ORIGINAL ARTICLE

Formulation and Evaluation of Lornoxicam Nanocrystals with Different Stabilizers at Different Concentrations

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Abstract

Aim: To develop and evaluate nanocrystals of lornoxicam to improve solubility by converting pure drug of lornoxicam which is in micronized form to nanosized form. **Materials and Methods:** Saturation solubility of lornoxicam was evaluated by adding excess of the drug in 5 mL of different media (0.1 N HCl, phosphate buffer pH 6.8 and pH 7.4). Nanocrystals of lornoxicam were prepared successfully using polyvinylpyrrolidone (PVP) and β-cyclodextrin (BCD) as stabilizers by antisolvent precipitation method. The prepared nanocrystals were evaluated for their physicochemical characteristics such as physical appearance, Fourier transform infrared (FTIR), differential scanning calorimetry, scanning electron microscopy, X-ray powder diffractometry, solubility studies, particle size distribution, zeta potential, and *in vitro* drug release studies. **Results and Discussion:** This research work has been made to improve solubility by converting pure drug of lornoxicam which is in micronized form to nanosized form. The FTIR spectroscopy was used to confirm compatibility and to rule out any possible interactions between drug and polymers. Six nanocrystal formulations (PF1, PF2, PF3, BF1, BF2, and BF3) consisting pure drug of lornoxicam (micronized form) with PVP and BCD used as stabilizers in the ratios of 1:1, 1:2, and 1:3, respectively, were prepared. *In vitro* drug release from nanocrystals was carried out in different buffers, and the data obtained were fit into different equations and kinetic models to explain release kinetics. Lornoxicam with PVP and BCD in 1:3 ratio formulations in 7.4 pH phosphate buffer showed better solubility and emerged to be an ideal formulation for lornoxicam nanocrystals. **Conclusion:** From the study results, it can be concluded that optimized nanocrystals formulation of has improved solubility as compared to pure drug. The developed nanocrystals of lornoxicam found useful to improve solubility of lornoxicam.

Key words: Antisolvent precipitation technique, β-cyclodextrin, lornoxicam, nanocrystals, polyvinylpyrrolidone

INTRODUCTION

Currently, one of the main applications of nanotechnology in drug delivery is to overcome the problem of poor water solubility of hydrophobic drugs. Approximately, 40% of all developmental new chemical entities are poorly water soluble and, therefore, are difficult or impossible to formulate. Rather than abandon what could be a promising candidate drug or struggle with a non-optimal formulation, a range of nanotechnology-based technologies can be employed to improve drug solubility. Many of these technologies work on the premise that when the particle size of a drug is reduced to the nanometer range, the surface area is significantly increased, thereby enhancing the solubility of the drug. Size reduction can occur via a number of means, including milling or homogenization techniques. Once drug nanoparticles are produced, many technologies involve the addition of stabilizers or further formulants to prevent re-agglomeration of drug particles. As many of these size reduction technologies involve the use of milling or homogenization techniques, they are most suited to robust small chemical entities rather than more delicate

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macromolecules. The overall target of these approaches is to achieve enhanced drug bioavailability as well as potentially reduced toxicity because less amount of drug is needed to ensure the optimal dose. For orally delivered drugs, improvements in solubility can also reduce variability resulting from food effects; that is, whether the patient is in the fed or fasted state.[1] It is important to improve the solubility and/or dissolution rate for poorly soluble drugs because these drugs possess low absorption and bioavailability.[2] As solubility is an important determinant in drug liberation, hence it plays a key role in its bioavailability. Any drug to be absorbed must be present in the form of an aqueous solution at the site of absorption.[3,4]

The term drug nanocrystals imply a crystalline state of the discrete particles but depending on the production method they can also be partially or completely amorphous. Drug nanocrystals can be produced by bottom-up technologies (precipitation methods) or alternatively by top-down technologies (size reduction methods). At present most of the industrially feasible methods, they are top-down technologies: all products in the market are made by size reduction.

Drug nanocrystals are particles made from 100% drug; typically, they are stabilized by surfactants or polymeric steric stabilizers.[5,6] Hence, these particles possess a 100% drug loading in contrast to matrix nanoparticles consisting, e.g. of a polymeric matrix[7] or a lipidic matrix nanoemulsions.[8-12] The high loading makes them very efficient in transporting the drug to or into cells, reaching a sufficiently high therapeutic concentration for the pharmacological effect.

The main aim of this study is to enhance the dissolution rate and solubility of the lornoxicam, a poorly aqueous soluble drug by preparing nanocrystals using antisolvent precipitation technique. The drug chosen for the present investigation is lornoxicam. Lornoxicam is poorly water-soluble drug, belonging to BCS Class II (i.e., low solubility and high permeability). Lornoxicam is highly bound (99%) to plasma proteins with low apparent volume of distribution (0.2 L/kg). Lornoxicam is extensively metabolized in the liver, to the inactive metabolite 5'-hydroxy-lornoxicam. Excretion is shared between the renal (42%) and fecal (51%) routes. Lornoxicam has a relatively short terminal plasma elimination half-life (mean 3-5 h in healthy young volunteers), with considerable inter-individual variability. Hence, there was a need to improve lornoxicam dissolution profile and in turn its bioavailability.

MATERIALS AND METHODS

Materials

Lornoxicam was gift sample from M/s Dr. Reddys Laboratory, Hyderabad, β-cyclodextrin (S.D Fine Chemicals), polyvinylpyrrolidone-K30 (PVP-K30) (S.D Fine Chemicals), and dimethyl sulfoxide (DMSO) (S.D Fine Chemicals) were procured from commercial sources.

Methods

Preformulation studies

Solubility studies

Saturation solubility of lornoxicam was evaluated by adding excess of the drug in 5 mL of different media (0.1 N HCl, Phosphate Buffer pH 6.8 and pH 7.4) in 10 mL of glass vials. These vials were then kept in orbital shaker for 24 h at 37°C. The solution was then filtered using syringe filter (0.22 μm), and the absorbance was taken using an ultraviolet (UV) spectrophotometer to determine the amount of drug dissolved.

Analytical method for lornoxicam estimation (UV method)

The ultraviolet/visible spectrophotometric method (UV-1601PC, Shimadzu Corporation, Japan) was selected for the estimation of lornoxicam. The diluted solution of pure drug was scanned in between the wavelength of 800-400 nm. 10 mg of lornoxicam was dissolved in 100 mL of 0.1 N HCl, phosphate buffer, pH 6.8, phosphate buffer, pH 7.4 (stock solution of 100 μg/mL). From these stock solutions, 0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL, 1 mL, 1.2 mL, 1.4 mL, and 1.6 mL were withdrawn using micropipette into 10 mL volumetric flasks, and the volume was made up to 10mL with respective buffers to get the concentration of 2, 4, 6, 8, 10, 12, 14, and 16 μg/mL. The absorbances of the samples were measured.

Formulation of nanocrystals

Nanocrystals of lornoxicam were prepared successfully using PVP and β-cyclodextrin (BCD) as stabilizers by antisolvent precipitation method. 100 mg of lornoxicam pure drug was dissolved in 10 ml of DMSO (Solution I). Stabilizing agents of PVP and BCD (100, 200, and 300 mg) were dissolved in 100 ml of double distilled water (Solution II). This Solution II was placed under propeller mixer at constant speed of 1400 rpm. Then, drug solution (Solution I) was injected into Solution II with the help of 25 mm syringe dropwise. Drug gets precipitated from the Solution II. It is allowed to stir for 2 h. Then, it is centrifuged for 10 min at a speed of 10000 rpm at 4°C, and then suspended in distilled water and sonicated for 10 min. After sonication, the suspension was filtered using vacuum filtration, then dried for 24 h at 70°C. The details of different ratios of formulations are tabulated in Table 1.

Evaluation of nanocrystals

Fourier transform infrared (FTIR) analysis of nanocrystals

Nanocrystals, which consists of purely 100% drug, FTIR spectroscopy was used for the confirmation of the presence

Table 1: Formulations with different ratios of drug: stabilizer

Formulation code	Stabilizer	Drug:Stabilizer ratio
PF1	PVP	1:1
PF2		1:2
PF3		1:3
BF1	BCD	1:1
BF2		1:2
BF3		1:3

PVP: Polyvinylpyrrolidone, BCD: β-cyclodextrin

of lornoxicam. The source provided a continuous spectrum of radiation ranging from 4000 to 500/cm. Intensities of absorption bands were expressed as % transmittance. Sample preparation was done using the potassium bromide pellet method. Component of analysis was added to powdered potassium bromide in the ratio of 1:100. The mixture was compacted under pressure (10 tons/cm^2) in vacuum to form a transparent pellet (13 mm in diameter) and was subjected to immediate analysis.

Surface morphology using scanning electron microscopy (SEM)

The average size and size distribution of lornoxicam and lornoxicam nanocrystals were determined by SEM (Oxford Instruments, model - INCA wave), in which the samples were mounted rigidly on the surface of a bronze-specimen holder called a specimen stub using a double-sided adhesive tape and coated with an ultrathin coating of electrically-conducting material, gold, deposited on the sample either by low vacuum sputter coating or by high vacuum evaporation with gold and observed under suitable magnification.

X-ray diffraction studies (XRD)

X-ray diffraction analysis was employed to detect the crystallinity of lornoxicam and lornoxicam nanocrystals, which was conducted using an XRD-6000 diffractometer (Shimadzu, Japan). The powder was placed in a glass sample holder. CuK radiation was generated at 30 mA and 40 kV. Samples were scanned from 5 to 50 with a step size of 0.05.

Differential scanning calorimetry (DSC)

DSC was performed using DSC-60, Shimadzu, Japan. The instrument comprised calorimeter (DSC 60), flow controller (FCL 60), Thermal analyzer (TA 60), and operating software TA 60. The samples (lornoxicam and lornoxicam nanocrystals) were placed in sealed aluminum pans and heated under nitrogen flow (30 mL/min) at a scanning rate of 5°C/min from 25°C to 260°C. Empty aluminum pan was used as a reference. The heat flow as a function of temperature was measured for the lornoxicam and lornoxicam nanocrystals.

In vitro drug release

In vitro drug release of the samples (lornoxicam and lornoxicam nanocrystals) was carried out using USP-type I dissolution apparatus (basket type). The volume was 900 ml of dissolution medium (0.1 N hydrochloric acid solutions and phosphate buffer solution pH 6.8, 7.4). The temperature of the medium was maintained at 37 ± 0.5°C. The apparatus was allowed to run for 50 rpm. Aliquots of 5 ml samples were withdrawn at various intervals. The samples were filtered through Whatman filter. The fresh dissolution medium (0.1 N hydrochloric acid solutions and phosphate buffer solution pH 6.8 and 7.4) was replaced every time with the same quantity of the sample. Collected samples were analyzed at λ_{max} of drug (376 nm in 0.1 N hydrochloric acid solutions and 376 nm in phosphate buffer solution pH 6.8 and 7.4, respectively).

Particle size determination

The size of particles and their distribution were determined using Zetasizer (Nano ZS Malvern Instruments, UK) using a process called dynamic light scattering (DLS). Samples were examined to determine the mean particle size, size distribution, and polydispersity index (PDI). This technique measures the time-dependent fluctuations in the intensity of scattered light, which occurs because the particles are under Brownian motion. Analysis of these intensity fluctuations enables the determination of the diffusion coefficient of the particles, which are converted into the size distribution. This instrument is equipped with a 633 nm, 4 mW helium/neon laser (red laser), and it measures the nanosuspension sample with non-invasive backscatter technology at a detection angle of 173°. The average particle size and PDI of the nanosuspension samples were determined at 25°C. The results are represented as an average diameter of the nanosuspension (Z-average mean) with the PDI. The particle size distribution (PSD) was characterized using PDI, which is a measure of the width of size distribution.

Zeta potential

Measurement of zeta potential of samples in the Zetasizer (Nano ZS Malvern Instruments, UK) was done using a combination of laser Doppler velocimetry (LDV) and phase analysis light scattering (PALS) by a patented technique called M-3PALS to measure the particle electrophoretic mobility. The nanosuspension samples were measured at 25°C for zeta potential.

RESULTS AND DISCUSSION

Saturation solubility of lornoxicam

The saturation solubility of lornoxicam was carried out in different buffer solutions to select a suitable dissolution

medium for *in vitro* release studies. The saturation solubility of lornoxicam in different media is shown in Table 2. Based on the saturation solubility, data of lornoxicam have good solubility in phosphate buffer (pH 7.4); however, it decreased in pH 6.8 and 0.1 N HCL in descending manner, respectively. This clearly showed that lornoxicam has pH dependent solubility, i.e. solubility increases in very high alkaline medium and low in acidic medium. Based on the solubility studies data, 7.4 pH phosphate buffer was selected as dissolution medium for *in vitro* release studies dissolution method for lornoxicam.

Table 2: Saturation solubility of lornoxicam in different media

Media	Concentration (mg/ml)
0.1 N HCL	0.053922
Phosphate buffer, pH 6.8	0.081967
Phosphate buffer, pH 7.4	0.016129

Analytical method for the estimation of lornoxicam using UV spectrophotometer

The standard calibration plots of the drug were prepared in 0.1 N hydrochloric acid solutions and phosphate buffer solution pH 6.8. The solutions of lornoxicam were scanned by the UV spectrophotometer at the wavelength range of 800-4000 nm. The λ_{max} of drug solution is 376 nm in 0.1 N hydrochloric acid solutions and phosphate buffer solution pH 6.8, pH 7.4.

Fourier transforms infrared spectroscopy

FTIR analysis was carried out for both pure drug and nanocrystals prepared using stabilizers PVP and BCD. The presence of characteristic peaks associated with specific structural characteristics of drug molecules was noted. Figure 1 illustrates the FTIR spectrum of pure drug and nanocrystals prepared using stabilizers PVP and BCD. The FTIR spectrum of lornoxicam has a characteristic peak at 3066/cm corresponding to –CH starching of heteroaromatic ring. 2186/cm and 2358/cm corresponding to –NH stretches vibration. Intense absorption peak was found at 1,613 cm^{-1} due to the stretching vibration of the C=O group in the primary amide. Other peaks were observed at 1591.06/cm, 1535.32/cm, and 1421/cm and were assigned to bending vibrations of the N–H group in the secondary amide. The stretching vibrations of the O=S=O group appeared at 1328. Other prominent peaks appeared at 861.94/cm corresponding to –CH aromatic ring bending and heteroaromatics and at 788.20/cm due to the C–Cl bending vibration. All these prominent peaks of lornoxicam were present in lornoxicam in combination with excipients. It clearly indicates that the drug has retained its identity without losing its characteristics.[13] Considerable changes in the IR peaks of the drug were not observed when nanocrystals prepared using PVP and BCD indicating the absence of interaction between drug and excipients.

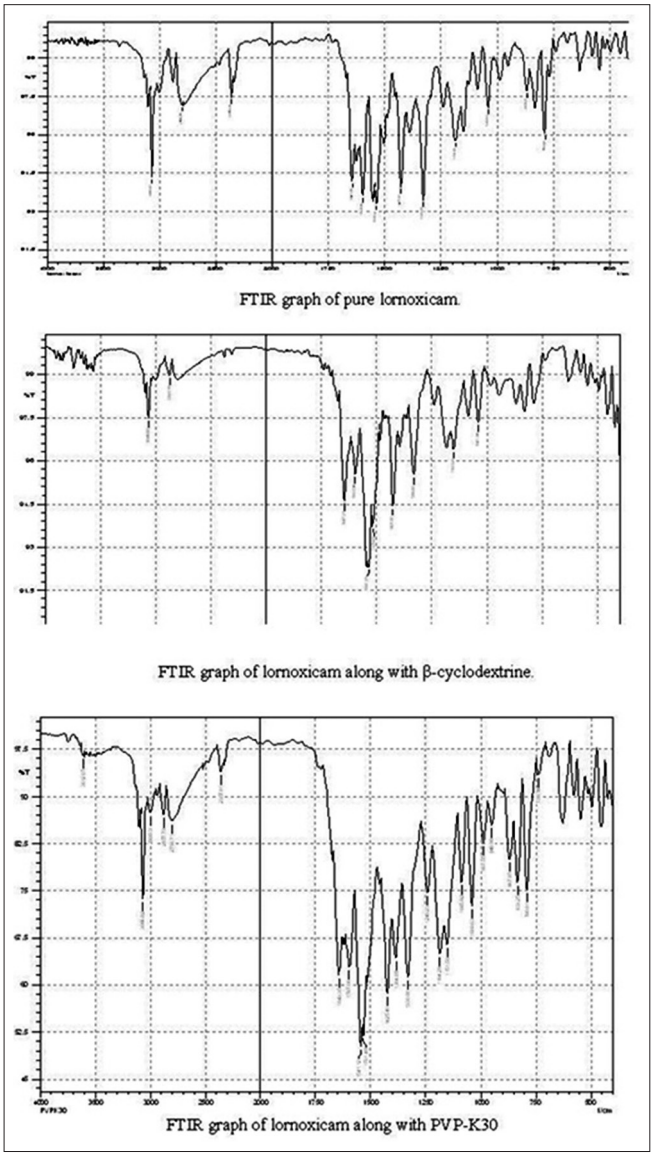

Figure 1: Fourier transform infrared spectrum of pure drug and nanocrystals prepared using stabilizers polyvinylpyrrolidone and β-cyclodextrin

DSC

The DSC thermograms of the pure drug and nanocrystals prepared using stabilizers PVP, and BCD excipients are shown in Figure 2. DSC curves for the lornoxicam and lornoxicam nanocrystals with a single sharp endothermic peak, attributing to the melting point of lornoxicam at 229.3°C, lornoxicam nanocrystal in PVP (PF3) at 221.5°C, and lornoxicam nanocrystal in BCD (BF3) at 221.6°C, respectively. There was no substantial change in the melting peak of drug in the nanocrystals when compared to pure drug. The small shift to the lower melting point after precipitation process may attribute to the reduction of particle size to nanometer range. By reduction of dimensions of particles from micron range or even bigger down to nano range, the surface-to-volume ratio increases significantly, and the surface energy substantially

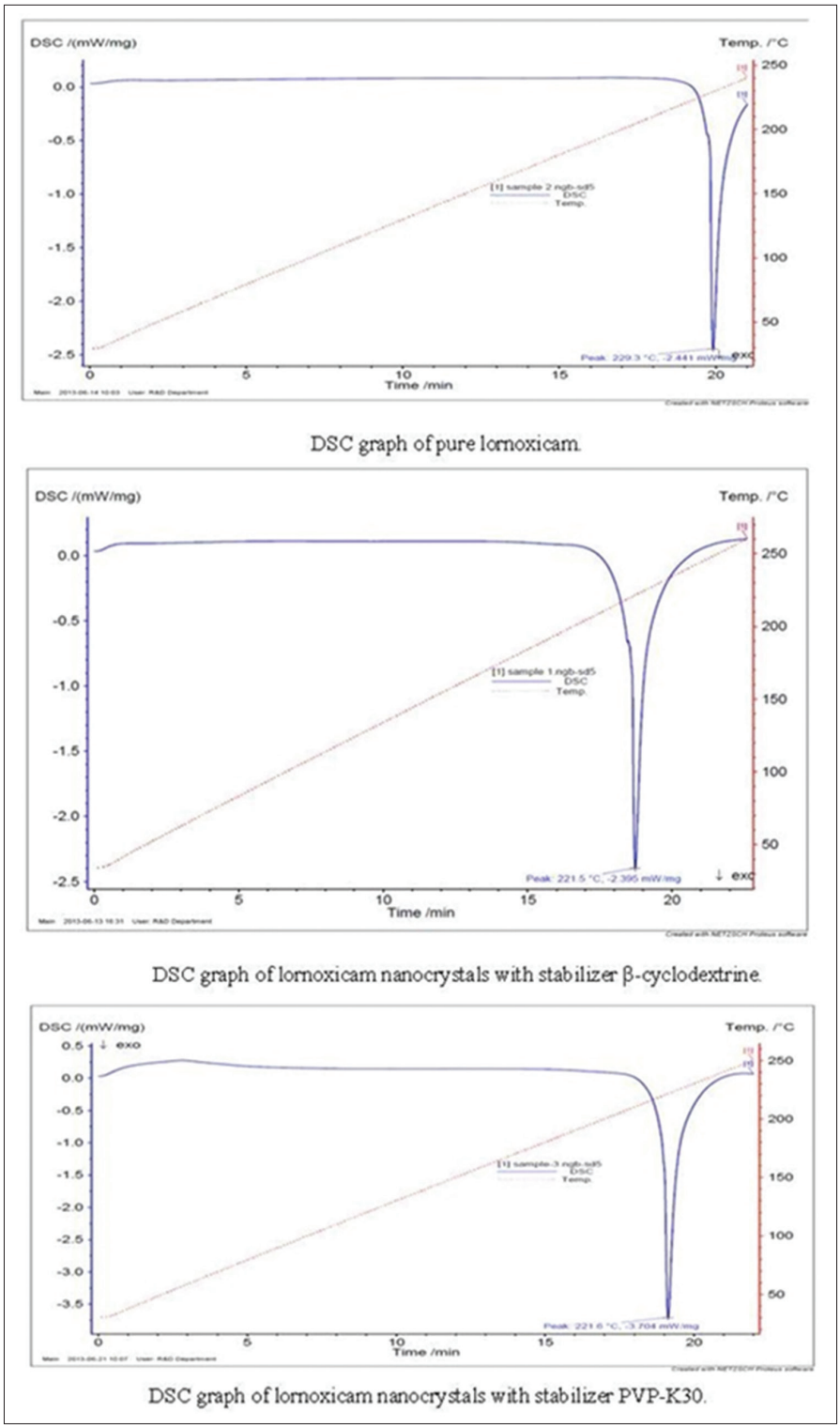

Figure 2: X-ray powder diffractometry patterns of pure drug and nanocrystals prepared using stabilizers polyvinylpyrrolidone and β-cyclodextrin

affects the interior ''bulk'' properties of the material. In other words, the nanosized small particles have a higher proportion of surface molecules with fewer nearest neighbors than larger particles, and thus are more weakly bound and less constrained in their thermal motion than molecules in the body of crystals, which is supposed to be responsible for the decrease of the melting point.

X-ray powder diffractometry (XRPD)

XRPD analysis was performed to analyze whether any potential changes happened in the inner structure of the lornoxicam nanocrystals in comparison to the lornoxicam pure drug. The XRPD patterns for the lornoxicam and lornoxicam nanocrystals are shown in Figure 3. It can be

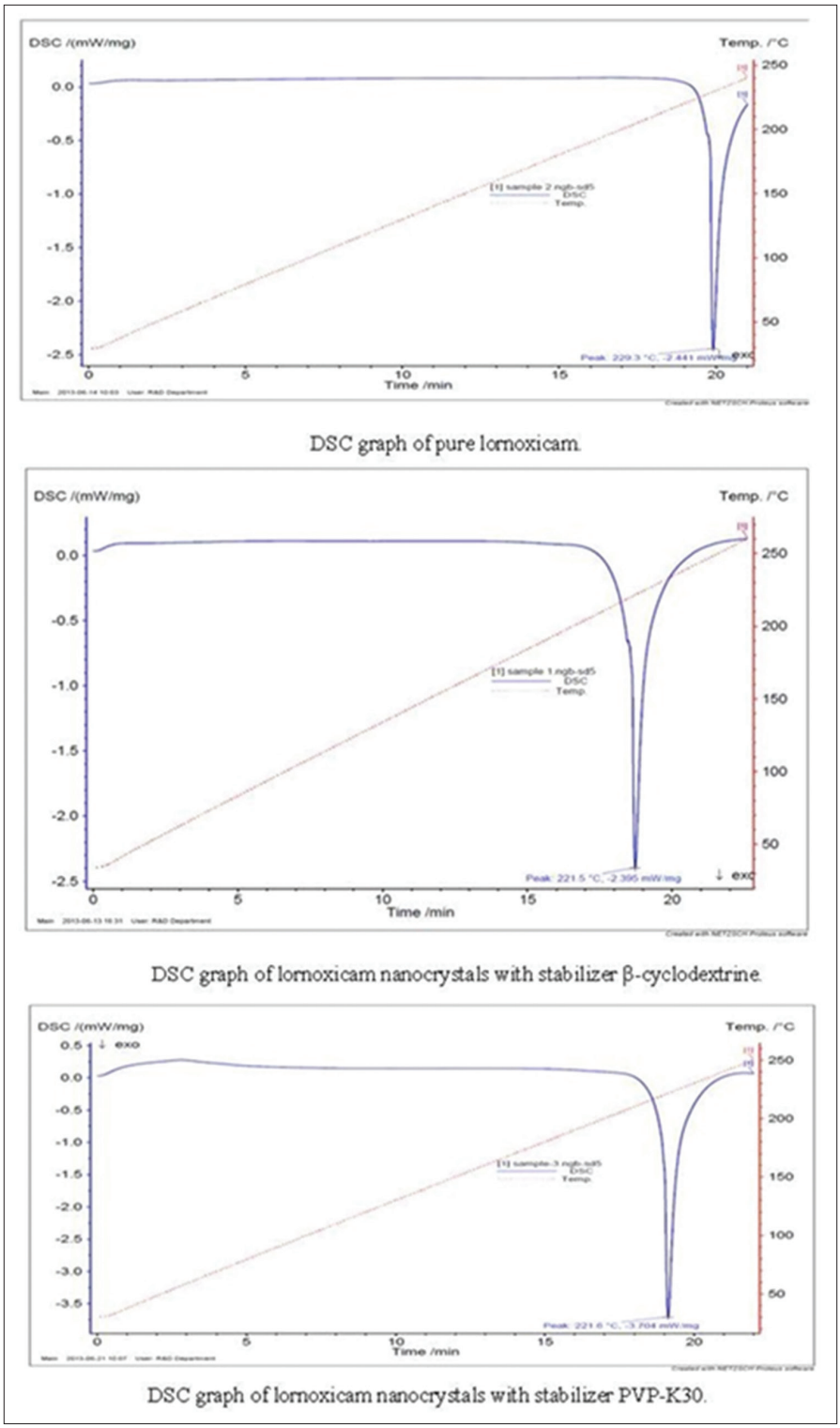

Figure 3: Differential scanning calorimetry curves of pure drug and nanocrystals prepared using stabilizers polyvinylpyrrolidone and β-cyclodextrin

observed that the characteristic peaks in X-ray diffraction pattern of the lornoxicam nanocrystals are the same as that of the lornoxicam pure drug, indicating the same crystalline modification. The identical 2 h peaks at 17, 19, 22, 22.5, 25, and 27.5 appeared in all XRPD. However, the degree of crystallinity, representing as intensity in XRPD diagram, was significantly decreased after preparation of lornoxicam nanocrystals by precipitation process. This could be attributed to so-called ''particle size broadening'' phenomenon in the XRPD analysis of crystalline materials <1 μm and the partially amorphous property of the lornoxicam nanocrystals.

PSD

The nanocrystals were characterized with respect to practical yield and particle size. The particle size and the width of the PSD are important characterization parameters as they govern saturation solubility, physical stability, dissolution rate, drug absorption, and biological performance of nanoparticles. The PSD of lornoxicam and lornoxicam nanocrystals are shown in Table 3. The PSD reports are shown in Figure 4. The results from clearly suggest that as drug: polymer ratio increased from 1:1 to 1:3, particle size decreased significantly from 818 to 349 nm PVP (PF3) as stabilizer and 276.6 to 149 nm in the case of BCD (BF3).

Table 3: Particle size and zeta potential values of lornoxicam and lornoxicam nanocrystals

Formulation	Zeta potential	Particle size (nm)
Pure	−15.6	3040
PF1	10.8	818
PF2	8.35	629
PF3	7.0	349
BF1	12.63	276.6
BF2	9.46	206
BF3	6.35	149

Zeta potential

The zeta potential of a particle is the overall charge that the particle acquires in a particular medium. It is used to predict the particle-particle interaction. Knowledge of the zeta potential of nanosuspension helps to assess the stability of formulation during storage. If it is not within the range, the attractive forces exceed the repulsive forces and this leads to aggregation of particles The nanoparticles possessing a zeta potential <−30 and >+30 mV are generally considered as stable. The charge on the surface of the nanospheres will influence their distribution in the body and extent of uptake into cells. There is greater electrostatic affinity for positively charged nanoparticles because cell membranes are negatively charged. The PSD reports are shown in Figure 5. All the formulations were analyzed for zeta potential during the formulation development process. The zeta potential values of the nanoparticles were within the

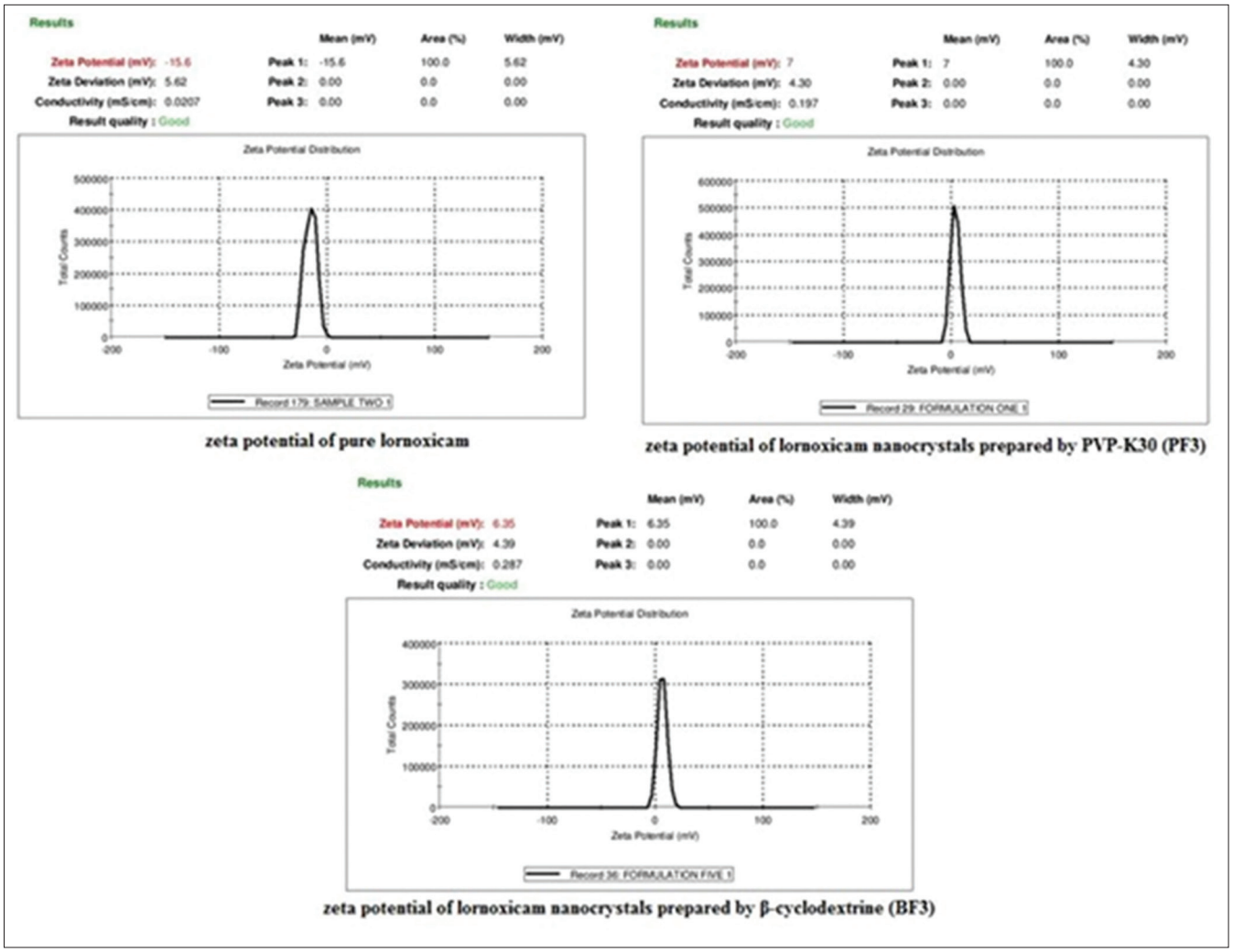

Figure 4: Particle size distribution of pure drug and nanocrystals prepared using stabilizers polyvinylpyrrolidone and β-cyclodextrin

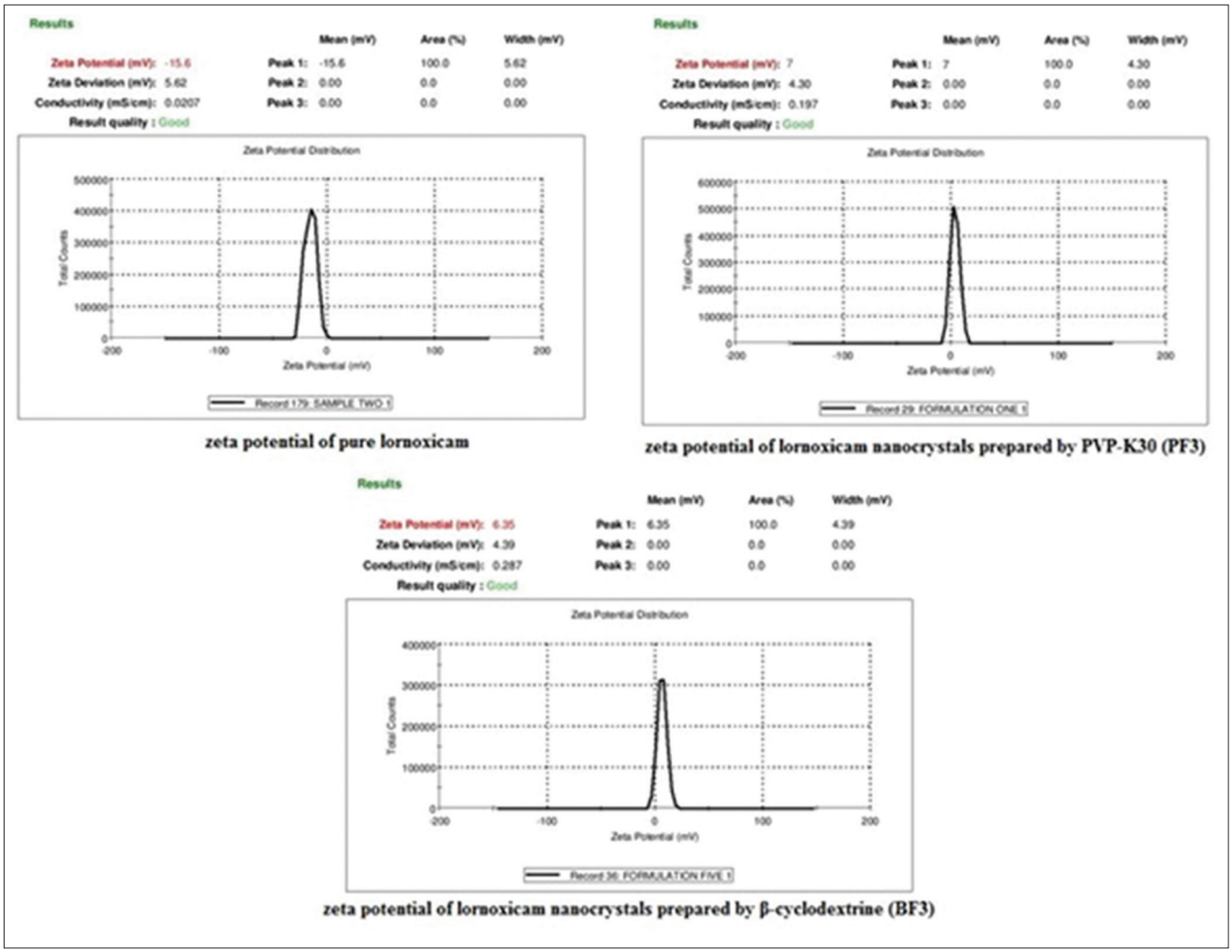

Figure 5: Zeta potential results of pure drug and nanocrystals prepared using stabilizers polyvinylpyrrolidone and β-cyclodextrin

range of −15.6 to +6.35 mV, indicating that the colloidal suspension is stable.

Surface morphology of nanocrystals: SEM

Particle shape is related to geometric shape and surface regularity. This will influence the surface area which, in turn, affects the dissolution of the particles. The nanocrystals prepared using PVP and BCD as stabilizers were observed for shape and surface morphology by SEM. The nanocrystals were slightly aggregated but they were almost spherical in shape, and size was found below 1 μm. The SEM photograph is shown in the Figure 6.

In vitro release studies

In vitro release studies of pure lornoxicam and nanocrystals were carried out in 0.1N HCl, Phosphate buffer pH 6.8, and phosphate buffer pH 7.4. Release data were presented in terms of dissolution profile curves Figure 7. From the release studies, it was found that pure drug dissolved more in the case of pH 7.4 phosphate buffer than pH 6.8 phosphate buffer than 0.1 N HCL. In 0.1 N HCL, pure drug showed 30.62% drug release at 60 min, whereas in nanocrystals prepared by PVP (PF3) formulation showed 50.56 % drug release at 60 min and in case of BCD (BF3) formulation showed 60.44% drug release at 60 min as the highest. In pH 6.8 phosphate buffer, pure drug showed 56.47% drug release at 60 min, whereas in nanocrystals prepared by PVP (PF3) formulation showed 73.18% drug release at 60 min and in case of BCD BF3 formulation showed 97.82 % drug release at 60 min as the highest. In pH 7.4 phosphate buffer, pure drug showed 78.68 % drug release at 60 min, whereas in nanocrystals prepared by PVP (PF3) formulation showed 99.29% drug release at 45 min and in case of BCD (BF3) formulation showed 100.161% drug release at 20 min as the highest. This might be because of high solubility of lornoxicam in the alkaline medium which was already reported in saturation solubility studies, and the drug was also not chemically changed by the use of stabilizer.

CONCLUSION

Lornoxicam is a non-steroidal anti-inflammatory drug (NSAID) of the oxicam class with analgesic,

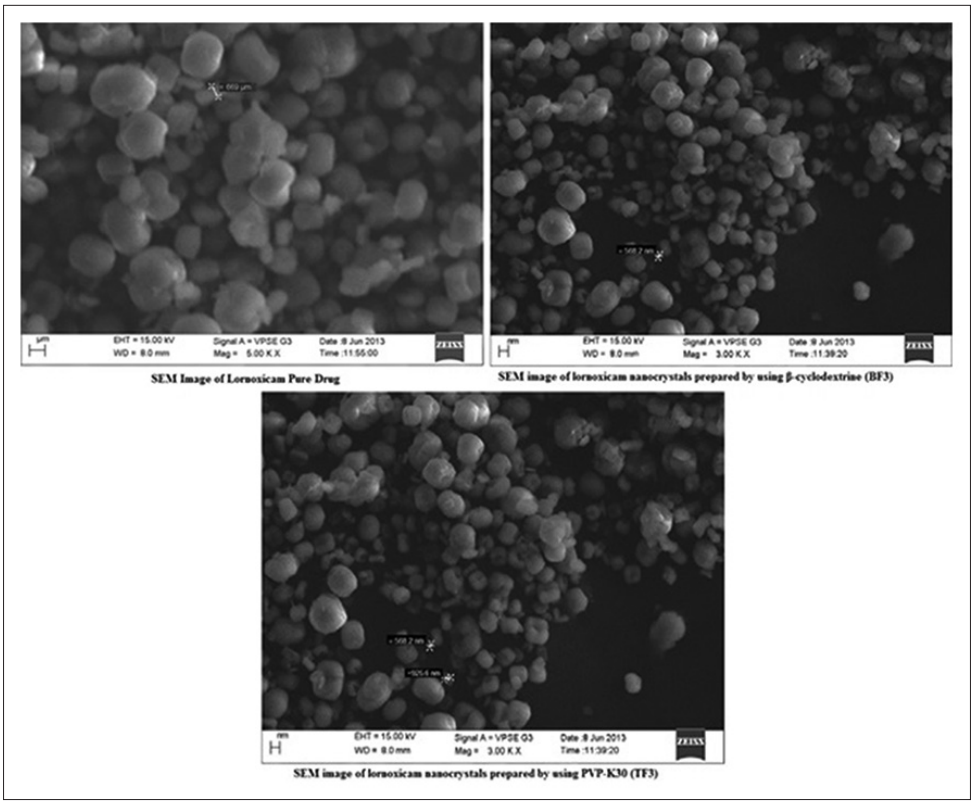

Figure 6: Scanning electron microscopy images of pure drug and nanocrystals prepared using stabilizers polyvinylpyrrolidone and β-cyclodextrin

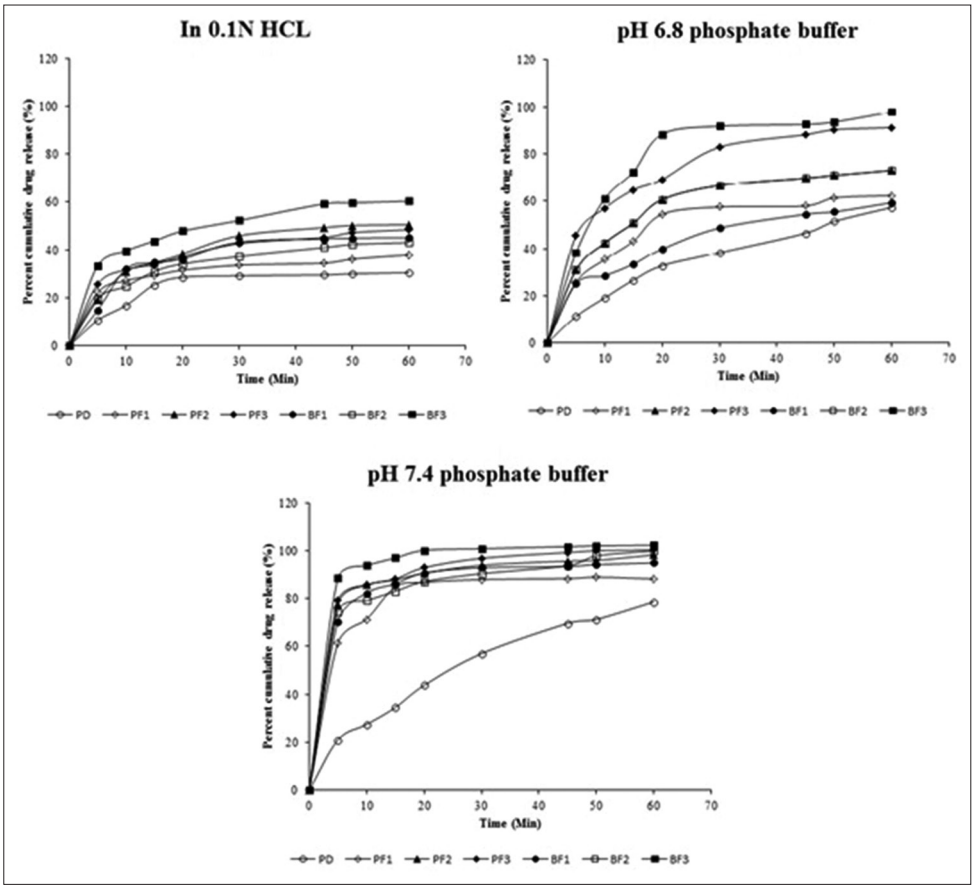

Figure 7: *In vitro* release profiles of pure drug and nanocrystals prepared using stabilizers polyvinylpyrrolidone and β-cyclodextrin

anti-inflammatory, and antipyretic properties. Lornoxicam belongs to BCS Class II (low soluble and high permeable). Therefore, an attempt has been made to improve solubility by converting pure drug of lornoxicam which is in micronized form to nanosized form. Nanocrystals of lornoxicam were developed with different ratios of PVP and BCD polymers as stabilizers using antisolvent precipitation technique. The FTIR spectroscopy was used to confirm compatibility and to rule out any possible interactions between drug and polymers. Six nanocrystal formulations (PF1, PF2, PF3, BF1, BF2, and BF3) consisting pure drug of lornoxicam (micronized form) with PVP and BCD used as stabilizers in the ratios of 1:1, 1:2, and 1:3, respectively, were prepared. All formulations carried using DMSO and double distilled water as antisolvent system. The prepared nanocrystals were evaluated for their physicochemical characteristics such as physical appearance, FTIR, DSC, SEM, XRD, solubility studies, PSD, zeta potential, and *in vitro* drug release studies. *In vitro* drug release from nanocrystals was carried out in different buffers, and the data obtained were fit into different equations and kinetic models to explain release kinetics. Lornoxicam with PVP and BCD in 1:3 ratio formulations in 7.4 pH phosphate buffer showed better solubility and emerged to be ideal formulation for lornoxicam nanocrystals. Hence, it can be concluded that optimized nanocrystals formulation of lornoxicam improved the solubility of lornoxicam as compared to pure drug.

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