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Smart nanocrystal of indomethacin: Nanonization and characterization through top down method of media milling

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Abstract: Indomethacin is potent and effective drug belongs to NSAID group having low bioavailability. To address this issue the novel method is Nanosuspensions which can be achieved through bottom up and top down methods. The drug concentration, batch size and crystallinity retention are the problems associated with bottom up method consequently top down method was applied. In current project batch size of 350 ml was prepared by mixing 3.5% of Indomethacin with polymer solution. Then it was introduced into Dena® having 0.2µm yttrium reinforced zirconium beads. The effect of milling time was observed for sixty minutes. Stable nanocrystals with particle size of 161nm ±1.90 with PDI of 0.229 ±0.06 were produced. The DSC and PXRD confirmed the crystallinity of created nanocrystals. The pattern of particle size reduction was initially abrupt and then gradual. The two months Stability studies at 4°C and at 25°C revealed that polymers combination (PVP-K30, HPMC-6cps, SDS) were effective in marinating the stability. The SEM and TEM studies unfastened that nanocrystals were homogenously distributed with discrete crystalline morphology. The fabricated nanocrystals demonstrated marked dissolution rate compared to the raw and marketed formulations. It is demonstrated that it is useful for industry due to high drug concentration, large batch size and retention of distinct characteristics.

Keywords: Dena®, indomethacin, milling time, nanocrystal

INTRODUCTION

Drugs having poor aqueous solubility show lower bioavailability upon oral delivery (Fridgeirsdottir *et al*., 2016) , resulting in their inferior therapeutic efficacy along with their toxic effects (Plakkot *et al*., 2011). Almost 40% of the marketed drugs and 40% of the active drugs candidate molecules are facing the problem of poor aqueous solubility (Kawabata *et al*., 2011, Ku and Dulin, 2012). Drug formulation scientists have developed various techniques for enhancing drugs solubility and dissolution. These include micronization (Fahim *et al*., 2014), solubilization (Rodriguez-Aller *et al*., 2015), émulsions (Dhillon *et al*., 2014), solids dispersions (Khadka *et al*., 2014), liposomes (Allen and Cullis, 2013) and inclusion of complexes as cyclodextrins (Mura, 2015). However, these techniques have reported to be associated with drawbacks like short term stability, production of ionizable groups and their limited solubility enhancing potentials (Wu *et al*., 2011, Huang and Dai, 2014). Currently, nanosuspensions have got greater scientific attentions for enhancing the solubility of poor aqueous soluble drugs due to their unique properties (Ghosh *et al*., 2011). Two different methods are exploited for the preparation of nanocrystals (Rabinow, 2004, Nasilowski *et al*., 2016). Indomethacin is potent and effective drug belongs to NSAID group having low bioavailability (Ungprasert *et al*., 2016). It is pale-yellow to yellow-tan, crystalline powder with odorless, or has slight odor (Allen Jr, 2016). Its melting point is 159-162°C. Indomethacin is classified as class-II drug owing to its deprived aqueous solubility and increased permeability (Semjonov, 2018). The indications include arthritis, fever, various headache syndromes and dysmenorrhea. Indomethacin is also used for closure of patent ductus arteriosus (Thiruvengadam *et al*., 2016).

The current study was undertaken for the preparation of Indomethacin nanosuspension using media milling method (Khan, 2019). The effects of the milling time on nanoparticles production were studied. Impaction and attrition forces play vital role in the reduction of particles size during milling process, thus enhancing the rate of dissolution (Kalepu and Nekkanti, 2016). The produced nanosuspension was further screened for its physicochemical characteristics such as particle size, PXRD, DSC, SEM, TEM and invitro dissolution.

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MATERIALS AND METHODS

Chemicals

Indomethacin (Batch no: BCBP0623V Sigma-Aldrich, UK), Hydroxypropylmethylcellulose viscosity; 6cps (B. No:8028213, BASF, Germany), PVP-K30 (B.No:08297052G0, BASF, Germany), Sodium dodecyl sulfate (B.No:MKBR3557V Sigma-Aldrich, UK). Laboratory Distilled water was acquired at University of Bradford Research laboratory.

Preparation of indomethacin nanosuspension

Indomethacin nanosuspension was prepared using size reduction system of Dena® DM100(BK Ltd, England (Ali et al., 2017). This system contains a fast rotating soft polymeric conical rotor sitting within conical polymeric sleeve. A narrow gap is formed between rotor and outer sleeve when rotor indentations are filled with grinding media (0.2 µm yttrium reinforced zirconium beads) (Ali *et al*., 2017). Increased turbulence and shear within the narrow gap are enough for the production of particles in nano/micro size range. Suspension formed is continually recycled in a stainless-steel screen, thus retains the milling media and prevents product contamination. The final nanosuspension is removed followed by its characterization. In this study, stabilizer solutions (250 ml) were prepared by mixing of 3 polymers i.e. 6 cps grade of HPMC (0.5% w/w), Sodium dodecyl sulfate (0.1% w/w) and PVP K30 (0.5% w/w) (Khan *et al*., 2018c). Indomethacin and water were dispersed in polymer solution by stirring for 5 min followed by sonication for further 5min. This gave a coarse suspension (350 ml) containing 3.5% w/w of Indomethacin. The coarse suspension was then fed in stock hopper of media milling machine and recycled in size reduction chamber. Milling time effects on size reduction was investigated by withdrawing samples of nanosuspenstion at various time intervals followed by characterization through different technique.

Particle size and zeta potential measurements

Photon correlation spectroscopy, PCS (Zetasizer® NanoS, Malvern Instruments, UK) was used for determination of Indomethacin in nanosuspension. Test sample without dilution was subjected to particle size analysis. Polydispersity index (PI) and Mean size were obtained from triplicate trail data (Khan *et al*., 2018b). For determination of zeta potential of Indomethacin nanocrystals, Malvern zetaszier was used. Sample was taken in disposable cells and was analyzed three times.

Scanning electron microscopy (SEM)

SEM (Quanta 400 SEM, FEI Company, Cambridge U.K) was used for investigating the surface morphology of unprocessed Indomethacin using different levels of magnification. In order to obtain clear image, Indomethacin gold coated particles were prepared through sputter coater (Shah *et al*., 2016).

Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) (JEM-1200EX, Japan Electron Optics Laboratory Corporation, Japan) operated at 100 kV was used for investigating the surface morphology of Indomethacin nanosuspension. Nanosuspension was dropped on copper grid surface, dried at room temperature followed by negative staining with 2% magnesium uranyl acetate aqueous solution (Ali *et al*., 2009).

Differential scanning calorimetry (DSC) analysis

Thermal characteristics of unprocessed Indomethacin and its naosuspension was investigated through Differential scanning calorimetry. Instrument calibration was carried out Zinc and indium 99% having 419.5°C and 156.6°C melting points respectively. Under nitrogen atmosphere, samples were scanned at 10°C/min scanning rate. Analysis was performed three times in 60-190°C range (Ali *et al*., 2011).

Powder X-ray diffraction (XRPD) assessment

X-ray powder diffraction (XRPD) of Indomethacin and its nanocrystals was passed out via Siemen D-8 diffractometer (Germany), using Cu Ka radiation *(X* = 1.5418 A). The trials were conducted at 0.05° step size, 5-50° angle range, 3s per step count time and 30 rpm rotation. Generator was fixed on 40kV and 30mA (Khan, 2019).

Stability studies

The Chemical stability of nanosuspension of Indomethacin was carried out for one week (Khan *et al*., 2018a) while the Physical stability was carried out at 4°C and 25 °C for a time period of two month. Samples were investigated for particle size growth and changes in polydispersity at various time intervals.

In-vitro dissolution

Indomethacin nanocrystals formulation was subjected to dissolution study and results were compared with raw drug, its micronized form and commercial formulations. Rotating paddle method using USP XXIV was employed for dissolution study. The samples were added to the vessels of dissolutions encompassing 900 ml phosphate buffer with pH 7.2 kept at 37ºC±0.5 at 100 rpm as reported previously (Khan *et al*., 2018a). Samples were withdrawn at pre-determined time intervals. Equal volume of buffer was added to the dissolution vessel after each withdrawal. Samples were read on U.V spectrophotometer (V-630 (JAS.C0) and Indomethacin was detected at 320nm.

STATISTICAL ANALYSIS

Data was calculated and presented as mean ± standard deviation using SPSS 18 (SPSS Inc., USA). The least significant difference test (LSD) and ($p<0.05$) One-way ANOVA test were employed.

RESULTS

Effect of milling time

Media milling method was used for the production of Indomethacin nanosuspension. During first 10 min, there was abrupt decrease in particle size of Indomethacin coarse suspensions followed by decrease in the particle size in a gradual manner. Minimum size for Indomethacin nanosuspension was achieved at 60 min as shown in fig. 1. Mean size for Indomethacin nanosuspension was found 161±1.90 nm with PDI of 0.229±0.06.

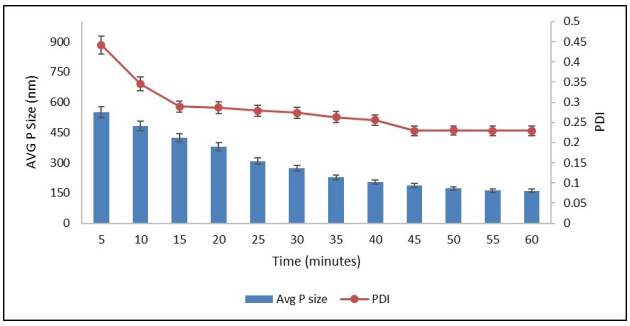

Fig. 1: Effect of milling time on Indomethacin nanoparticles. Values expressed as mean ±SEM.

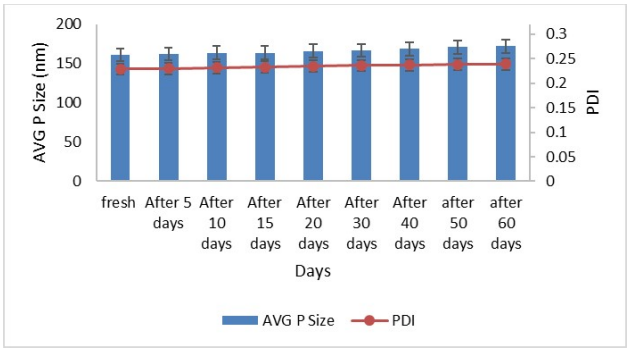

Fig. 2: Indomethacin nanoparticle stability at 2-8ºC

Stability studies

Physical and chemical stabilities of the produced nanosuspension were evaluated. Physical stability was investigated for 60 days while chemical stability of the formulation was carried out for 7 days. The formulation revealed no degradation. Physical stability of nanosuspension was investigated at two different temperatures i.e. fridge temp (2-8ºC) and room temperature (25ºC). Results confirm the stability of Indomethacin nanosuspension at both the tested temperature as shown in figs. 3&4.

Thermal and X-ray analysis

DSC and PXRD were used for investigating the crystallinity of Indomethacin nanocrystals produced through milling and results were compared with its raw form. Both the Indomethacin and its nanocrystals revealed sharp melting endotherms. Reduction in Indomethacin nanocrystal melting point was observed when compared to raw drug. Raw Indomethacin revealed a melting point of 161.6ºC which decreased to 158.0ºC upon conversion to its nanocrystals (fig. 4). XRD investigation established the crystallinity of Indomethacin. fig. 5 revealed sharp and high intensity peaks for raw Indomethacin. Upon conversion to nanocrystals, the peaks intensity got markedly decreased.

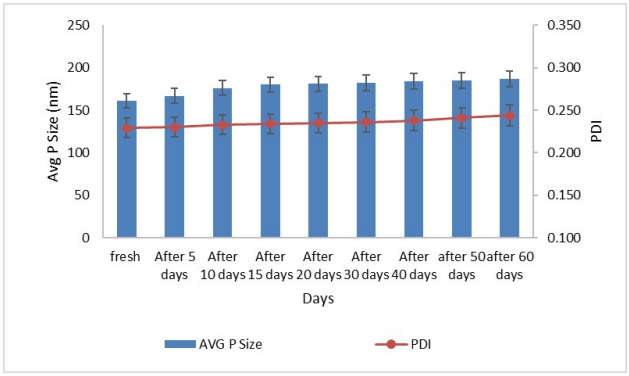

Fig. 3: Indomethacin nanoparticle stability at 25ºC

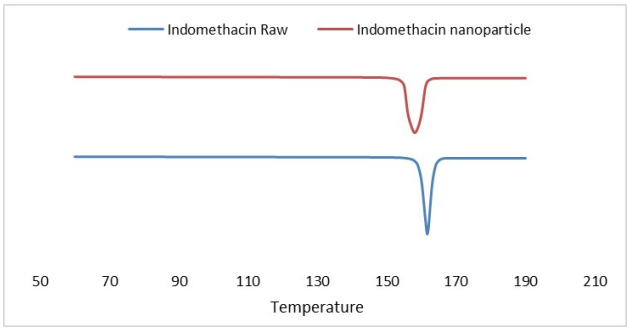

Fig. 4: DSC analysis of nanocrystal and un-processed Indomethacin

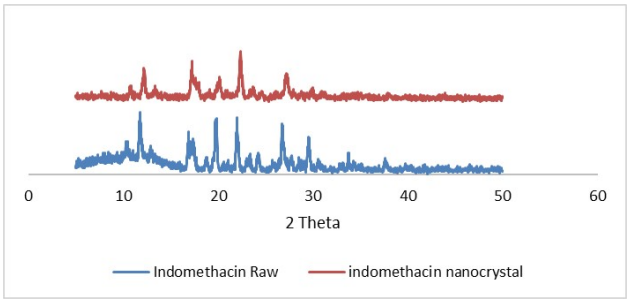

Fig. 5: X Ray Diffractogram of raw and nanocrystals of Indomethacin

Morphological studies

TEM and SEM were used for investigating the surface morphologies of both raw Indomethacin and its nanocrystals. fig. 6(a) shows the SEM images of raw Indomethacin indicating them to be crystalline in nature. Indomethacin raw particles have been found to be irregular and triangular in shape (Dixit *et al*., 2012). TEM images of nanocrystals obtained {fig. 6(b)} indicate they are below 200 nm with in almost homogenous population.

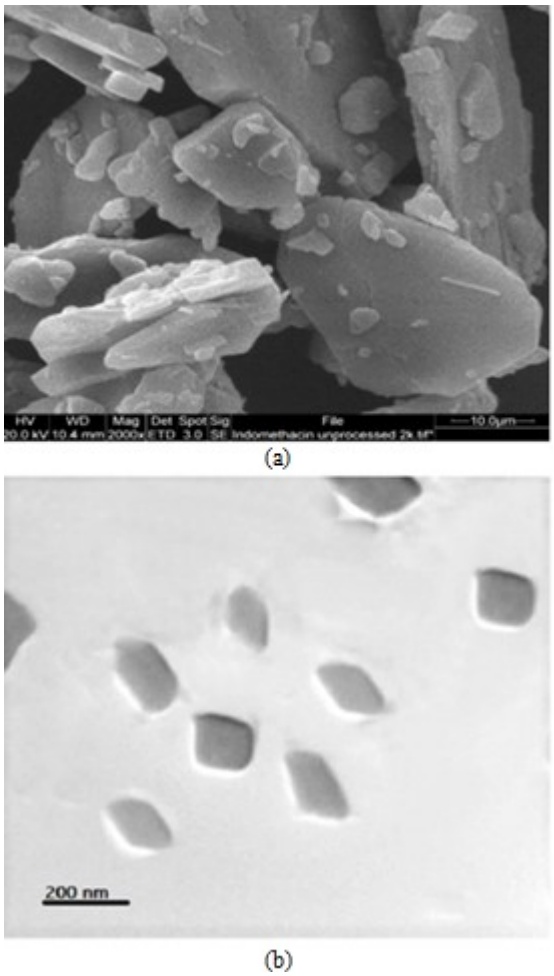

Fig. 6: SEM and TEM images of (a) Raw Indomethacin (b) Indomethacin nanocrystal

In-vitro dissolution

Indomethacin nanocrystals revealed increased dissolution in comparison of its raw and marketed tablets, i.e. 25 mg. As shown in figs. 8, about 85.6% Indomethacin nanocrystals got dissolved within first 5 min of the study. In comparison, raw Indomethacin revealed 18.3% dissolution in first 5 min while its commercial tablets showed 29.2 % dissolution.

DISCUSSION

The results of the study (figs. 1-7) confirm that Indomethacin nanocrystals were successfully produced in nano range using media milling method. Basically the turbulent, shearing of particles and milling media generate enough energy that result in ultimate reduction in particle size, producing nano size particles having high surface area and increased free surface energy (Van Eerdenbrugh *et al*., 2008). Attrition and impaction are among the main mechanisms responsible for size reduction in top down approach (Lu *et al*., 2015). The comparison of the current data with published data shows that the effect of the milling i.e. pattern of decrease in particle sizes of indomethacin nanoparticle is similar (abruptly decrease in first few minutes and then there is gradual decrease in size) with the ketoprofen nanoparticles (Khan *et al*., 2019) while Dexibuprofen nanoparticle (Khan *et al*., 2018c) have different pattern (decreases gradually only).

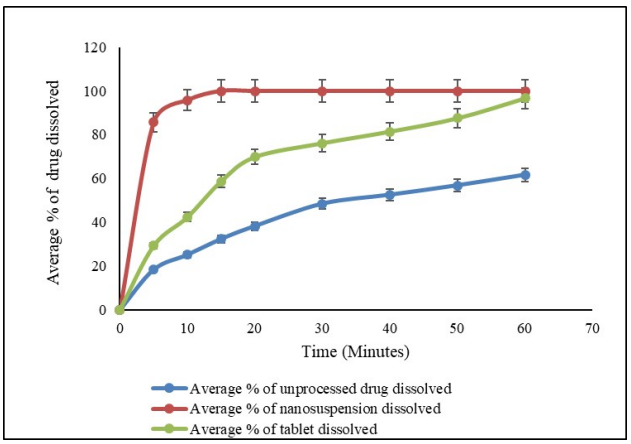

Fig. 7: Dissolution studies of raw, nanoparticle and marketed product of Indomethacin.

The physical stability of the formulation can be linked to the polymers combination (HPMC-PVP-SDS) which has been already reported for such stable formulation (Plakkot *et al*., 2011). Similarly, such polymers combination adsorbs nanocrystals surfaces and thus prevent them from agglomeration (Khan *et al*., 2014). Moreover, PDI of the formulation indicates that the particles are well distributed and thus prevents the process of Ostwald ripening. This in turn results in the particles agglomeration prevention and suspension remains stable (Deng *et al*., 2010).

The decrease in melting point (fig. 4) can be linked to smaller size of crystal and lesser packing density of nanoparticles (Patravale and Kulkarni, 2004, Valleri *et al*., 2004). Moreover, slight angle reflection over the nanoparticles results in shifting the peak intensity towards lower levels, while, nanosizing also broadened the nanocrystal peak correlated to unprocessed material (fig. 7). The diffraction patterns (fig. 5) were found to little bit variation for nanocrystals as compared to its raw form as peak intensity got moved to lesser step owing to slight angle reflection through nanparticles (Bunjes *et al*., 2000). TEM images further confirm a distinct crystalline morphology for the nanocrystals (fig. 6). This can predict a faster distribution pattern for the drug. The faster dissolution rate ($P<0.05$) of Indomethacin can be linked to smaller size and increased surface area nanocrystals (Khan *et al*., 2013, Plakkot *et al*., 2011, Junghanns and Müller, 2008) and it is also a predicting tool for the absence of large particle or its agglomeration as maximum surface area available during dissolution (Junyaprasert and Morakul, 2015).

CONCLUSION

Indomethacin was formulated into nanocrystals, retained all its characteristics using media milling (DENA®) with a large batch size of 350 ml and 3.5% drug concentration. Milling time effects revealed initial abrupt size reduction followed by gradual size reduction. Nanocrystals exhibited 161 nm average sizes upon milling for 60 min. The nanosuspension of the drug was highly stable for a period of 60 days. The drug dissolution rate increased upon conversion to its nanocrystals as compared to its raw or commercial formulation. The increased dissolution rate of the nanocrsytals confirms the nano size with homogenous size distribution and increased surface area. The results of the study clearly reveal that the adopted method for the formulation of Indomethcin nanocrystals is applicable for its large scale production at industrial levels and encourages the scientists for in-vivo investigations in experimental animals and clinical trials.

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