and prepared solid dispersions of carriers, physical mixtures

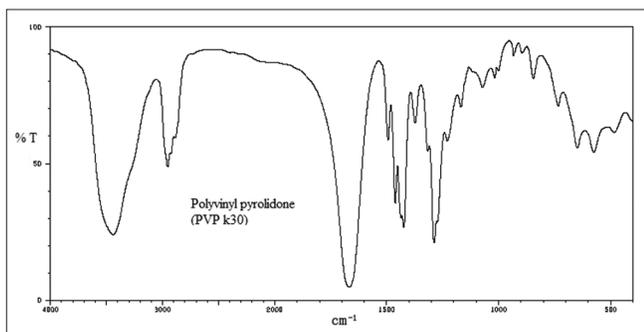**Figure 3:** Fourier transform infrared spectra of febuxostat

Figure 4: Fourier transform infrared spectra of poly vinyl pyrrolidone K30
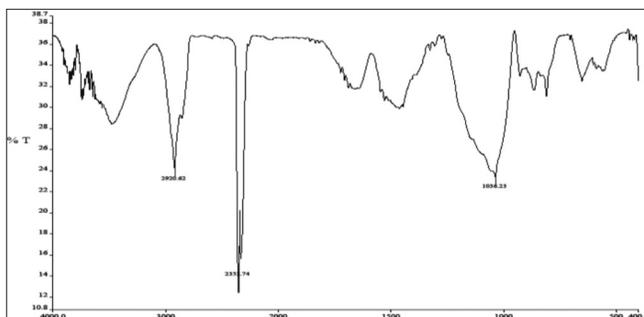

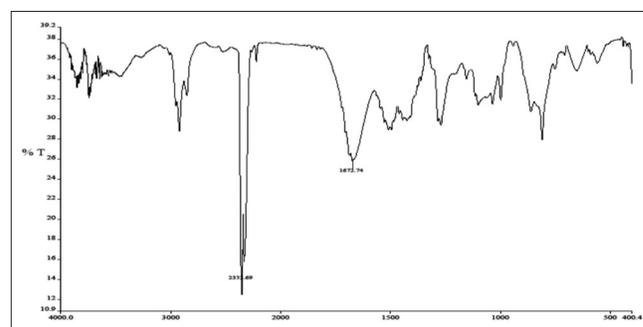**Figure 5:** Fourier transform infrared spectra of hydroxy propyl methyl cellulose E15

Figure 6: Fourier transform infrared spectra of solid dispersions of poly vinyl pyrrolidone k30 (1:1 ratio)
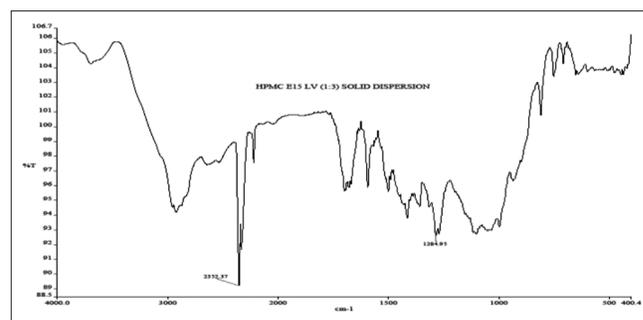

Figure 7: Fourier transform infrared spectra of solid dispersions of febuxostat and hydroxy propyl methyl cellulose E15 (1:3 ratio)
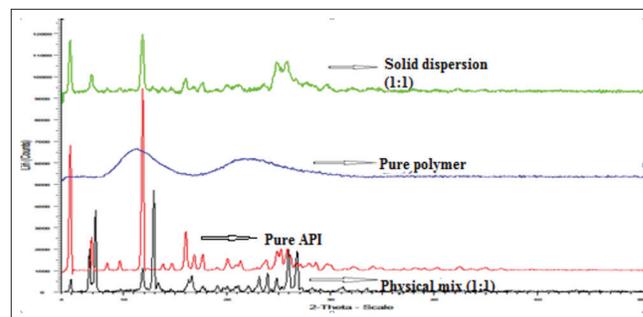

Figure 8: Overlaying of powder X-ray diffraction patterns of various compounds of poly vinyl pyrrolidone K30

were studied by XRD as shown in Figures 8 and 10. PVP being amorphous did not show any sharp peaks. The powder diffraction patterns of pure FBX showed characteristic high-intensity diffraction peaks at 2θ values of 4.788, 6.857, 8.363, 11.79, 15.98, 16.78, 17.58, 20.001, 25.16, and 25.77, whereas the spectroscopy of PVP K30 and HPMC E15 do not show any characteristic diffraction peak. The high-intensity diffraction peaks are very prominently preserved in case of physical mixtures, whereas these characteristic peak intensities were drastically reduced in 1:1 and 1:3 ratios of drug and polymers owing to the complete encapsulation and amorphization of the drug. The findings of XRD are in line with that of DSC findings.

DSC analysis

The DSC thermograms for pure FBX, polymer, and solid dispersions were shown in Figures 9 and 11. The DSC thermogram of pure FBX shows the sharp endothermic peak at around 200-220°C, confirming the crystallinity of the drug. During scanning of PVP K30, a broad endotherm ranging from 60 to 120°C with a peak of 98.86°C was observed indicating the presence of residual moisture owing to the hygroscopicity of the polymer. The DSC thermogram of the solid dispersion FBPVP in 1:1 ratio showed the presence of broaden peaks with no characteristic peaks of the drug. A broad endotherm ranging from 77.99 to 123.25 with a peak value of 98.74 was obtained for HPMC E15. In case of 1:3 ratio of FBMC also broadening of peaks with no characteristic peaks of the drug were observed, which means that in both the ratios the drug is in the form of amorphous nature or solid solution.

SEM

SEM photomicrographs obtained for pure FBX, PVP K30, HPMC E15, and their physical mixtures and solid dispersions are shown in Figures 12 and 13 in selected magnifications. From the photomicrograph of pure drug FBX, it is clear that the drug is present as needle-shaped crystals. In the solid dispersion, drug particles were entrapped in the carrier matrix and the crystalline appearance of the drug was reduced and became more amorphous confirming the FTIR, XRD, and DSC data analyses.

In vitro dissolution studies

Dissolution of pure FBX and all other prepared systems (solid dispersions and physical mixtures) were carried out in phosphate buffer of pH 6.0. DP_{45min} (percent drug dissolved within 45 min) values were reported in Table 1. From these data, it is evident that the onset of dissolution of pure FBX is very low (8.29%). Dissolution profiles of pure FBX, its physical mixtures and solid dispersions with PVP K30 and HPMC E15 over a period of 45 min were shown in Figures 14 and 15, respectively. It can be clearly observed that the dissolution rate of pure FBX is 41.8% in 45 min solid dispersions FBVP
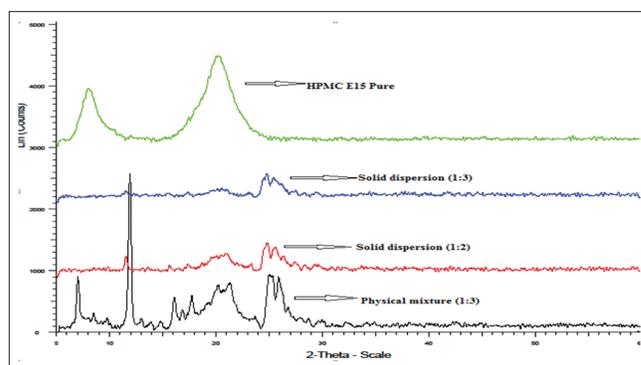

Figure 9: Overlaying of differential scanning calorimetry thermograms of various solid dispersions of poly vinyl pyrrolidone K30
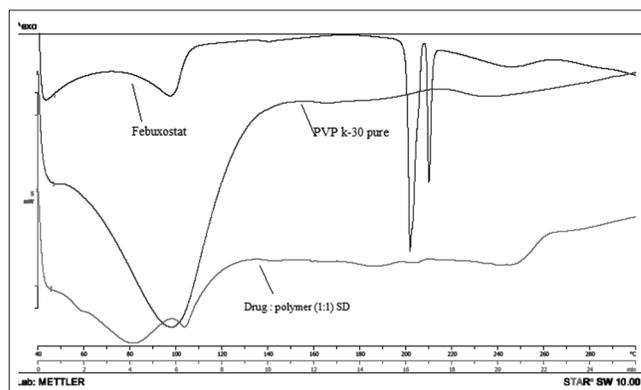

Figure 10: Overlaying of differential scanning calorimetry thermograms of drug, polymer, and solid dispersion of poly vinyl pyrrolidone K30
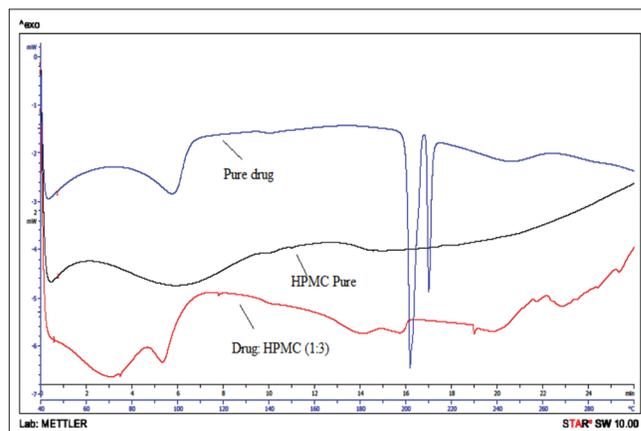

Figure 11: Overlaying of differential scanning calorimetry thermograms of drug, polymer and solid dispersion of hydroxy propyl methyl cellulose E15

and FBMC significantly enhanced the dissolution rate of FBX (100% release in 30 min in case of both PVP K30 and HPMC E15) as compared to physical mixtures as well as pure FBX. The highest improvement was observed in solid dispersions of FBVP 1:1 ratio while in case of solid dispersions prepared with HPMC E15, the improvement in dissolution rate was higher for FBMC 1:3 ratio.
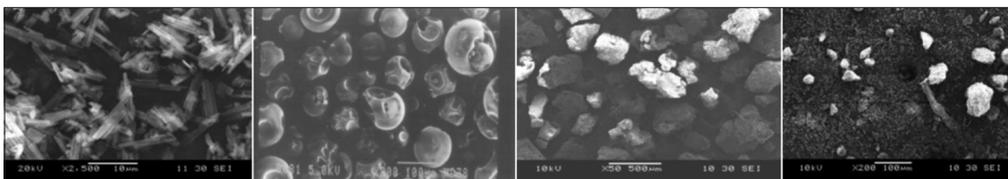
Figure 12: Scanning electron microscopy of febuxostat, poly vinyl pyrrolidone K30, solid dispersion of 1:1 ratio and physical mixture of 1:1 ratio
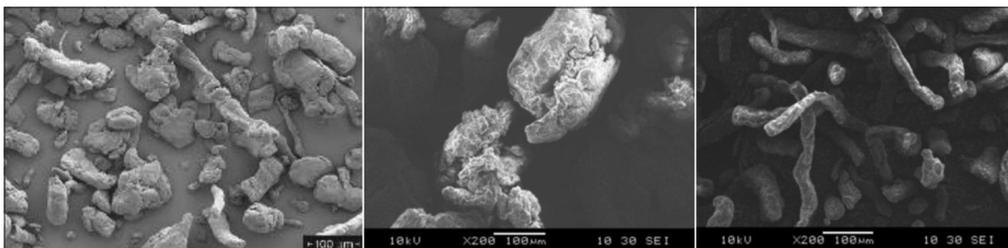

Figure 13: Scanning electron microscopy of hydroxy propyl methyl cellulose E15, solid dispersion of 1:3 ratio and physical mixture of 1:3 ratio
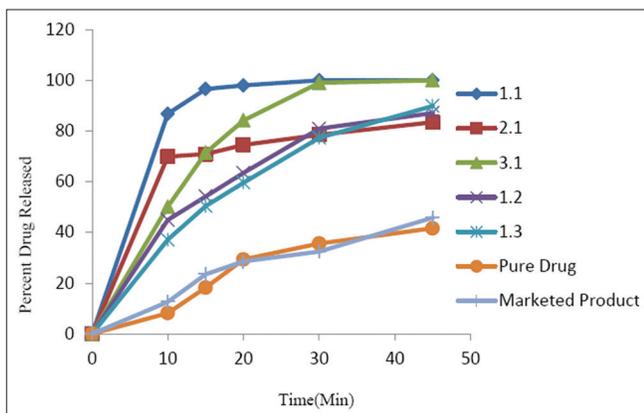

Figure 14: Drug release profiles of different ratios of solid dispersions with febuxostat and poly vinyl pyrrolidone k30, pure drug and marketed preparation
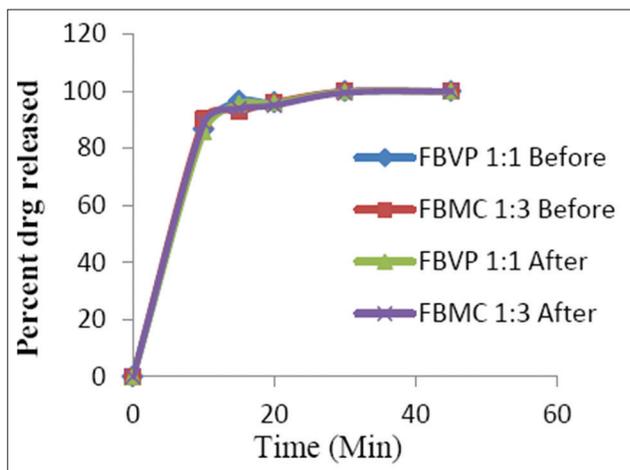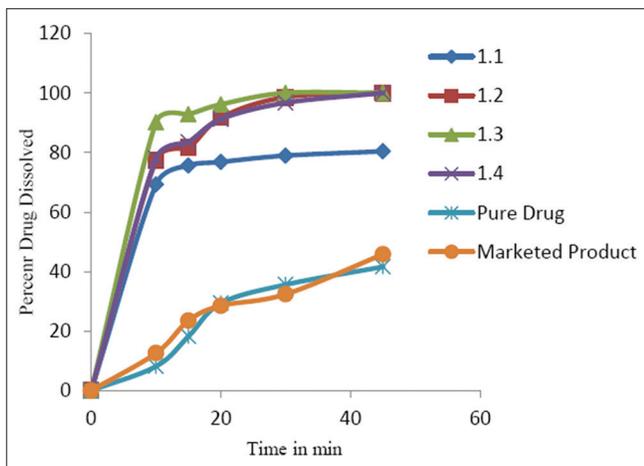

Figure 15: Drug release profiles of different ratios of solid dispersions with febuxostat and hydroxy propyl methyl cellulose E15, pure drug, and marketed preparation

Figure 16: Drug release profiles of selected solid dispersions before and after stability

Drug content

The percent drug content values of various solid dispersions prepared are given in Table 2. There was no loss of drug during the preparation and all the solid dispersions contained the drug equal to the theoretical drug content based on the proportion of drug and carrier taken. Low standard deviation and coefficient of variation (<2.0) in the percent drug content values indicated that the drug content was uniform in a batch of a solid dispersion in all the cases.

Stability studies

The selected solid dispersions of both the polymers, i.e., FBVP 1:1 and FBMC 1:3 were kept for stability studies, and the preparations were evaluated for drug release studies after stability. In the stability studies, the formulations did

Table 1: Percent drug released from different ratios of solid dispersions prepared, marketed preparation, and pure drug

Time (min)	Percent drug released										
	FBVP 1:1	FBVP 2:1	FBVP 3:1	FBVP 1:2	FBVP 1:3	FBMC 1:1	FBMC 1:2	FBMC 1:3	FBMC 1:4	Marketed preparation	Pure drug
10	86.8	69.9	50.09	44.87	37.10	69.27	77.5	90.17	78.02	12.6	8.16
15	96.6	70.9	71.47	54.22	50.36	75.57	81.6	92.81	83.52	23.5	18.23
20	96.14	4.54	84.20	63.47	59.64	76.81	81.7	96.14	91.2	28.5	29.34
30	100	78.50	99.99	80.93	77.36	78.94	98.6	100	96.7	32.4	35.61
45	100	83.50	100	87.11	89.92	80.39	100	100	100	45.8	41.8

Table 2: Ratios of solid dispersions prepared and the drug content values

Ratios of solid dispersions prepared (drug:polymer)	Percent drug content
FBVP 1:1	100
FBVP 2:1	95.9
FBVP 3:1	98.7
FBVP 1:2	97.2
FBVP 1:3	93.4
FBMC 1:1	96.5
FBMC 1:2	98.8
FBMC 1:3	100
FBMC 1:4	98.5

Table 3: Percent drug release of the finalized formulations before and after stability

Time (min)	Before stability		After stability	
	FBVP 1:1	FBMC 1:3	FBVP 1:1	FBMC 1:3
10	86.8	90.17	85.7	88.82
15	96.6	92.81	95.62	93.91
20	96.14	96.14	95.9	95.1
30	100	100	99.8	99.5
45	100	100	100	100

not show any significant changes in the drug release profile which were also as per the DSC and XRD studies which prove that the drug had retained its amorphous form and did not convert into crystalline form on storage. The drug release profile in stability studies were shown in Figure 16 and listed in Table 3.

CONCLUSION

Solid dispersions of FBX with PVP K30 gave higher intrinsic dissolution rates. In contrast to physical mixture, FBX in the solid dispersions was present in amorphous form and was found to interact with PVP suggesting greater stability

for the drug. The most important findings were that FBVP 1:1 and FBMC 1:3 ratios showed the best dissolution rate. The XRD, DSC, SEM studies of drug, carrier polymers, and solid dispersions prepared indicated the entrapment of drug in a carrier matrix. In these systems, the drug-carrier interactions were shown in FTIR. The results showed the suitability of carrier polymers in preventing crystallization of FBX. The increased dissolution rates in systems containing carrier polymers were due to surface tension lowering effect of polymers to the medium, resulting in the wettability of hydrophobic and BCS Class II drug-like FBX and thus increase in dissolution rates.

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