Enhanced dissolution rate of Ketoprofen by fabricating into smart nanocrystals

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Abstract: The low bioavailability of Ketoprofen is associated with its hydrophobic nature that can be solved by nanonization. For this purpose, a polymeric solution with drug concentration of 3.5% w/w was formulated. The produced solution was milled for 60 minutes in DENA® mill which contains 0.2µm yttrium reinforced zirconium beads. The Physicochemical properties, characterization including stability studies of the prepared nanoparticles were carried out using pharmacopeial techniques of zeta potential, PXRD, DSC, SEM and TEM. Results suggest that stable crystalline nanocrystals with a size of 169±1.98nm with PDI of 0.194±0.04 and zeta potential of -22.0±2.25mV were produced. Moreover, enhances *in-vitro* release rate of 78.6% for the processed Ketoprofen was achieved in first 5 min as compared to raw form and marketed drug which released only 22.9% and 33.1% of drug respectively. The 60 days stability studies at 4°C & 25°C revealed that polymers PVP-K30-HPMC-6cps-SDS were effective in stabilizing the nanocrystals. Comparatively stable ketoprofen nanocrystals were successfully produced by DENA® mill with marked enhanced dissolution rate. It proved a useful for commercialization technique due to high drug concentration and retention of distinct characteristics at large scale.

Keywords: Nanocrystals, Ketoprofen, DENA®, Milling time, characterization.

INTRODUCTION

Currently the drugs are facing bioavailability problems which is erected from poor water solubility (Fridgeirsdottir *et al*., 2016) and consequently produce unwanted toxic effects (Dalvi *et al*., 2015, Plakkot *et al*., 2011). The newly applicant drugs and marketed drugs are also poor soluble and these drugs %age are 70 and 40 respectively (Kawabata *et al*., 2011, Ku and Dulin, 2012). The continuous struggle of the drug delivery scientists have established and fabricated a variety of procedure to boost up the solubility and dissolution of poor water soluble active compounds including emulsions (Dhillon, 2014), solubilization (Rodriguez-Aller, 2015) and solids dispersions (Khadka, 2014). The insufficient improvement in solubility, stability issues and ionizable groups are the problems associated with these methods and consequently have restricted applications (Loh *et al*., 2015, 1999, Huang and Dai, 2014). Current literature emphasis on nanosuspensions to address the issue of hydrophobic drugs (Ghosh *et al*., 2011). Nanocrystals can be created by two methods (Nasilowski *et al*., 2016). The issue associated with these approaches include possible changes in crystalline state of the produced nanocrystals, stability problems and change in physical properties (Mariano *et al*., 2014,Wu *et al*., 2011).

Ketoprofen belongs to the group of nonsteroidal anti-inflammatory drugs (NSAID) (Ungprasert *et al*., 2016). The well-known pharmacological uses of this drug include uses in chronic and acute inflammatory diseases, rheumatoid arthritis, spondylitis, osteoarthritis and severe abdominal pain/cramps linked with menstruation (Stock *et al*., 2015). However this drug is poor water soluble, which results in erratic bioavailability and low absorption (Dobrek *et al*., 2015).

This study aimed to produce nanosuspension of Ketoprofen through media milling method and to investigate the impact of milling time on production of nanoparticles. Industries normally utilizes top down method (Zhang *et al*., 2015). Furthermore, the special characteristics of nanocrystals in nanosuspensions were investigated through different physicochemical characterization technique.

MATERIALS AND METHODS

Chemicals

Ketoprofen (Batch no: ILKT140005 infinity lab India), PVP-K30 (B.No:08297052G0, BASF, Germany),

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Hydroxypropylmethylcellulose viscosity; 6cps (HPMC-6cps) (B.No: 8028213, BASF, Germany), Sodium dodecyl sulfate (B.No:MKBR3557V Sigma-Aldrich, UK). Laboratory Distilled water was acquired at University of Bradford Research Laboratory.

Preparation of Ketoprofen nanosuspension

Nanosuspension of Ketoprofen was prepared through a size reduction system called Dena®DM100 (Sulaiman, 2007,). The system of Dena®DM100 (BK Ltd, England) consists of soft polymeric fast rotating conical rotor sitting within conical polymeric sleeve. In between the rotor and outer sleeve, a narrow gap is formed upon filling the rotor indentations with grinding media (0.2µm yttrium reinforced zirconium beads). Within the narrow gap, the production of high shear and turbulence provides potential for particles rupturing and shearing which results in ultrafine product formation in the size ranges of sub-micron/Nano (Shete *et al*., 2016). During the process, a suspension is produced which is recycled incessantly through a screen of stainless-steel retaining the milling media and preventing contamination of product. Finally, desired nanosuspension are collected and characterized. In the current study, 250 ml stabilizer solutions were prepared by mixing sodium dodecyl sulfate (0.1%w/w), PVP-K30 (0.5%w/w) and 6cps grade of HPMC (0.5%w/w) in water (Khan *et al*., 2018c). Then ketoprofen was dispersed in polymer solution for 5minutes in stirring and then sonicated for 5min. Upon mixing of stabilizer solution and drug materials gives 350ml coarse suspension which contains the ketoprofen as 3.5%w/w. The obtained coarse suspension was then kept in the feed stock hopper of media milling machine. Inside the size reduction chamber, the suspension was recycled. The effect of the milling time on nanoparticle size was determined by taking samples from the media milling machine at different interval of time and characterization through different technique (Ali *et al*., 2017).

Measurements of particle size and zeta potential

The nanosuspension was assessed by photon correlation spectroscopy, (NanoSZetasizer®, Malvern, UK) for the measurements of Particle size and zeta potential (Lievonen *et al*., 2016). Moreover, no dilution of the samples was carried out before size analysis. Three measurements were taken for average PS and PDI determination. Furthermore, Zeta potential measurements were carried out in clear disposable zeta cells.

Scanning electron microscopy (SEM)

SEM (Quanta 400SEM, U.K) was used for the morphological studies of unprocessed Ketoprofen by applying gold coating through sputter coater (Ali *et al.,* 2017).

Transmission electron microscopy (TEM)

Produced nanosuspension was characterized for external morphology through TEM (JEM, Japan) operated at 100kV. The drop of nanosuspension was poured to surface of copper grid and then dried out at ambient temperature. For negative staining, 2% aqueous magnesium uranyl acetate solution was used (Pathak *et al*., 2016).

Differential scanning calorimetry (DSC) analysis

Raw ketoprofen and produced nanocrystal were assessed through differential scanning calorimeter for thermal characteristics. Standard materials used for DSC calibration were 99% Zinc and indium having melting points of 419.5°C and 156.6°C respectively. The experimental condition includes scanning rate of 10°C/min and nitrogen atmospheric condition. The scanning was done in range of 0 to 120°C. Samples analysis was performed in triplicate (Khan *et al*., 2018a)

X-ray powder diffraction (XRPD) analysis

The Crystallinity of the raw ketoprofen and produced nanocrystals was determined through D-8diffractometer (Bruker, Germany) using Cu Ka radiation (X = 1.5418 A). The measurement angle scale was 5-50° with step size of 0.05°, count time was 3s per step and rotation was 30rpm. The generator was kept on 40kV and 30mA

Physical stability

The Ketoprofen nanosuspension was evaluated for Chemical and Physical stability studies. The Chemical stability was conducted for one week (Khan *et al*., 2018a) while Physical stability was conducted for 60daysat 25°C and 4°C. The particles size and polydispersity index were monitored at a regular time of intervals for particle growth.

In-vitro dissolution

Comparative study of the dissolution rate of the raw ketoprofen, marketed formulations and produced nanocrystals was conducted with rotating paddle as per USP XXIV method (D'Souza, 2014). All the samples were transferred to dissolution vessels comprising of 900ml phosphate buffer with pH of 7.2 and kept at 37ºC±0.5ºC with stirring speed of 100rpm (Khan *et al*., 2018b). The sample were collected after 5,10,15,20,25,30,40,50,60minutes and were replaced with equivalent quantity of fresh solution of phosphate buffer. The samples were measured at 260 nm on U.V spectrophotometer (V-630 JAS.C0).

STATISTICAL ANALYSIS

Experimentally data was statistically analyzed by SPSS 18 (SPSS Inc., USA). Collection of data was done in triplicate. Mean ± standard Deviation was used for data. The test performed was One-way ANOVA ($p<0.05$) and least significant difference.

RESULTS

Effect of milling time

The crystal sizes of the coarse suspensions of ketoprofen decreases abruptly in first 10 minutes and then there is gradual decrease. After 60 minutes the optimum nanoparticle size was achieved (fig. 1). The obtained particle size of ketoprofen was 169±1.98nm with PDI of 0.194±0.04. The fig. 2 shows the particle size distribution.

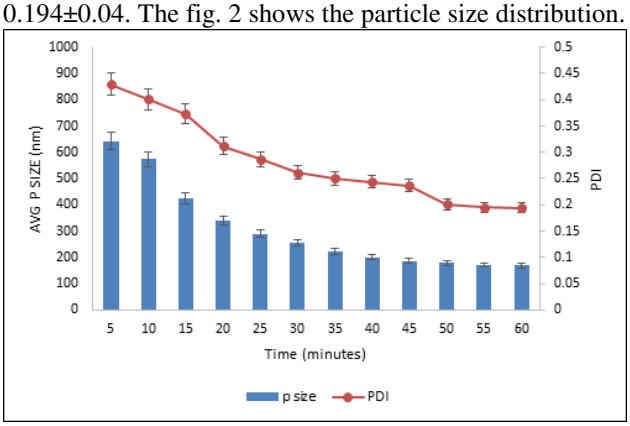

Fig. 1: Effect of milling time on ketoprofen nanoparticles

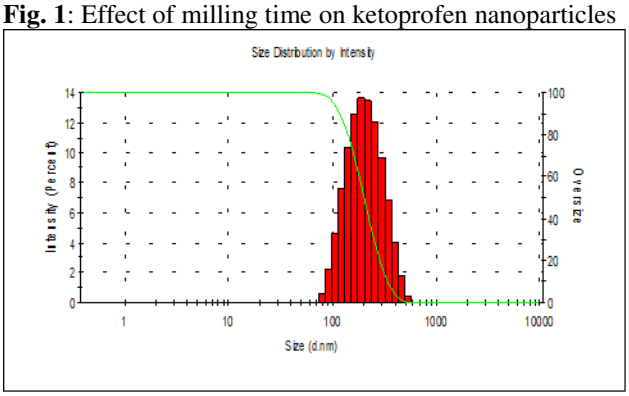

Fig. 2: ketoprofen nanoparticle particle size distribution

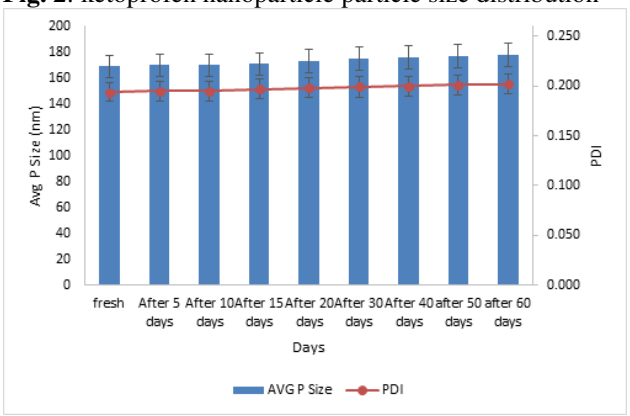

Fig. 3: ketoprofen nanoparticle stability at 4 ºC

Zeta potential

The achieved zeta potential value of the ketoprofen was-22.0±2.25 mV which is more than the testified accepted level. This zeta potential value has been recognized as accepted value for stability of the nanosuspension (Ali *et al*., 2009, Khan *et al*., 2018a).

Stability studies

Chemical stability was assessed for one week and there was no sign of degradation while physical stability was measured for 60days at 4ºC and 25ºC and nanosuspensions were stable at both the temperatures (figs. 3 & 4).

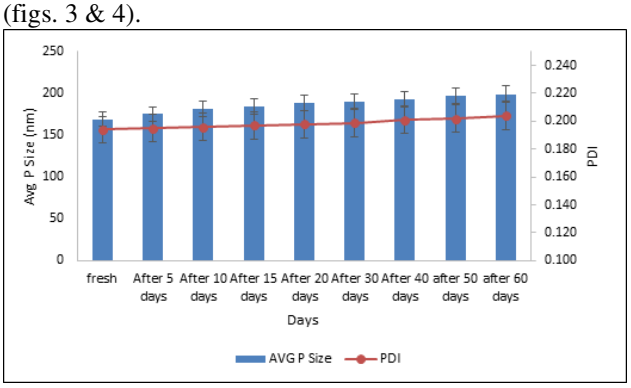

Fig. 4: Ketoprofen nanoparticle stability at 25ºC

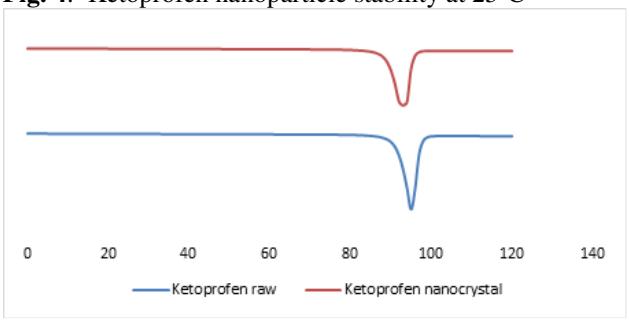

Fig. 5: DSC analysis of nanocrystal and unprocessed Ketoprofen

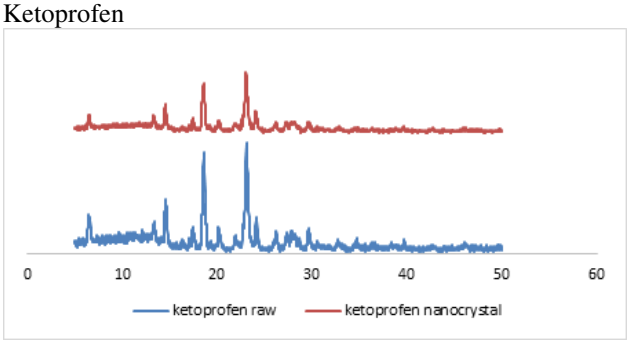

Fig. 6: X Ray Diffractogram of raw and nanocrystals of ketoprofen

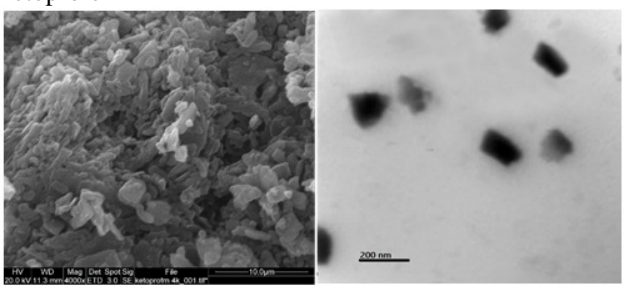

Fig. 7: SEM and TEM images (a) Raw Ketoprofen (b) Ketoprofen nanocrystal

Thermal and X-ray analysis

The raw drugs and nanocrystals have sharp DSC melting endotherm. The melting points of raw and nanocrystal

were 95°C and 93°C respectively (fig. 5). The XPRD Diffract grams of the raw ketoprofen was found sharp with high intensity peaks while for nanocrystal there is decrease in intensity of the peaks (fig. 6).

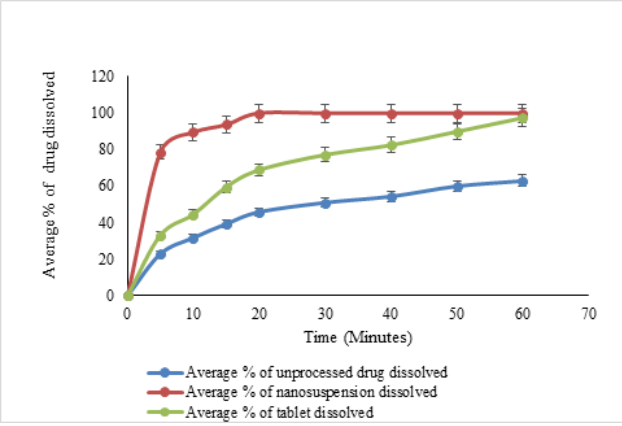

Fig. 8: Dissolution studies of raw, nanoparticle and marketed product ofketoprofen

Morphological Studies

SEM images (fig. 7a) shows that raw drug is crystalline in nature with triangular and irregular in shape. Moreover, TEM images (fig. 7b) clearly shows that the nanoparticles are below 200nm with homogenous size distribution.

In-vitro dissolution

Then nanocrystals demonstrated enhanced *in-vitro* dissolution rate compared to marketed ketoprofen tablets and unprocessed drug. It is clear from figs. 8 that in first 5 minutes approximately 78.6% of ketoprofen nanocrystals were dissolved in first 5minutes while the raw ketoprofen and marketed tablets showed the dissolution rate around 22.9%, and 33.1% respectively.

DISCUSSION

In current study an attempt was made to produce stable nanocrystals of Ketoprofen by top-down approach. The top-down approach is also known as stepwise design or decomposition which is essentially the breaking down of a system to gain insight into its compositional sub-systems in a reverse engineering fashion. In this case energy is generated by shearing and turbulent of the milling media and particles with consequent in particles reduction (Nigam *et al*., 2014).

The results indicate (figs. 1 & 2) that shear stress has been increased with increase in milling time with consequent further decrease of particle sizes (Cheng *et al*., 2015). Moreover, assessment of zeta potential is significant in projection of stability and achieved zeta potential of the ketoprofen nanocrystal (-22.0±2.25 mV) was in accepted level (Mikolajczyk *et al*., 2015). The stability studies are important because nanoparticles have high surface area with high surface free energy (Van Eerdenbrugh *et al*., 2008) and tries to agglomerate to decrease surface free energy. The produced nanosuspension of Ketoprofen was stable for two months at $4C^0$ & 25 C^0 (fig. 3 & 4). Basically, it has been observed that HPMC-PVP-SDS combination adsorbs on nanocrystal surface and stabilizes it (Shah *et al*., 2016). Furthermore, the PDI value indicates the consistent distribution of particles which avoids Ostwald ripening, so particle size is retained (Deng *et al*., 2010). Moreover, the DSC results shows reduction of melting point from 95 C^0 to 93 C^0 and the basic reason for this is lower packing density and small particle size of the nanocrystals lattice as compared to the unprocessed particles (Jermain *et al*., 2018).

Furthermore, XPRD proved the crystallinity of Ketoprofen nanocrystals and due to the effect of smaller particle size, the peak for the nanocrystal was also found little broadened compared to the raw drugs and also due to minor angle reflection, the peak intensity of nanocrystal has been shifted to lower level (Bunjes *et al*., 2000). TEM images of Ketoprofen clearly demonstrates the defined morphology indicative of crystalline materials. The significant increase ($P<0.05$, one way ANOVA) in dissolution rate of ketoprofen nanocrystals displayed that produced nanocrystals has smaller sizes with increased surface area and also did not agglomerated (Khan *et al*., 2013, Plakkot *et al*., 2011, Junghanns and Müller, 2008).

CONCLUSION

The current research demonstrates the production and optimization of stable nanocrystal of Ketoprofen below 200 nm particle sized, successfully fabricated by media milling (DENA®) in large scale. The pattern of reduction in particle size was initially abrupt and then gradual. Stability studies proved that produced nanosuspension was robust stable. Additionally, enhanced dissolution rate of nanoparticle was achieved as compare to raw and marketed brands. This shows that the fabricated nanocrystals have low particle size with high surface area, confirming the homogenous distribution of crystals instead of cluster of particles. The present research outcome is a beneficial technique for Ketoprofen industrial manufacturing and commercialization.

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