

# Formulation of Ibuprofen Nanoparticles and Nanosuspensions with Enhanced Dissolution Rate using Ultra-Homogenization Technique

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## Abstract

**Introduction:** Ibuprofen (IBU), a well-known drug with dissolution rate-limited bioavailability was formulated as nanoparticles and nanosuspensions by ultra-homogenization technique, employing a series of hydrophilic polymers/surfactants. **Materials and Methods:** Various nanosuspensions were prepared using different hydrophilic polymers, freeze-dried into nano-particles and evaluated for relevant physicochemical characteristics. Based on the *in vitro* dissolution and particle size, the optimized drug: Polymer ratio was re-formulated into nanosuspensions using Tween-80 to study any further reduction in particle size and enhancement in dissolution as compared with marketed suspensions. **Results and Discussion:** All the suspensions prepared with the different drugs: Polymer ratio (1:1, 1:2 and 1:3) showed a significant decrease in particle size with increase in the number of homogenization cycles and pressure. Polyvinyl pyrrolidone K30 (PVP-K30) served as the best polymer to enhance the aqueous solubility of IBU, based on stability constant (K) and Gibbsfree energy ( $\Delta G_o$ ) values. IBU: PVP-K30 (1:2 w/w) nanosuspensions gave the least particle size ( $527 \pm 31.04$  nm) with a narrow polydispersibility index and high negative zeta potential value. Nanoparticles produced by freeze drying the nano-suspension were amorphous in nature as confirmed by differential scanning calorimetry and Fourier transform infrared and also showed enhanced release rate than the pure drug. Furthermore, a combination of IBU: PVP-K30:Tween 80 (T80) (1:2:2 w/w) homogenized nanosuspensions exhibited much smaller particle size ( $127 \pm 6.18$  nm) and two-fold faster release compared to the marketed products ( $DP_{15\text{ min}} = 95\%$ ). Other polymer combinations either did not show any significant change in particle size or exhibited agglomeration and crystal growth. **Conclusion:** IBU/PVP-K30/T80 nanosuspensions were successfully prepared by high-pressure homogenization technique, with enhanced solubility and dissolution properties which could reduce the required dose and gastrointestinal side effects of the pure drug. This, in turn, is expected to increase the therapeutic benefits of IBU and other similar drugs belonging to Class II of Biopharmaceutics Classification System system, as well as to decrease/abolish the ulcerative effect. The proposed technique also has the potential of commercial scale-up to formulate safe products with higher bioavailability.

**Key words:** Dissolution, ibuprofen, nanosuspension, particle size, ultra-homogenization

## INTRODUCTION

The number of drugs with poor water solubility still continues to increase during the drug discovery process. The majority of synthesized drugs belongs to Class II of the Biopharmaceutics Classification System (BCS) and is characterized by low solubility and high permeability through biological membranes. That is why the solubility and/or dissolution rate is the limiting step to oral absorption for all BCS Class II drugs. Therefore, improvement in solubility and/or dissolution rate is considered as a key factor for enhancing their bioavailability.<sup>[1]</sup>

Structural modifications such as prodrug,<sup>[2]</sup> formation of a salt or different polymorph<sup>[3]</sup> and pharmaceutical technologies such as micronization,<sup>[4]</sup> formation of solid dispersions,<sup>[5-8]</sup>

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or inclusion compounds,<sup>[9]</sup> solvent co-precipitation,<sup>[10]</sup> spray drying,<sup>[11]</sup> grinding<sup>[12,13]</sup> have been widely used to enhance the solubility and/or dissolution rate. Recently, the use of the crystalline sodium salt and polymeric precipitation inhibitors to improve pharmacokinetic profile of IB through supersaturation has also been reported.<sup>[14]</sup>

Pharmaceutical literature reviews about dissolution enhancement of hydrophobic drugs indicate that comminution has evolved as an effective method to prepare drug nanoparticles. Although nano-comminution has advantages, such as cost effectiveness and easy scale-up, the processing is significantly sensitive to the selection of a polymeric stabilizer.<sup>[15]</sup> The instability of the finely divided particles and their tendency to agglomerate is due to increase the effective surface of the drug particles, which is associated with a proportional vast increase of the surface energy.<sup>[16]</sup> Among the preparation methods of nanoparticles, nano-comminution has conveniently been utilized to prepare solid nanoparticles due to simplicity and cost-effectiveness.<sup>[17,18]</sup> Furthermore, an additional advantage of using wet comminution is that no organic solvent or harsh environment is needed and scaling-up to industrial level is easy. This is reflected into production of nanoparticle-based solid dosage forms, such as Emend® (Merck & Co.).

However, the proper choice of polymeric stabilizers effective in reducing the particle size to nanometers is relatively limited and dependent of the nature of the drug in question. Therefore, the additional use of small molecular weight surfactants is a common method to further decrease the particle size.<sup>[19,20]</sup> However, the choice is also based on empirical approaches. Many authors concluded that the preparation of drug nanoparticles via nano-comminution requires a series of trial and error experiments because there is a lack of systematic knowledge of the process.<sup>[15]</sup> High-pressure homogenization technology was found as a simple, effective, well-established technique and can be scaled up to an industrial level.<sup>[21]</sup> This method was found previously to be an efficient technique to prepare stable nanosuspensions of several drugs such as diclofenac,<sup>[22]</sup> paclitaxel,<sup>[23]</sup> budesonide,<sup>[24]</sup> clofazimine,<sup>[25]</sup> nifedipine,<sup>[26]</sup> bupravaquone,<sup>[27]</sup> amphotericin B,<sup>[28]</sup> and spirinolactone.<sup>[29]</sup>

Considering these general advantages of nanotechnology, the main objective of the present investigation was to prepare nanosuspensions of ibuprofen (IBU) which is a widely used non-steroidal anti-inflammatory drug. As its serum concentration and pharmacological effects are correlated, rapid drug absorption is a prerequisite for the quick onset of its analgesic action. Because of its low aqueous solubility and high membrane permeability, its dissolution from the dosage forms is the rate limiting step for its absorption. Thus, the improvement of IBU dissolution for its immediate release at absorption site in the gastrointestinal tract following oral administration is desirable. Furthermore, high concentrations of undissolved drug at the absorption site lead to undesirable

side effects; *viz.*, peptic ulcers and gastric bleeding after chronic use. It was thus considered of interest to design nano-sized IBU formulations by high-pressure homogenization. The choice of the hydrophilic polymer for homogenization was based on their influence on the solubility of IBU. The effect of the applied pressure and number of cycles during homogenization was investigated to achieve optimum size reduction and prevent re-aggregation of formed nanoparticles. The physicochemical properties of the produced particles was assessed in regard to particle size, zeta potential, dissolution rate, crystal/amorphous changes and drug-carrier interactions based on differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) studies. Furthermore, the *in vitro* drug release of the best nanosuspension was compared to that of marketed IB suspensions.

## MATERIALS AND METHODS

### Materials

IBU - Mallinckrodt Pharmaceuticals, USA; Poloxamer (PXM) 188, 338 and 407 - Spectrum Chemicals, USA; polyvinyl pyrrolidone K30 (PVP-K30); PVP-K15; polyethylene glycol (PEG) 4000, 10,000 and 20,000; ortho-phosphoric acid (OPA) - Fluka, Germany; beta cyclodextrin (BCD); hydroxy propyl BCD (HPBCD) - Sigma, Germany; sodium lauryl sulfate (SLS) - Loba Chemie, India; Gelita Sol P (GLP) - Gelita AG, Germany; vitamin E d-alpha tocopheryl PEG (TPG 1000 succinate) (vitamin E-TPG) - Isochem (France); acetonitrile (ACN) - HPLC grade; T80 - Merck, Germany; Ultra-pure water - Millipore, USA.

### Phase solubility studies

Phase solubility studies were carried out according to the method reported by Higuchi and Connors<sup>[30]</sup> to investigate the influence of various hydrophilic polymers (*viz.*; PVP-K15, PVP-K30, PXM-188, PXM-338, PXM-407, PEG-4000, PEG-10,000, PEG-20,000, BCD, HPBCD, SLS, GLP and vitamin E-TPG) on solubility of IBU. Excess amounts of IBU (>50 mg) were loaded in screw-capped conical flasks containing 25 mL of aqueous solution of each polymer at different concentrations (0, 0.5, 1, 2, 5 and 10% w/v) in ultra-pure water. The suspensions were continuously stirred at 250 rpm on an orbital shaker (Excella E5 Platform Shaker, New Jersey, USA) at 20°C ± 1°C for 24 h (this duration was found to be sufficient to reach equilibrium). The suspensions were filtered through Whatman No. 40 filter (Whatman Ltd., UK). The filtrates were suitably diluted with water and analyzed spectrophotometrically (Shimadzu UV-1601, UV/Vis spectrophotometer, Shimadzu Corp, Japan) for the dissolved drug at 222 nm ( $\lambda_{max}$ ). All analyses were performed in triplicates. Blank polymer samples at different concentrations used in the study were analyzed to rule out interference.

## Preparation of nanosuspensions and optimization of homogenization

To produce nanosuspensions of IBU alone and in combination with various polymers, the ultra-homogenization technique was used. IBU in micronized form (sieved through 60# size screen) was used for all the suspensions. The polymer was first dissolved in distilled water using a magnetic electric stirrer (Cole-Parmer Instrument Company, USA). The micronized drug was then dispersed in the aqueous polymer solution. Coarse suspensions were prepared using an Ultra-Turrax T25 basic overhead homogenizer (IKA, Germany) for 2 min. at 10,000 rpm. This served as a pre-milling step to avoid blocking of the homogenization gap by large suspended particles. The suspensions were then transferred to a high-pressure ultra-homogenizer (Emulsiflex C5, Avestin, Ottawa, Canada). The samples were homogenized at 500 bar for 15 cycles, followed by 750 bar for 15 cycles and finally 1250 bar for 20 cycles. The number of homogenization cycles and pressure were optimized based on the particle size analysis.

## Particle size analysis

The average particle size of each suitably diluted suspension was measured at 20°C by photon correlation spectroscopy (PCS) using N4 Plus Coulter Counter (Beckman Coulter, USA). PCS yields the mean particle diameter and the width of the particle size distribution (polydispersibility index [PI]).

## Measurement of Zeta potential

The physical stability of all nano-suspensions was determined by measurement of zeta potential using zeta meter 3.0 + (Zeta-Meter Inc., USA). All measurements were performed in triplicates in distilled water with conductivity adjusted to 50  $\mu$ S and field strength 75 V/cm.

## Lyophilization

The nanosuspensions were freeze dried to study drug-polymer interaction and effectiveness of nanosuspension formulation on IBU dissolution rate. The suspensions were rapidly frozen below -50°C in a deep freezer (Kelvinator Scientific, USA) and lyophilized using IL Shin Freeze dryer (Korea) at a pressure <10 mTorr at -40°C for 48 h. The dry samples were stored in airtight containers for further studies.

## Liquid chromatography analysis of IBU content

Analysis of drug content was performed using ultra-fast liquid chromatography (UFLC). The chromatographic system comprised a prominence UFLC pump equipped with a photodiode array detector (Shimadzu, Japan). The data were acquired and processed using Shimadzu LC Solutions software. Pre-filtered samples (10  $\mu$ L) were injected into

a Shim-pack XR-ODS column (3.0 mm I.D., 30 mm L, 2.2 mm particle size) maintained at 30°C. The mobile phase system consisted of ACN: Water: OPA (50:50:0.1 v/v) and was run in isocratic mode at a flow rate of 0.25 mL/min. The run time was 8 min. per injection and the elute was monitored at a wavelength of 222 nm. The developed UFLC method was validated for linearity (5-50  $\mu$ g/mL), repeatability, precision (intra-day and inter-day), accuracy, analyte stability, asymmetry, limit of quantification (LOQ), and limit of detection (LOD). Samples containing an equivalent of 50 mg of IBU were dispersed in a suitable quantity of ACN and sonicated (Elma Transonic, 460/H, Germany) for 15 min. The samples were filtered through 0.45  $\mu$  PTFE syringe filters (Membrane Solutions, China), suitably diluted with the solvent (ACN: Water [50:50 v/v]), loaded in an autosampler tray in glass vials and measured for drug content.

## Thermal analysis

DSC study was performed on IBU, PVP-K30, and optimized freeze-dried samples. Each sample (10 mg) was accurately weighed in an aluminum pan, crimped and sealed non-hermetically. An empty aluminum pan served as the reference. The analysis was performed using DSC 141 Setaram Group Thermal Analyzer (France) under a nitrogen flow of 40 mL/min and heating rate of 10°C/min in a 20-250°C temperature range.

## FTIR spectroscopy

Infra-red spectra of IBU, PVP-K30, and optimized freeze-dried samples were carried out using Thermo Scientific Nicolet iS50 FT-IR (USA) using attenuated total reflectance sampling station. The samples were scanned from 4000 to 400/cm at room temperature. Scans were obtained at a resolution of 0.5  $\text{cm}^{-1}$ .

## *In vitro* dissolution studies of lyophilized IBU: PVP-K30 nanoparticles

A dissolution study was performed on micronized IBU and lyophilized nanoparticles equivalent to 50 mg of the pure drug. The study was conducted for 60 min in 500 mL distilled water using USP XXV Type II (paddle) dissolution apparatus (Erweka DT 80, GmbH, Germany). The samples were stirred at a speed of 100 rpm, and the temperature was maintained at 37°C  $\pm$  0.5°C. Aliquots (5 mL) were withdrawn at predetermined time intervals, filtered through Whatman No. 40 filter paper (Whatman Ltd., UK) and measured at 222 nm spectrophotometrically, after suitable dilution with the dissolution medium if needed, to determine the amount of drug released. An equal volume of fresh dissolution medium kept at the same temperature was replaced after each sampling to maintain the sink condition. All studies were performed in triplicates.

## Preparation of IBU: PVP-K30:T80 nanosuspension and comparison of *in vitro* release with marketed suspensions

It was considered of interest to prepare and study the dissolution pattern of IBU: PVP-K30:T80 nanosuspensions, because of the extremely low particle size exhibited by them. Nanosuspensions were prepared using the same method and parameters described earlier for IBU: PVP-K30. Lyophilization of nanosuspensions containing T80 to study the drug release from the solid nanoparticle was not possible due to the oily nature of T80. Thus, they could not be converted to dry nanoparticle form. *In vitro* release of IBU from the homogenized nanosuspension of different ratios was studied, and the optimized one was compared to three different marketed products using USP XXV Type II (paddle) dissolution apparatus (Erweka DT 80, GmbH, Germany). The study was conducted for 60 min in 500 mL distilled water maintained at  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$  and at a stirring speed of 100 rpm. The suspensions were shaken for 15 min at 250 rpm (Stuart Reciprocating Shaker, UK) and samples equivalent to 100 mg IBU were withdrawn with volumetric pipettes and added to the dissolution vessels. Aliquots (5 mL) were withdrawn at predetermined time intervals, filtered through  $0.45 \mu\text{m}$  PTFE syringe filters (Membrane Solutions, China) and analyzed for IBU released by the UFLC method mentioned above, after suitable dilution with the dissolution medium. An equal volume of fresh dissolution medium kept at the same temperature was replaced after each sampling to maintain the sink condition. All studies were performed in triplicates.

## RESULTS AND DISCUSSION

### Phase solubility studies

The study involved an initial screening of various hydrophilic carriers (*viz.*; PVP-K15, PVP-K30, PXM-188, PXM-338, PXM-407, PEG-4000, PEG-10000, PEG-20000, BCD, HPBCD, SLS, GLP, and vitamin E-TPG) to study their influence on IBU solubility. The aqueous solubility of IBU at different polymer concentration following phase solubility studies was analyzed spectrophotometrically. The mean calibration curve of IBU (regression equation:  $y = 0.043x + 0.0013$ ) in water over a concentration range of 4-20  $\mu\text{g/mL}$  at 222 nm ( $\lambda_{\text{max}}$ ) was found to be linear ( $n = 6$ ) with a correlation coefficient of  $r^2 = 0.9993$ . The data were treated statistically using linear least square regression and the apparent 1:1 ratio stability constant (K) and the Gibbs free energy ( $\Delta G^{\circ}$ ) were calculated from the phase-solubility diagram using equations (1) and (2), respectively.

$$K = \frac{\text{Slope}}{\text{y-intercept (1-slope)}} \quad (1)$$

Where the y - intercept corresponds to the intrinsic solubility of IBU at  $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ .

$$\Delta G^{\circ} = -RT \ln K \quad (2)$$

Where, R is the ideal gas constant and T is the absolute temperature.

The plots of drug solubility against increasing polymer concentrations investigated for the different polymers were constructed and used for calculation of the K and  $\Delta G^{\circ}$  values. The solubility of pure IBU in water at  $20^{\circ}\text{C}$  was found to be only 47  $\mu\text{g/mL}$ , indicating that the drug is practically insoluble in water (as per the USP solubility classification system). The solubility curve was classified as  $A_L$  type according to Higuchi and Connors.<sup>[30]</sup> The extent of interaction between the drug and the hydrophilic polymers in aqueous media was characterized by the apparent K values, calculated according to equation 1. Higher K values confirm solubility enhancement with an increase in polymer concentration and possible physical interaction between the drug and the polymer in an aqueous state. A negative  $\Delta G^{\circ}$  value is also indicative of a spontaneous solution process. Based on these physical parameters, it was evident that PVP-K30 [Figure 1] with a K value of 48.217/M and  $\Delta G^{\circ}$  value of  $-9.452 \text{ KJ/mole}$  served as the best polymer to enhance the aqueous solubility of IBU as compared to other hydrophilic carriers and hence was selected as the polymer of choice for further studies.

### Optimization of homogenization process

Before starting preparation of IBU nanosuspensions, it was preferred to use the drug in a uniform size fine powder form; hence, IBU was sieved through 60# sieve before the ultra-homogenization process. To avoid blocking during the homogenization process, it was considered important to perform pre-milling using a high-speed over-head homogenizer. As IBU is practically insoluble in water and extremely hydrophobic in nature, it was necessary to disperse it in the aqueous polymeric solution.

Three different ratios (1:1, 1:2, 1:3 w/w) of IBU: PVP-K30 were selected to study the influence of ultra-homogenization

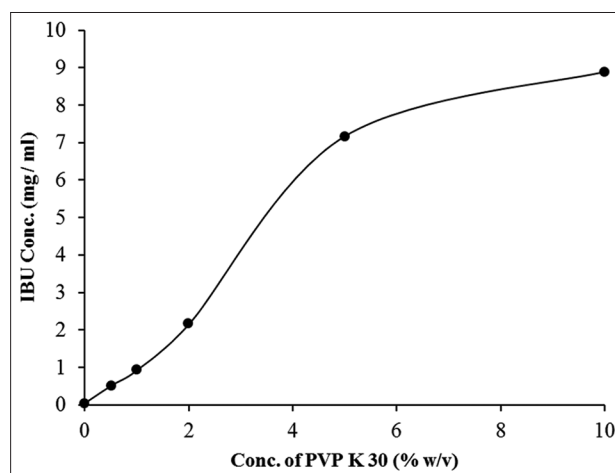


Figure 1: Phase solubility plot of ibuprofen with PVP-K30

on particle size and *in vitro* dissolution in the presence of the polymer. The number of homogenization cycles and pressure was gradually increased and optimized based on the output velocity and decrease in particle size, while the suspension passes the very small homogenization gap with a very high velocity. Due to the narrowness of the gap, the streaming velocity of the suspension increases tremendously, which means the dynamic fluid pressure increases. The cavitation forces are strong enough to break the drug microparticles to nanoparticles. Accordingly, an increase in pressure and number of cycles can lead to a decrease in particle size. However, the applied pressure was increased gradually to avoid blocking of the piston gap. To obtain a narrow size distribution, it was necessary to run several cycles through the homogenizer. A total number of 50 homogenization cycles (500 bar for 15 cycles, followed by 750 bar for 15 cycles and finally 1250 bar for 20 cycles) was considered to be practically feasible and optimum for this study.

### Particle size analysis

The mean particle size and PI of various homogenized drug: Polymer combinations after 50 homogenization cycles are summarized in Table 1. An increase in the drug: Polymer ratio from 1:2-1:3 did not show any significant positive influence on particle size, hence 1:2 ratio was considered to be optimum which gave an average particle size of  $527 \pm 31.04$  nm and a narrow PI (0.21). Attempts were also made to study the synergistic effect of polymers/surfactants on further drug particle size reduction. A superior effect on particle size reduction was observed from IBU: PVP-K30:T80 (1:2:2 w/w) nanosuspension, which showed mean particle size of  $127 \pm 6.18$  nm and excellent re-dispersibility. The other polymer combinations either did not show any significant change in particle size or exhibited agglomeration and crystal growth.

### Zeta potential measurement

Zeta potential determines the physical stability of a nanosuspension. It is a measure of particle surface charge

and an indirect measurement of the thickness of the diffusion layer, i.e., it can be used to predict long-term stability. An increase in the absolute value of zeta potential is correlated with a lesser tendency to aggregate/flocculate and is indicative of the colloidal stability of the nanosuspension. To obtain a nanosuspension exhibiting good stability, an electrostatically stabilized nanosuspension should have a minimum zeta potential of  $\pm 30$  mV.<sup>[31]</sup> It is evident from the data given in Table 1 that all the nanosuspensions prepared with PVP-K30 and T80 showed good stability (Zeta potential  $> -30$  mV).

### Analysis of drug content

IBU content of nanosuspensions and lyophilized nanoparticles was determined by a validated UFLC method. The mean calibration curve ( $n = 6$ ) of IBU (regression equation:  $y = 22026x - 2158.46$ ) was obtained by plotting the peak area of IBU versus concentration. It was found to be linear in the concentration range of 5-50  $\mu\text{g/mL}$  ( $r^2 = 0.9999$ ), with a typical chromatogram and a retention of IBU at 4.93 min. The drug peak was symmetrical with a tailing factor of 1.08. The developed UFLC method showed good reproducibility with relative standard deviation (RSD) values  $< 3\%$  for precision and high accuracy between 99.5% and 101.8%. The analyte samples were stable when stored for 3 days under refrigeration ( $8^\circ\text{C} \pm 0.5^\circ\text{C}$ ) with a mean % RSD  $< 2$ . The LOQ of IBU was 75 ng/mL (C.V  $< 3\%$ ), and the minimum detectable level (LOD) was 25 ng/mL. These results ensure that the analytical method could be appropriately used for IBU analysis with good sensitivity and precision. Results confirmed the presence of IBU in all the homogenized products to a level of 97.5-102% [Table 2].

### Thermal analysis

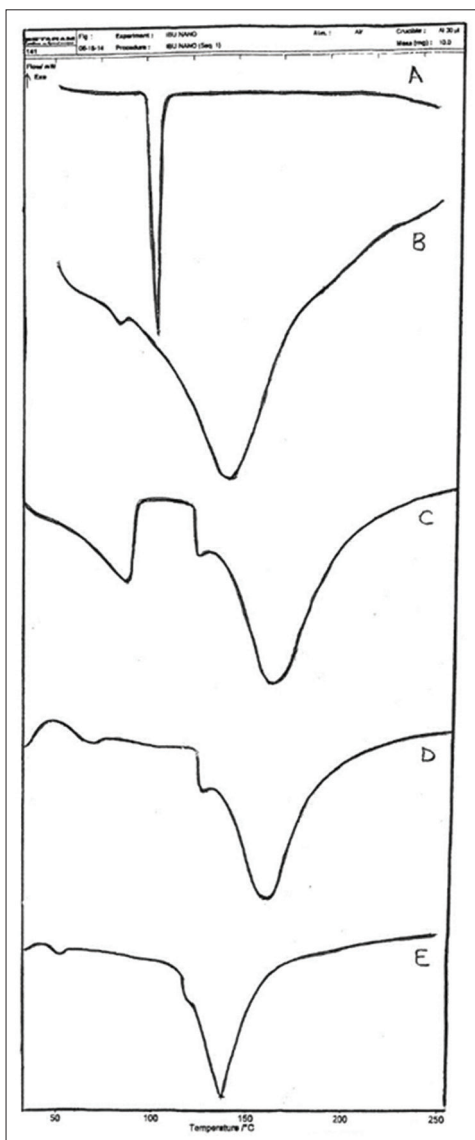
To understand the possible interactions between the drug and PVP-K30 which might also be responsible for improved drug dissolution in addition to size reduction, DSC thermogram of the drug, polymer and lyophilized nanoparticles were recorded [Figure 2]. The DSC graph of untreated IBU

**Table 1:** Mean particle size and polydispersibility index values of IBU nanosuspensions prepared by ultra-homogenization

S. No.	Sample name	Mean particle size (nm) $\pm$ standard deviation*	PI	Mean zeta potential (mV) $\pm$ standard deviation*
1.	IBU	$1026 \pm 158.19$	1.32	$-46.4 \pm 0.632$
2.	IBU: PVP-K30 (1:1)	$670 \pm 76.39$	0.65	$-39.2 \pm 0.976$
3.	IBU: PVP-K30 (1:2)	$527 \pm 31.04$	0.21	$-58.9 \pm 0.503$
4.	IBU: PVP-K30 (1:3)	$539 \pm 28.67$	0.36	$-44.4 \pm 1.414$
5.	IBU: PVP-K30:T80 (1:1:1)	$193 \pm 11.53$	0.83	$-39.0 \pm 1.585$
6.	IBU: PVP-K30:T80 (1:2:2)	$127 \pm 6.18$	0.24	$-42.4 \pm 1.187$
7.	IBU: PVP-K30:T80 (1:3:3)	$121 \pm 9.34$	0.38	$-41.9 \pm 1.065$

\*All reported values are mean  $\pm$  standard deviation ( $n=3$ ). IBU: Ibuprofen, PI: Polydispersibility index

showed a sharp endothermic peak at 75.6°C, which is indicative of its melting temperature. The thermogram of untreated PVP-K30 depicts a melting endotherm near 124.8°C. The DSC pattern of IBU: PVP-K30 lyophilized



**Figure 2:** Differential scanning calorimetry thermogram of ibuprofen (IBU) nanoparticles; A - IBU, B - PVP-K30, C - IBU: PVP-K30 (1:1), D - IBU: PVP-K30 (1:2), E - IBU: PVP-K30 (1:3)

nanoparticles (1:1 w/w) shows the presence of peaks of both the drug and polymer; however, the intensity of IBU endotherm was significantly reduced due to their partial interaction. The thermogram of IBU: PVP-K30 lyophilized nanoparticles (1:2 and 1:3 w/w) exhibited almost complete disappearance of the endothermic peak characteristic of IBU; which can be attributed to its amorphous character in the fused state; strongly indicating that the drug is well dispersed in the polymer matrix and its recrystallization is restrained. PVP-K30 has been found also to be a strong crystallization inhibitor for numerous drugs including captopril,<sup>[32]</sup> zolmitriptan,<sup>[33]</sup> and sulfamerazine.<sup>[34]</sup>

### FTIR spectroscopy

The interaction between the drug and its carrier often leads to identifiable changes in the infrared profile of the final product. FTIR spectral studies were employed to confirm the interaction between IBU and PVP-K30. The intense peaks appearing in the spectra of IBU and PVP-K30 are due to the asymmetric stretching vibrations of their functional groups. The IR spectrum of IBU [Figure 3] shows an intense, well-defined infrared band at around 1769  $\text{cm}^{-1}$  (carbonyl stretching of the iso-propionic acid group) as well as prominent peaks at 2953  $\text{cm}^{-1}$  (carboxylic acid O-H stretching), 3053  $\text{cm}^{-1}$  (aromatic C-H stretching), and 1506  $\text{cm}^{-1}$  (aromatic C-C stretching). The IR spectrum of PVP-K 30 [Figure 4] shows characteristic principle peaks at 1695  $\text{cm}^{-1}$  (carbonyl stretching), 1453  $\text{cm}^{-1}$  (C-H alkane bend), and 2920  $\text{cm}^{-1}$  (-C-H-alkane stretch). The spectra of the nanoparticles [Figure 3] show a drift and broadening of the carbonyl peak at 1680  $\text{cm}^{-1}$ . This peak broadening indicates possible hydrogen bonding between IBU and PVP-K30. Decrease in intensity and shifts are also visible in the peaks of the aromatic -C-H-(2925  $\text{cm}^{-1}$ ) and aromatic -C-C-(1489.0  $\text{cm}^{-1}$ ) stretching vibrations of the benzene ring, suggesting that these groups are taking part in the hydrogen bonding process. These chemical shifts could be attributed to the physical interaction of IBU with PVP-K30 which in turn enhances wettability, aqueous solubility and dissolution of the drug.<sup>[5]</sup> The spectra of the nanoparticles, however, did not show any new peaks, indicating that there was no new chemical bond formation between the drug and the polymer.

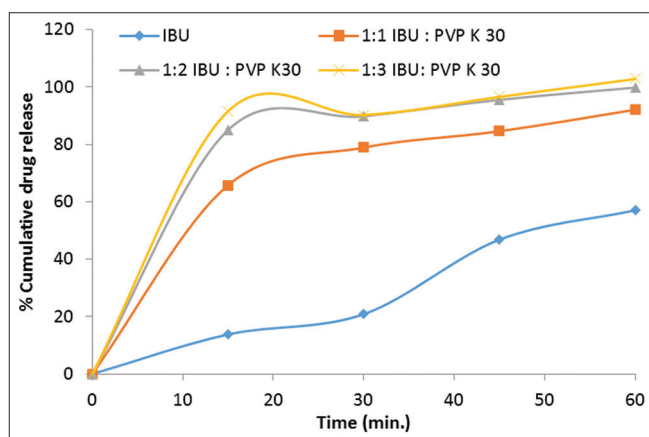
**Table 2:** Percentage of IBU content in ultra-homogenized samples

S. No.	Sample	Percentage IBU content $\pm$ standard deviation
1.	IBU: PVP-K30 (1:1)	99.86 $\pm$ 1.554
2.	IBU: PVP-K30 (1:2)	99.67 $\pm$ 0.863
3.	IBU: PVP-K30 (1:3)	98.94 $\pm$ 1.323
4.	IBU: PVP-K30:T80 (1:1:1)	100.46 $\pm$ 1.117
5.	IBU: PVP-K30:T80 (1:2:2)	101.25 $\pm$ 0.656
6.	IBU: PVP-K 30:T 80 (1:3:3)	99.84 $\pm$ 1.175

IBU: Ibuprofen

### ***In vitro* dissolution of IBU from lyophilized IBU: PVP-K30 nanoparticles**

The dissolution profiles of untreated IBU and lyophilized IBU: PVP-K30 nanoparticles prepared in different w/w ratios are depicted in Figure 3. The mean calibration curve of IBU (regression equation:  $y = 0.043x + 0.0013$ ) in the dissolution medium over a concentration range of 4-20  $\mu\text{g/mL}$  at 222 nm ( $\lambda_{\text{max}}$ ) was found to be linear ( $n = 6$ ) with a correlation coefficient of  $r^2 = 0.9993$ . The *in vitro* release profiles were evaluated, on the basis of percentage of drug dissolved at 15 min and 60 min ( $DP_{15}$  and  $DP_{60}$ ). The reported values [Table 3] were obtained by calculating the arithmetic mean of three measurements. The dissolution rate of untreated IBU was very slow with only about 13.78% of the drug dissolved in

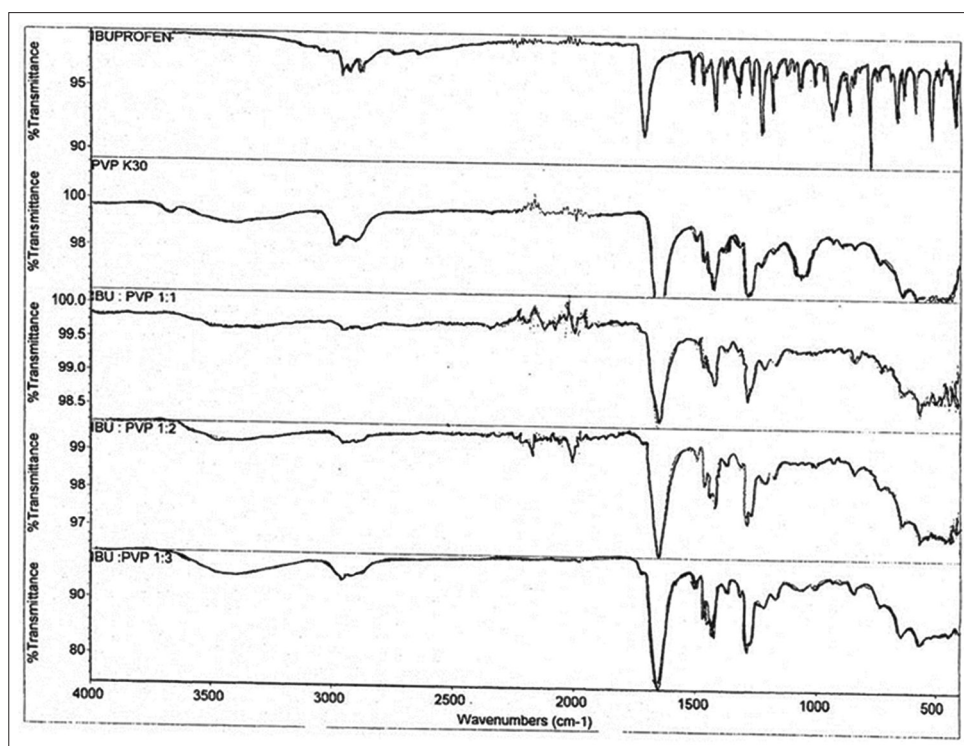


**Figure 3:** Dissolution profile of ibuprofen (IBU) and IBU: PVP-K30 nanoparticles

15 min and 57.06% dissolution at the end of 1 h. Significantly rapid dissolution was observed through the ultra-homogenized lyophilized nanoparticles as compared with the untreated drug. This improvement in the dissolution rate could be attributed to the reduction in particle size in sub-micron range, amorphous nature of the material and hydrophilic carrier properties. It can be summarized from the results depicted in Table 3, that IBU: PVP-K30 (1:2 w/w) nanoparticles showed optimum release compared to the other nanoparticles.

### **Comparison of *in vitro* dissolution of IBU: PVP-K30:T80 nanosuspension and marketed suspensions**

It was considered of interest to prepare and study the dissolution pattern of IBU: PVP-K30:T80 nanosuspensions, because of the extremely low particle size exhibited by them. The dissolution profiles of nanosuspensions (1:1:1, 1:2:2, and 1:3:3; IBU: PVP-K30:T80, respectively) are shown in Figure 5. IBU: PVP-K30:T80 (1:1:1 w/w) nanosuspension showed 82% drug release in 15 min., as compared to IBU: PVP-K30:T80 (1:2:2 w/w) nanosuspension which showed 95% release in first 15 min. This enhancement in dissolution rate could be attributed to further reduction of particle size, increased surface area of the exposed drug, and increase in saturation solubility due to the presence of hydrophilic polymers and formation of a colloidal solution. Similar observations were documented by other investigators.<sup>[35,36]</sup> Since there was no significant difference observed in particle size [Table 2] and release pattern [Table 3] of nanosuspensions in the ratios 1:2:2 and 1:3:3 of IBU: PVP-K30:T80, therefore (1:2:2 w/w)



**Figure 4:** Fourier transform infrared spectra of ibuprofen (IBU), PVP-K30 and IBU: PVP-K30 nanoparticles

**Table 3:** DP<sub>15</sub> and DP<sub>60</sub> parameters for pure IBU, nanoparticles and nanosuspension

Compound <sup>†</sup>	Dissolution parameters <sup>††</sup>	
	DP <sub>15</sub>	DP <sub>60</sub>
Untreated IBU	13.78±3.61	57.06±1.94
IBU: PVP-K30 (1:1 w/w)	65.78±1.85	92.09±0.97
IBU: PVP-K30 (1:2 w/w)	85.01±1.09	99.89±1.18
IBU: PVP-K30 (1:3 w/w)	89.55±0.77	102.83±1.46
IBU: PVP-K30:T80 (1:1:1 w/w)	82.56±1.81	93.67±0.94
IBU: PVP-K30:T80 (1:2:2 w/w)	95.38±1.28	100.81±1.94
IBU: PVP K30:T80 (1:3:3 w/w)	96.75±1.13	101.26±1.58

<sup>†</sup>NP: Indicates nanoparticles prepared by ultra-homogenization,

<sup>††</sup>n=3, DP: Dissolution parameters. IBU: Ibuprofen

**Table 4:** DP<sub>5</sub>, DP<sub>15</sub> and DP<sub>60</sub> values of optimized IBU nanosuspension and marketed suspensions

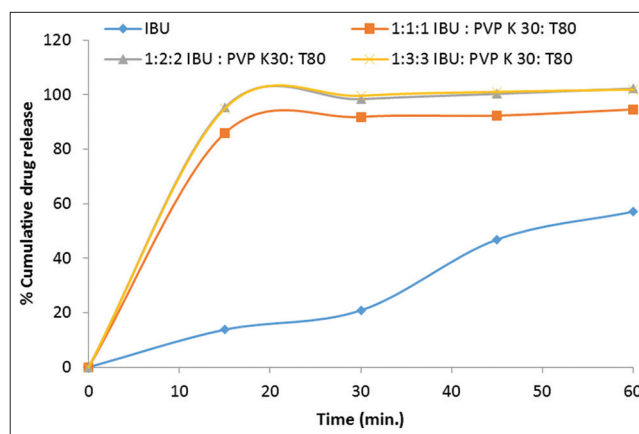
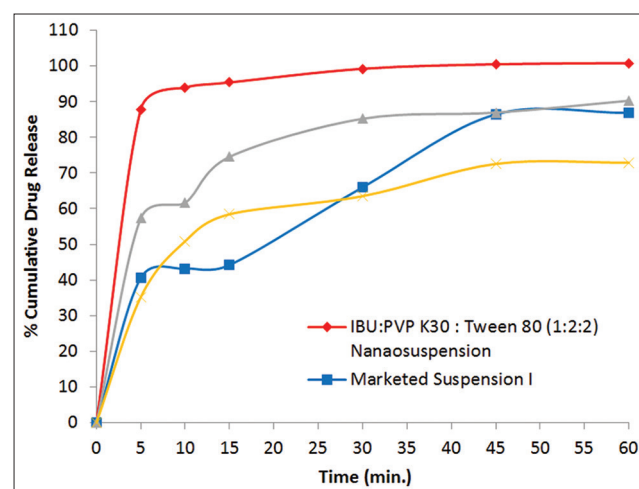
Compound	Dissolution parameters <sup>†</sup>		
	DP <sub>5</sub>	DP <sub>15</sub>	DP <sub>60</sub>
IBU nanosuspension	87.87±1.61	95.38±1.28	100.81±1.94
Marketed suspension I	40.60±2.38	44.17±1.05	86.86±0.98
Marketed suspension II	57.32±1.91	74.51±1.43	90.27±1.57
Marketed suspension III	35.22±1.87	58.42±2.16	72.89±1.91

<sup>†</sup>n = 3, DP: Dissolution parameters, IBU: Ibuprofen

nanosuspension was selected for further comparison with marketed IBU suspensions. The rate of drug dissolution from the formulations under study was compared at 5 min intervals in the first 15 min, followed by 15 min interval for a period of 1 h [Figure 6]. It is evident from Table 4 that the nanosuspension showed almost two-fold faster release compared to the marketed products in first 5 min and 95% release in 15 min, which would induce faster rate of absorption, rapid onset of action and improved patient safety by decreasing gastric irritation after oral administration.

## CONCLUSION

IBU nanosuspensions were successfully prepared by high-pressure homogenization technique with different hydrophilic polymers. There was a significant decrease in particle size with increase in the number of homogenization cycles and pressure. PVP-K30 resulted in maximum aqueous solubility of IBU with sub-micron particles ( $527 \pm 31.04$  nm) characterized by a narrow PI and a high negative zeta potential value. Nanoparticles produced by freeze drying the nano-suspension were amorphous in nature and showed higher release rate than the pure drug. Nanosuspensions containing Tween

**Figure 5:** Dissolution profile of ibuprofen: PVP-K30:T80 nanosuspensions**Figure 6:** Dissolution profile of ibuprofen: PVP-K30:T80 (1:2:2 w/w) nanosuspension and three marketed suspensions

80 (IBU: PVP-K30:Tween 80; 1:2:2 w/w) exhibited much smaller particle size ( $127 \pm 6.18$  nm) and two-fold faster release, compared to 3 marketed products. Other polymer combinations either did not show any significant change in particle size or exhibited agglomeration and crystal growth. The proposed technique also has the potential of commercial scale-up to formulate safe products with higher bioavailability.

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