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Research Article

FORMULATION, OPTIMIZATION, AND *IN VITRO* EVALUATION OF POLYMERIC NANOSUSPENSION OF FLURBIPROFEN

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ABSTRACT

Objective: At present, more than 40% of drugs are poorly water-soluble that leads to reduced bioavailability. The objective of the present investigation was to overcome the issue of poor aqueous solubility of drug; therefore, stable flurbiprofen (FBF) nanosuspensions were developed by nanoprecipitation method.

Materials and Methods: Based on particle size, zeta potential, and entrapment efficiency, the polymeric system of hydroxypropyl methylcellulose E15 and poloxamer 188 was used effectively. The prepared formulations were evaluated for Fourier transform infrared spectroscopy, transmission electron microscopy, differential scanning calorimetry, powder X-ray diffraction, saturation solubility, entrapment efficiency, particle size, zeta potential, dissolution profile, and stability.

Results: The resultant FBF nanosuspensions depicted particles in size range of 200–400 nm and were physically stable. After nanonization, the crystallinity of FBF was slightly reduced in the presence of excipients. The aqueous solubility and dissolution rate of all FBF nanosuspensions were significantly increased as compared with FBF powder.

Conclusion: This investigation demonstrated that nanoprecipitation is a promising method to develop stable polymeric nanosuspension of FBF with significant increase in its aqueous solubility.

Keywords: Nanosuspension, Nanoprecipitation, Flurbiprofen, Hydroxypropyl methylcellulose E15, Lyophilization.

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INTRODUCTION

The large number of active pharmaceutical ingredients emerging from the drug discovery process exhibits poor aqueous solubility resulting in a low dissolution rate and oral bioavailability [1,2]. Solubility, dissolution, and permeability of drugs are rate-limiting parameters for its oral absorption [1,2]. Various physicochemical and physiological parameters of drug affect the oral bioavailability of drugs [1,2]. Size reduction of drugs improves oral bioavailability of drug by increasing its effective surface area and thus increasing solubility and dissolution rate of drugs [1,2]. High log p value and molecular weight of the substance are important factors regarding nanosuspension of less aqueous solubility of drugs [2]. Nanosuspension is the novel approach to overcome the problem of low dissolution rate and compromised oral bioavailability and reduce the delivery issues by maintaining the drug in preferred crystalline state [3-8]. Nanosuspension signifies sufficient safety and efficacy [4-6]. According to Nernst-Brunner diffusion layer model, the peripheral layer of the solid particle gets saturated by small portion of an adjacent solvent. Afterward steady-state mass transfer takes place into the bulk solution [8-12]. The formulation can be achieved by top-down (fracturing larger particles to smaller particles) or bottom-up (generation of smaller particles by precipitation at molecular level) approaches [1,9-13]. Nanoprecipitation is one of the promising techniques for the development of nanosuspension of low water-soluble drug molecules [14]. However, particle agglomeration and crystal growth due to Van der Waals forces or Ostwald ripening can be prevented by addition of one or more stabilizer (s) [15]. The selection of polymers and stabilizers is very crucial in the development of nanoformulations. Hydroxypropyl methylcellulose E15 (HPMC E15) and poloxamer 188 (Pluronic F68) are steric stabilizers provide stabilized dispersion by steric hindrance [1,13]. Nanosuspension formulations of several drugs such as Rapamune (sirolimus) and Tricor (fenofibrate) are already successfully marketed [16].

Flurbiprofen (FBF) is a phenylalkanoic acid derivative (Fig. 1), nonsteroidal anti-inflammatory and classified as Biopharmaceutics Classification System Class II drug due to its practical insolubility in water. Its oral bioavailability is affected by low aqueous solubility having p*K*a value ~ 4.03. The high log p value of FBF is an important feature in the development of its nanosuspension [17,18].

This study was focused to develop stable polymeric nanosuspension for enhancement of dissolution and oral bioavailability of FBF. The solidification of formulations was carried out by freeze-drying.

MATERIALS AND METHODS

Materials

FBF, HPMC E15, and poloxamer 188 (Pluronic F68) were kindly gifted by Sun Pharma Pvt., Ltd., Ahmednagar. Polyvinylpyrrolidone K30 (PVP K30), polyethylene glycol 6000 (PEG 6000), and sodium dodecyl sulfate (SDS) were procured from BASF Ltd. All used supplementary chemicals and reagents were of analytical grade and utilized without additional purification. Double distilled water was used during the experimental work.

Methods

Screening of stabilizer based on settlement volume ratio

To select the optimal stabilizer, the FBF (0.5% w/v) nanosuspensions were prepared using different stabilizers (0.5% w/v) such as PVP K30, PEG 6000, SDS, and poloxamer 188, respectively, by nanoprecipitation technique. The obtained nanoformulations were analyzed by settlement volume ratio (F) for a week, and suitable stabilizer was selected based on the stability of the system [19].

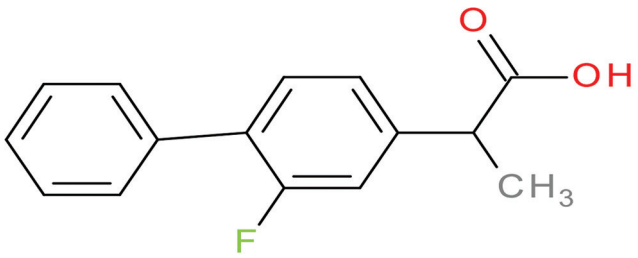

Fig. 1: Chemical structure of flurbiprofen

Table 1: Settlement volume ratio for FBF nanosuspensions with different stabilizers

Stabilizer	PVP K30	PEG 6000	SDS	Poloxamer 188
F	0.19	0.13	0.41	0.73

PVP K30: Polyvinylpyrrolidone K30, PEG 6000: Polyethylene glycol 6000, SDS: Sodium dodecyl sulfate, FBF: Flurbiprofen

Table 2: Results of statistical analysis of the experimental design

Responses	Sources		
	Model p value	Adj-R^2	Lack of fit test p value
Entrapment efficiency	-	-	0.2371
Particle size	0.0488	0.8415	0.9727
Zeta potential	0.0081	0.952	0.5359

Screening of stabilizer based on solubility study

The solubility of FBF was determined in the solutions of stabilizers. Briefly, excess amount of FBF was added to the stabilizer solution (5 ml) in sealed glass vials. The vials were shaken at rotary shaker (Remi RS BL) at 75 rpm (rotations per minute) for 72 h at 37°C and centrifuged at 40,000 rpm for 10 min at 4°C (Remi C30 PLUS) to remove non-dissolved drug. The supernatant was suitably diluted with distilled water and was analyzed for FBF by ultraviolet (UV) spectrophotometer (Shimadzu UV Spectrophotometer 1800) at 247 nm. Each experiment was carried out in triplicate.

Experimental design

Initial screening studies were carried out to check the effect of process parameters and formulation parameters on FBF nanosuspension as well as its stability. The stirrer speed of mixing was identified as a critical process parameter and polymer:surfactant ratio as a critical formulation parameter. The design of experiment was used systematically to evaluate and optimize the selected process and formulation parameters at three levels (−1, 0, and +1) using 3^2 factorial design to find out their effects on critical quality attributes of nanosuspension. The batch size 100 ml, drug concentration (0.1% w/v), HPMC E15 as a polymer and poloxamer 188 as a stabilizer, drug:polymer ratio (1:1), and stirring time (1 h) were kept constant in experimental process.

Preparation of a physical mixture of FBF, HPMC E15, and poloxamer 188

A physical mixture of FBF, HPMC E5, and poloxamer 188 was prepared by mixing them in same proportion and used for comparison with optimized formulation. The homogenous mixture was obtained by mixing in mortar, passed through 40# mesh sieve, and stored in desiccator.

Preparation of FBF nanosuspension

Nanoprecipitation technique was used to prepare FBF loaded nanosuspensions. Nanosuspensions were obtained using 500 mg FBF at different HPMC E15:poloxamer 188 ratios and stirring speed. Accurately weighed 500 mg of FBF and 500 mg of HPMC E15 were dissolved in 10 ml of methanol (cosolvent) by sonication. The prepared organic phase of drug was added in 100 ml distilled water containing poloxamer 188 at different ratios using a syringe (26 G) with constant speed (0.5 ml/min). Stirring was continued for 1 h by mechanical stirrer at different speeds of stirrer. An excess amount of methanol was evaporated by air drying. The resulting nanosuspension was sonicated for 15 min. The optimized formulation FB8 was lyophilized without cryoprotectant.

Lyophilization of optimized batch (FB8) of nanosuspension

An optimized batch of nanosuspension (FB8) was converted into the dry powder without mannitol using laboratory scale lyophilizer (Christ, Alpha, 12 LD PLUS) by freeze-drying for 48 h at predetermined conditions. The freeze-dried product was placed in airtight container for further characterization.

Evaluation of optimized FBF nanosuspension

Particle size distribution and zeta potential

The mean particle size analysis and polydispersity index PDI of suspended particles in nanosuspensions were carried out using Nanoparticle Analyzer SZ-100 with a Zetasizer (Horiba Scientific, Japan). Zeta potential is an index of the stability of suspension depending on the surface charge of particles. The zeta potential was determined by a laser Doppler anemometer coupled with Nanoparticle Analyzer SZ-100 (Horiba Scientific, Japan). The samples were properly diluted with de-ionized water before analysis. Each sample was assessed in triplicate [13].

Determination of % drug entrapment efficiency

FBF nanosuspensions (10 ml) were centrifuged at 10,000 rpm and 6°C using a cooling centrifuge (Remi C30 PLUS) for 30 min. The supernatant was separated out, and the absorbance was measured for the free drug content by UV spectrophotometer (Shimadzu UV Spectrophotometer 1800) at 247 nm. Entrapment efficiency was determined by subtracting the amount of free drug from the initial amount of drug. The % drug entrapment efficiency calculated by the following equation [20],

$$\%\text{Entrapment efficiency} = \frac{\text{Initial drug concentration} - \text{Final drug concentration}}{\text{Initial drug concentration}} \times 100$$

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were recorded on the infrared spectrophotometer (Alpha T Bruker). Samples about 2–3 mg were mixed with dry potassium bromide and scanned over the range 4000–400 cm^{-1}.

Differential scanning calorimetry (DSC)

The DSC thermograms of FBF, HPMC E15, poloxamer 188, physical mixture, and lyophilized powder of optimized nanosuspension (FB8) were recorded on DSC (Mettler Toledo, Staresw 920) at a rate of 10°C/min over a temperature range of 25–200°C in an atmosphere of nitrogen having flow rate of 40 ml/min.

Powder X-ray diffraction (PXRD)

PXRD patterns of FBF, HPMC E15, poloxamer 188, physical mixture, and lyophilized powder of optimized nanosuspension (FB8) were recorded on diffractometer (Miniflex 600 X-ray diffractometer, Rigaku Corporation, Japan). The samples were scanned from 5 to 80° 2θ at a scan rate of 2°/min.

Transmission electron microscopy (TEM)

Surface morphology of suspended nanoparticles in FBF nanosuspensions was observed by TEM (A Hitachi H-7500, Japan). The images were viewed at different magnifications.

Saturation solubility study

The saturation solubility of FBF, physical mixture, and lyophilized powder of optimized nanosuspension FB8 was determined using three different mediums. Briefly, excess amount of each crude FBF, physical mixture, and lyophilized powder was added to each 5 ml of 0.1 N HCl (pH 1.2), phosphate buffer (pH 4.5), and phosphate buffer (pH 7.2), respectively, in sealed glass vials at 37±0.5°C. All vials were agitated at 100 rpm for 72 h using rotary shaker (Remi RS-BL). The obtained dispersions were centrifuged (Remi C30 PLUS, Mumbai, India) at 40,000 rpm for 10 min at 4°C. The supernatant was filtered and analyzed by UV spectrophotometer at 247 nm. Each experiment was performed in triplicate [21].

Dissolution testing

In vitro dissolution study of crude FBF, physical mixture and lyophilized powder of optimized nanosuspension FB8 were investigated by USP 37 Type II dissolution test apparatus (Electrolab EDT-08 Lx) in three different dissolution media 0.1 N HCl (pH 1.2), phosphate buffer (pH 4.5), and phosphate buffer (pH 7.2), respectively. The samples equivalent to 100 mg of FBF were added in 900 ml of dissolution medium maintained at 37±0.5°C. The paddle speed was 75 rpm. Samples (5 ml) were collected after 5, 10, 20, 30, 45, and 60 min with immediate replacement by fresh dissolution medium to maintain the sink condition. All samples were filtered through 0.10 µm PTFE filter and analyzed by UV spectrophotometer at 247 nm. Each experiment was performed in triplicate [22-24].

Physical stability

The physical stability of optimized nanosuspension FB8 was evaluated at 4°C and 25°C for a period of 3 months. Small aliquots of nanosuspension were withdrawn after 3 months of storage for analysis of particle size, PDI, and zeta potential. Each sample was analyzed in triplicate.

RESULTS AND DISCUSSION

Optimization

Formulation optimization by stabilizer

The selection of suitable stabilizer is an important aspect of the stability of nanosuspension. In the present investigation, settlement volume ratio (F) and aqueous solubility of FBF in the presence of different stabilizers were used as stability index for the nanosuspension.

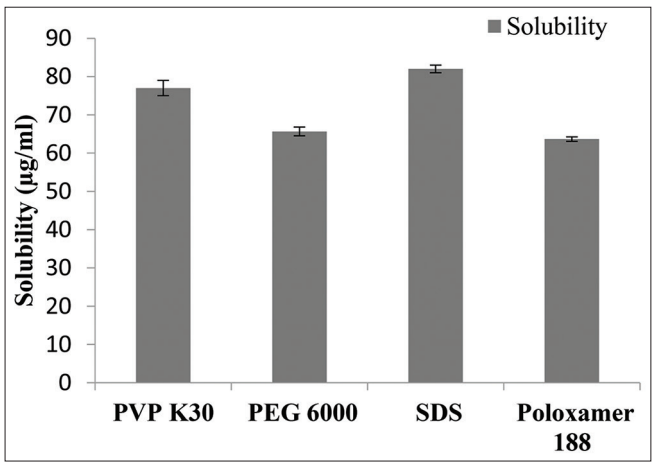

Fig. 2: Aqueous solubility of flurbiprofen in the presence of different stabilizers

Selection of stabilizer by settlement volume ratio (F) method

Settlement volume ratio (F) is the ratio of the volume of sedimentation after and before for a given time. Larger the F value more is the stable suspension. The settlement volume ratios for FBF loaded nanosuspensions formulated with different stabilizers are recorded in Table 1.

The stabilization effect observed was followed by Pluronic F68 >SDS >PVP K30 >PEG 6000. From the results, it could be concluded that reasonably poloxamer 188 should be selected for development of stable nanosuspension.

Selection of stabilizer by solubility study

Fig. 2 represents the apparent solubility profiles of FBF in the presence with different stabilizers. FBF represents higher aqueous solubility in the presence of SDS. This could be due to SDS micelle formation. Increased solubility can exert Ostwald ripening, resulting in increased particle size during storage. Comparatively minimum solubility was observed in the presence of poloxamer 188; therefore, it was selected as a steric stabilizer.

Experimental design

Regression and graphical analysis of data acquired from the experimental runs generated following equations in which F ratios were statistically significant ($p<0.05$) with Adj-R^2 value in the range of 0.8–1 (Table 2) and with a statistically non-significant lack of fit values ($p>0.05$). These model equations fitted the data well. A positive sign indicates a synergistic effect, while negative sign indicates an antagonistic effect.

$$\text{Entrapment efficiency} = +83.93 \text{ (Surface mean model)} \quad (1)$$

$$\text{Particle size} = 271.8 - 9.45 \times X_1 - 28.02 \times X_2 + 18.1 \times X_1.X_2 + 20.25 \times X_1^2 + 16.75 \times X_2^2 \text{ (Quadratic model)} \quad (2)$$

$$\text{Zeta potential} = -11.65 + 2.69 \times X_1 + 1.11 \times X_2 - 0.4 \times X_1.X_2 - 0.098 \times X_1^2 + 0.23 \times X_2^2 \text{ (Quadratic model)} \quad (3)$$

Where X_1 and X_2 are FBF: HPMC E15:poloxamer 188 ratio and stirring speed, respectively.

Equations (2) and (3) represent that increase in stirring time decreases particle size and increase in the concentration of surfactant to certain level increases entrapment efficiency. An increased concentration of surfactant decreases zeta potential.

Effect of formulation parameter and process parameter on entrapment efficiency, particle size, and zeta potential

Fig. 3a and c displays that the ratio 1:1:2 of drug, polymer, and surfactant, respectively, showed greater entrapment efficiency. An increase in the concentration of surfactant above optimum level depicted decreased entrapment efficiency probably due to surface adsorption of drug by it. A small decrease in zeta potential was observed with increase in concentration of surfactant. This could be explained by the reality that micelle formation and increased surface adsorption of drug. As shown in Fig. 3b, the increase in stirring speed gradually decreases the particle

Table 3: Observed and predicted values of responses of optimized nanosuspension (FB8)

Factors		Predicted value			Observed value*		
FBF: HPMC E15:poloxamer 188	Stirring speed (rpm)	Entrapment efficiency (%)	Mean particle size (nm)	Zeta potential (mV)	Entrapment efficiency (%)	Mean particle size (nm)	Zeta potential (mV)
1:1:2	1500	83.92	271.8	−11.65	89.83±0.93	263.3±2.73	−10.2±0.42

*All values are mean±SD (n=3). FBF: Flurbiprofen, HPMC E15: Hydroxypropyl methylcellulose E15, SD: Standard deviation

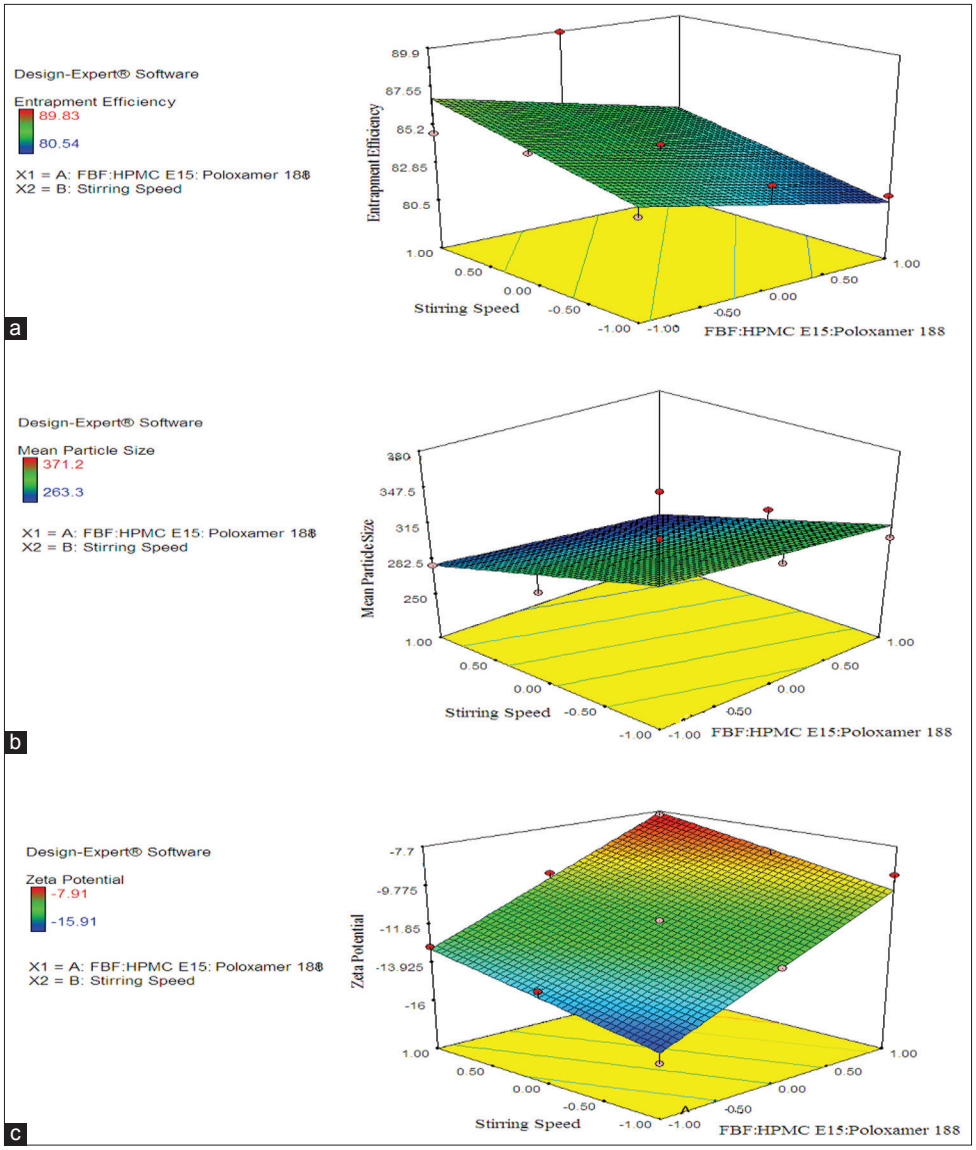

Fig. 3: Response surface plots showing the effect of flurbiprofen:hydroxypropyl methylcellulose E15:poloxamer 188 concentrations and stirring speeds on (a) entrapment efficiency, (b) mean particle size, and (c) zeta potential

Table 4: Preparation of FBF nanosuspensions using 3^2 factorial design

Formulation code	Independent variables				Dependent variables*		
	Formulation variable		Process variable		Y_1	Y_2	Y_3
	X_1	FBF: HPMC E15:poloxamer 188	X_2	Stirring speed (rpm)	Entrapment efficiency (%)	Mean particle size (nm)	Zeta potential (mV)
FB1	−1	1:1:1	−1	500	83.47±1.12	371.2±2.08	−15.91±0.08
FB2	0	1:1:2	−1	500	83.61±1.07	315.6±2.28	−12.7±0.13
FB3	+1	1:1:3	−1	500	81.37±0.77	303.4±0.93	−9.18±0.88
FB4	−1	1:1:1	0	1000	85.19±0.94	289.7±1.59	−13.93±0.94
FB5	0	1:1:2	0	1000	84.29±1.08	270.0±3.11	−11.6±0.18
FB6	+1	1:1:3	0	1000	80.54±0.87	296.2±2.21	−9.63±0.18
FB7	−1	1:1:1	+1	1500	84.74±0.82	277.1±1.16	−13.03±0.17
FB8	0	1:1:2	+1	1500	89.83±0.93	263.3±2.73	−10.2±0.42
FB9	+1	1:1:3	+1	1500	82.31±1.13	281.7±0.73	−7.91±0.37

*All values are mean±SD (n=3). FBF: Flurbiprofen, HPMC E15: Hydroxypropyl methylcellulose E15, SD: Standard deviation

size. It could be due to increased attrition and counter diffusion of particles during nanoprecipitation, and additional mechanical energy induces the repulsion between nanocrystals.

Model verification

The desirability function was evaluated by Design-Expert software to obtain the optimized FBF nanosuspension. The model verification results

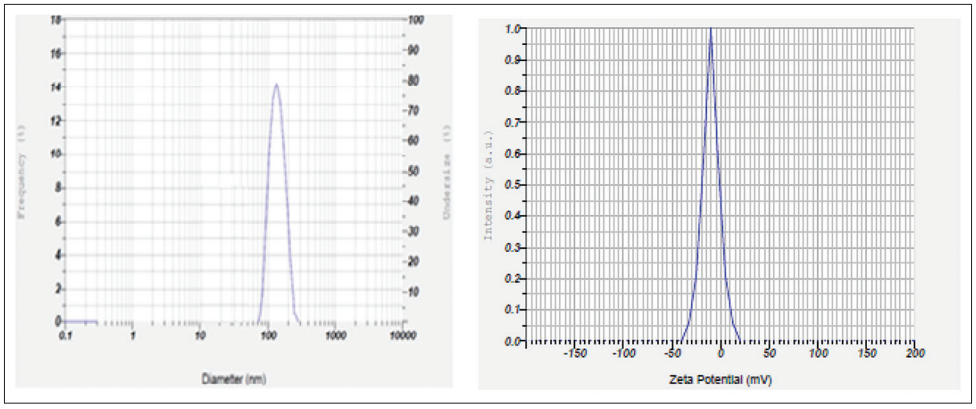

Fig. 4: Particle size and zeta potential distribution of an optimized batch of nanosuspension (FB8)

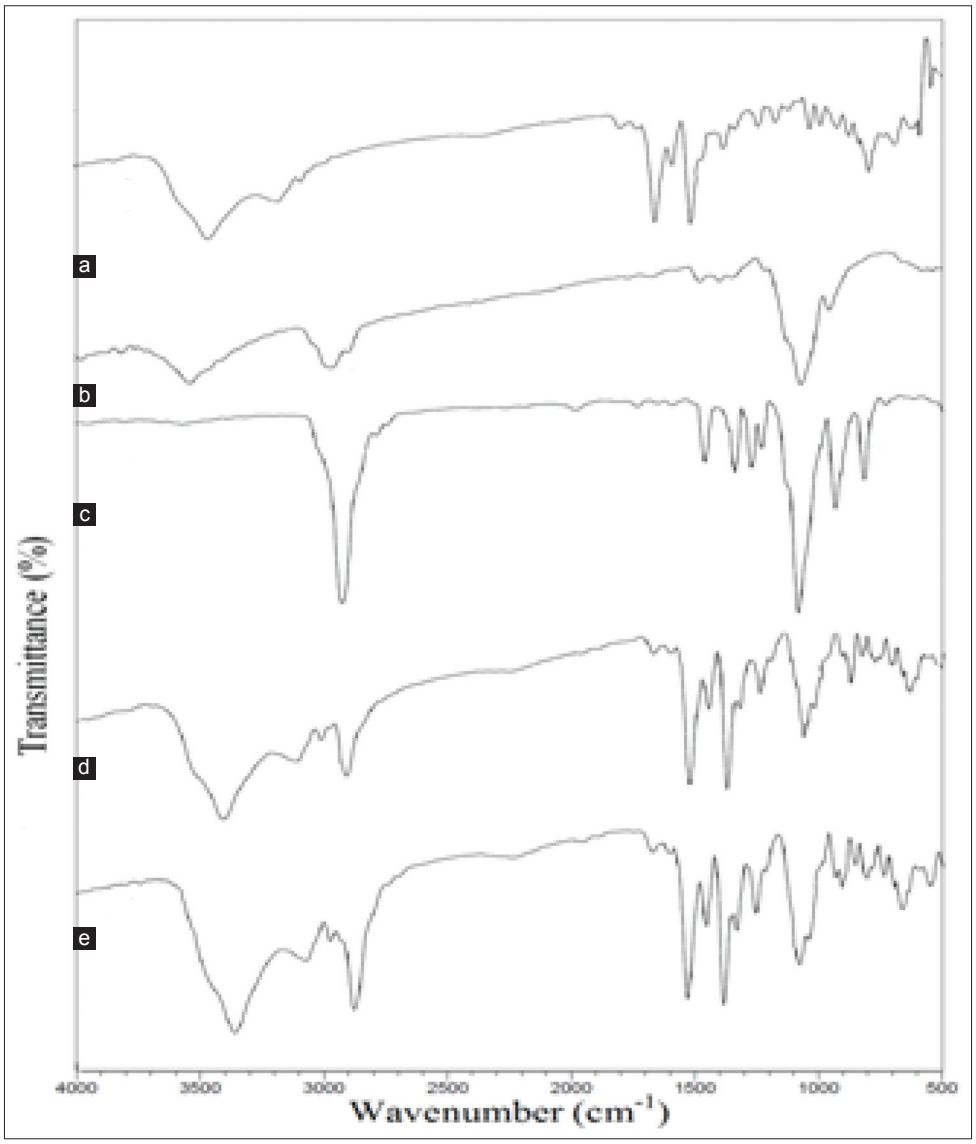

Fig. 5: Fourier transform infrared spectroscopy spectra of pure flurbiprofen (a), hydroxypropyl methylcellulose E15 (b), poloxamer 188 (c), physical mixture (d), and lyophilized nanosuspension FB8 (e)

are displayed in Table 3 that compare observed and predicted values of entrapment efficiency, particle size, and zeta potential using model equations.

Evaluation of optimized FBF nanosuspension

Particle size and zeta potential analysis

Table 4 depicts mean particle size, % entrapment efficiency, and zeta potential data of all formulations. The observed mean particle size of raw FBF powder was 16.41±2.41 µm while that of optimized nanosuspension (FB8) was 263.3±2.73 nm which represents significant (62-fold) reduction in particle size. The PDI of all batches of nanosuspension was found to be in the range of 0.258±0.01–0.411±0.02 indicates monodispersity of nanosuspensions. The PDI

Table 5: Kinetic profiles of *in vitro* drug release of optimized FBF nanosuspension FB8

Dissolution medium	Zero-order	First-order	Higuchi model	Hixson-Crowell	Korsmeyer–Peppas		Diffusion mechanism
	R^2				R^2	Release exponent (n)	
0.1 N HCl	0.998	0.992	0.985	0.967	0.970	0.225	Fickian diffusion
Phosphate buffer pH 4.5	0.993	0.989	0.988	0.991	0.993	0.240	Fickian diffusion
Phosphate buffer pH 7.2	0.990	0.863	0.979	0.975	0.995	0.239	Fickian diffusion

FBF: Flurbiprofen

Table 6: Particle size, PDI, and zeta potential values of optimized FBF nanosuspension (FB8) during 3 months of storage at 4°C and 25°C

Parameter	At 4°C		At 25°C	
	Initial*	After 3 months*	Initial*	After 3 months*
Particle size (nm)	263.3±2.73	271.12±0.93	263.3±2.73	293.12±1.09
PDI	0.266±0.02	0.272±0.01	0.266±0.02	0.274±0.01
Zeta potential (mV)	−10.2±0.42	−10.41±0.77	−10.2±0.42	−11.16±0.45

*All values are mean±SD (n=3). PDI: Polydispersity index, FBF: Flurbiprofen

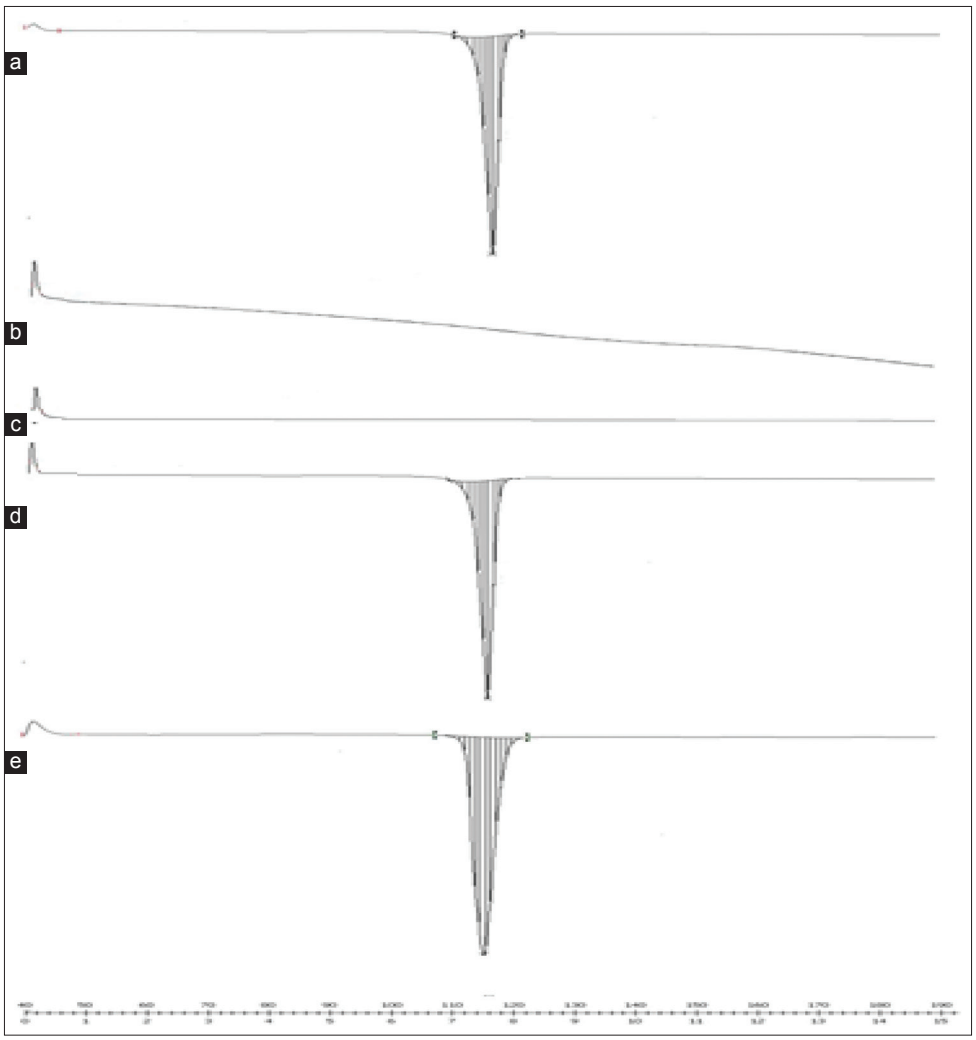

Fig. 6: Differential scanning calorimetry curves of crude flurbiprofen (a), hydroxypropyl methylcellulose E15 (b), poloxamer 188 (c), physical mixture (d), and lyophilized nanosuspension FB8 (e)

showed that optimized nanosuspension (FB8) was 0.266±0.02. Zeta potential is the stability index of prepared nanosuspension. Sterically stabilized systems show lower zeta potential values with sufficient stabilization [12]. Zeta potential of all batches of nanosuspension was found to be in the range from −15.91±0.08 mV to−7.91±0.37 mV. The zeta potential (Fig. 4) depicted that optimized nanosuspension (FB8) was −10.2±0.42 mV.

Drug entrapment efficiency (%) analysis

Entrapment efficiency is the percentage of the actual mass of drug entrapped in the polymeric carrier compared to the initial amount of loaded drug. The results (Table 4) suggested that the formulation FB8 having FBF: HPMC E15:poloxamer 188 (1:1:2) ratio showed increased drug entrapment efficiency due to formation of polymeric matrix with optimum viscosity. The drug entrapment efficiency of

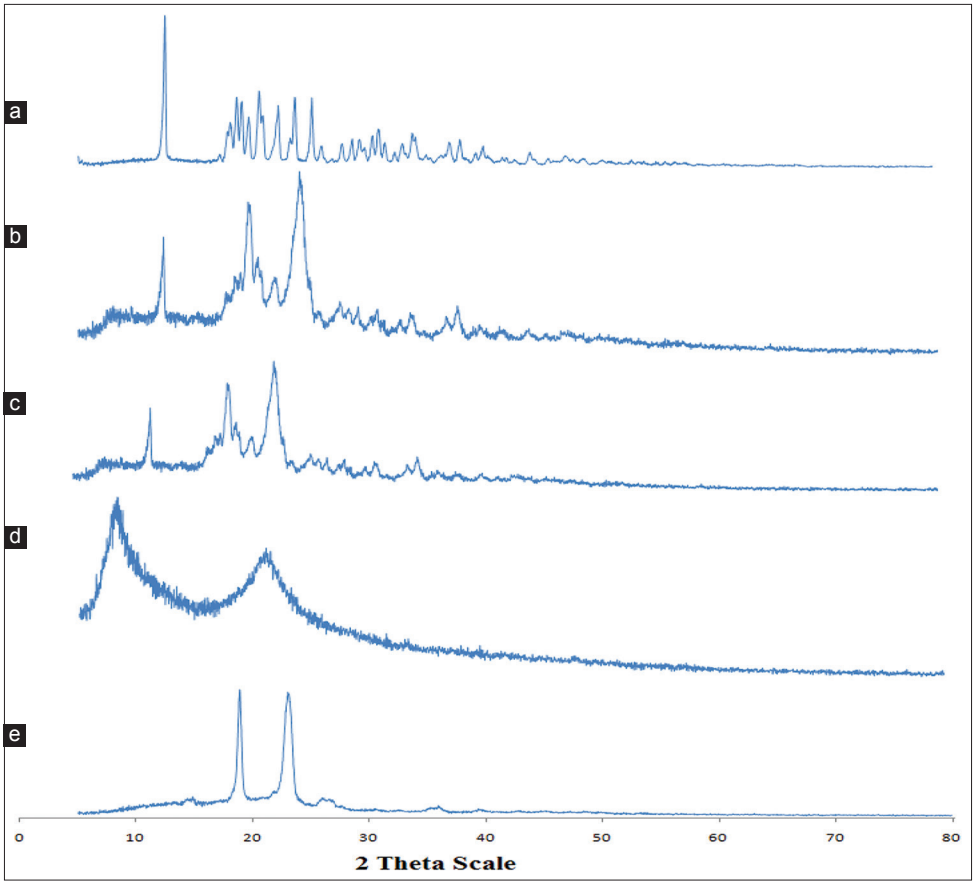

Fig. 7: Powder X-ray diffraction spectra of crude flurbiprofen (a), physical mixture (b), lyophilized nanosuspension FB8 (c), hydroxypropyl methylcellulose E15 (d), and poloxamer 188 (e)

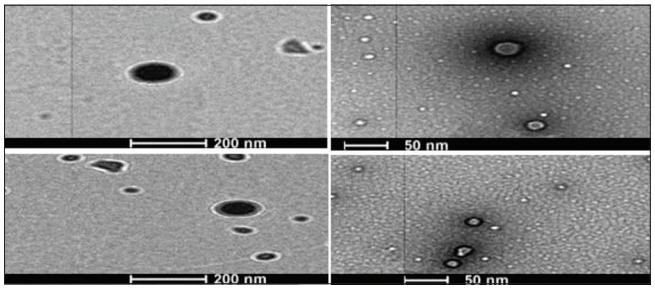

Fig. 8: Transmission electron microscopy images of optimized formulation of nanosuspension FB8

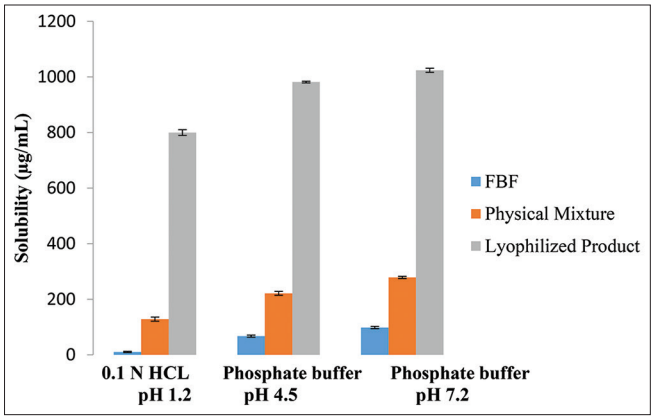

Fig. 9: Saturation solubility results of flurbiprofen, physical mixture, and lyophilized product. (All values are mean±standard deviation [n=3])

formulation (FB8) is statistically significant ($p<0.05$) when compared with FB6 by one-way ANOVA followed by Dunnett's test.

FTIR analysis

FTIR spectra of FBF and its nanosuspension (Fig. 5) depicted a typical broad peak of FBF in the range of 3000–3500 cm^{-1} due to hydrogen bonding in the compound which was broadened in the spectra of lyophilized formulation. The distinctive sharp peak at 1404 cm^{-1} represents stretching of C-F and peak at 1687 cm^{-1} represents the carbonyl stretch (C = O) in the FBF. The O-H stretch of carboxylic acid group represented by peak at 3072 cm^{-1} and C-H stretch characterized by peaks at 2945 cm^{-1} and 2981 cm^{-1}. Slight shifting of peaks in lyophilized nanosuspension FB8 from 2976 cm^{-1} to 2981 cm^{-1} and 3072 cm^{-1} to 3074 cm^{-1} indicates an enhancement of hydrogen bonding. The spectra of physical mixture and lyophilized nanosuspension showed the same absorbance pattern indicating the compatibility of drugs and polymers. Therefore, no shifting of position of the functional groups and no major interaction between FBF and polymers have been observed. Results likewise indicated that upgrade in the dissolution rate of FBF was not caused because of any chemical interaction among FBF and polymers.

DSC analysis

Fig. 6 represents the DSC thermograms of crude FBF, HPMC E15, poloxamer 188, physical mixture, and lyophilized nanosuspension. FBF demonstrated a sharp endotherm at 117.88°C ascribed to its crystalline nature. The peak of FBF (117.54°C) was maintained in the physical mixture of FBF with HPMC E15 and poloxamer 188 which noticeably indicates the absence of the physical interaction between drugs and excipients. Lyophilized nanosuspension revealed a sharp endotherm at 117.08°C which may represent the decreased particle size or miscibility of drugs with excipients. The above results signify the fact that there

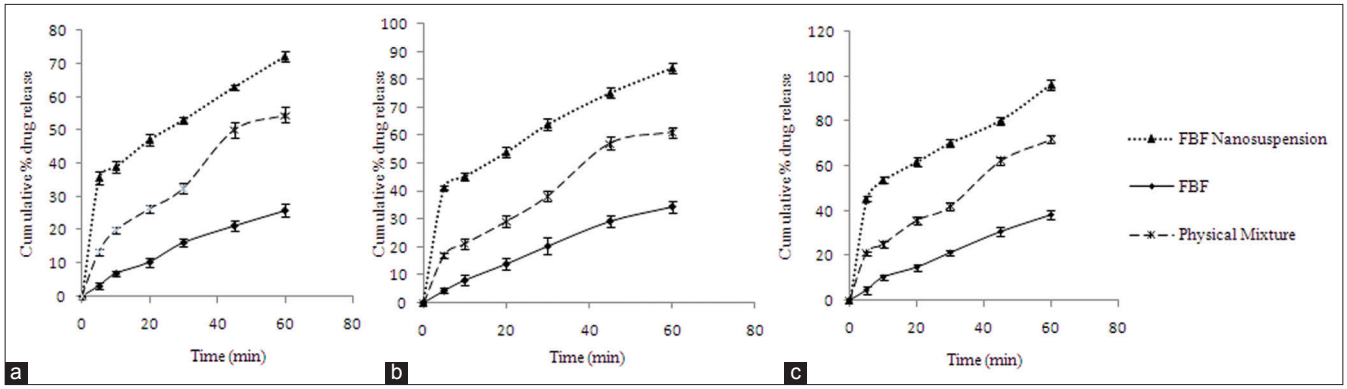

Fig. 10: *In vitro* drug release profile of pure flurbiprofen (FBF), physical mixture, and optimized FBF nanosuspension (FB8) in 0.1 N HCl pH 1.2 (a), in phosphate buffer pH 4.5 (b), and in phosphate buffer pH 7.2 (c)

is no significant change in crystallinity during the formulation of FBF. Results of DSC were verified by XRD analysis.

PXRD analysis

X-ray diffraction study (Fig. 7) of crude FBF, HPMC E15, poloxamer 188, physical mixture, and lyophilized nanosuspension FB8 was conducted to investigate the effect of excipients and method of formulation on the crystallinity of FBF. Pure FBF exhibited sharp and distinctive peaks at 2θ values of 12.1°, 18.0°, 19.8°, 19.9°, 22.7°, and 24.1° indicating its crystalline nature. The halo XRD pattern of HPMC E15 and poloxamer exhibited their amorphous nature. Distinctive peaks of FBF were evident in physical mixture and lyophilized nanosuspension, but their intensities were slightly reduced. The slight change in crystallinity could be due to the minor interactions of the added excipients with drug at specific angles.

Surface morphology by TEM study

The morphology of FBF nanoparticles suspended in nanosuspension was illustrated by TEM analysis, and obtained TEM micrographs of optimized nanosuspension are shown in Fig. 8. The images indicate spherical shapes with better dispersity of particles and level of particle agglomeration.

Saturation solubility study

Saturation solubility data of FBF, physical mixture and lyophilized powder in 0.1 N HCl (pH 1.2), phosphate buffer (pH 4.5), and phosphate buffer (pH 7.2) is presented in Fig. 9. The saturation solubility of lyophilized powder was significantly increased over 77 times (799.7±10.3 µg/ml vs. 10.3±2.1 µg/ml) in 0.1 N HCl, over 14 times (981.3±3.2 µg/ml vs. 67.33±3.7 µg/ml) in phosphate buffer pH 4.5, and over 10 times (1023.7±7.4 µg/ml vs. 98.22±4.1 µg/ml) in phosphate buffer pH 7.2 as compared with pure FBF. As being acidic in nature, FBF exhibited an increase in solubility with an increase in pH. A significant enhancement in solubility was observed in 0.1 N HCl. FBF is acidic in nature having p*K*a value ~ 4.03 has revealed an increase in solubility with an increased pH. Considerable enhancement in solubility was observed in 0.1 N HCl. Thus, an enrichment of solubility has the potential to increase its bioavailability. A significant increment in saturation solubility of FBF in physical mixture was observed might be due to the wetting action of surface stabilizers.

Dissolution study

FBF loaded nanosuspension is illustrated in Fig. 10 biphasic drug release outline. Rapid release of drugs was observed in the first phase due to the presence of free drugs not entrapped in polymer system. The second phase of slow release of drugs was observed due to slow diffusion of FBF through the polymer matrix. All the batches were optimized on the basis of minimum initial burst release and maximum release profile of drug. The batches with smaller particle sizes and lower entrapment of drugs showed high burst effect. The dissolution profile of optimized FBF nanosuspension in all three mediums displayed remarkable increase in dissolution rate compared with the physical mixture and pure FBF. Nanosuspension FB8 showed 96.33%, 84.31%, and 72.09% drug release compared with 38.21%, 34.41%, and 25.9% drug release by pure FBF in phosphate buffer (pH 7.2), phosphate buffer (pH 4.5), and 0.1 N HCl (pH 1.2), respectively. This stamped increment in dissolution rate might be because of decrease in particle size and wettability of the polymers. As indicated by the Noyes and Whitney equation, increment in the effective surface area builds the dissolution rate generally. HPMC E15 and poloxamer 188 are steric stabilizers which avert accumulation of particles and give wettability for better drug dissolution.

Table 5 indicates that *in vitro* drug release profile of optimized FBF nanosuspension (FB8) was best fitted with zero-order kinetics for dissolution in 0.1 N HCl and phosphate buffer pH 4.5 based on regression coefficient values (R^2=0.998 and R^2=0.993) respectively. It was best fitted with Korsmeyer–Peppas model for dissolution in phosphate buffer pH 7.2 (R^2=0.995). The release exponent (n) values of Korsmeyer–Peppas model for all FBF nanosuspensions were below 0.45 which indicates that the drug release follows Fickian diffusion mechanism. When dissolution pattern of FB8 in 0.1 N HCl was compared with that of in phosphate buffer pH 7.2; the difference factor (f_1) and similarity factor (f_2) were found to be 14 and 64, respectively, exhibiting similar pattern of drug dissolution.

Physical stability study

Physical stability is a fundamental problem in the development of nanosuspension due to aggregation of nanoparticles and Ostwald ripening effect. Ostwald ripening is the process of growth of larger particles from smaller particles that contribute to instability of nanosuspension. The physical stability study of optimized FBF nanosuspension was performed at 4°C and 25°C over 3 months. The minor increase in particle size and PDI was seen at both storage conditions after the completion of 3 months (Table 6). Therefore, the increase in particle size was insignificant. The initial and at the end of 3 months absolute zeta potential values of nanosuspension remained in between −15.91±0.08 mV and −7.91±0.37 mV indicating that nanosuspensions were physically stable. It might be due to the presence of HPMC E15 and poloxamer 188, which probably served as crystal agglomeration inhibitor by adsorbing onto the surface of FBF nanoparticles.

CONCLUSIONS

In the present work, FBF nanosuspensions were successfully developed by the nanoprecipitation method. Prepared nanosuspensions exhibited a great degree of aqueous solubility, dissolution rate, and stability. The resultant FBF nanosuspensions were physically stable with mean particle size range of 200–400 nm. There was a slight reduction in crystalline nature of FBF in the nanosuspensions due to the presence

of excipients. Hence, the polymeric system of HPMC E15 and poloxamer 188 was proved effective in the development of stable nanosuspension of FBF by exerting steric stabilization.

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