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Fabrication, characterization, process investigation and optimization of experimental conditions for the production of Indomethacin nanocrystals using microfluidic channel reactor

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Abstract: Indomethacin a Non-steroidal anti-inflammatory drug (NSAID) demonstrates low bioavailability. To counter the problem an attempt was made by preparing indomethacin nanocrystals in a micro-channel fluid reactor using different concentrations of the polymers. Indomethacin crystals were optimized by determining the particle size of 380 nm ± 5.0 with PDI of 0.29±0.05. The crystalline nature was confirmed with the help of Powder X-Ray diffraction and Differential scanning calorimetry. A high antisolvent volume (2.0/0.5ml/min), low inlet angle (10°) and mixing time of 60 min with 15 min sonication at a high rate of stirring were the key parameters that produced Indomethacin nanocrystals with good particles size. A high anti-solvent volume (2.0/0.5ml/min), low inlet angle (10°) and mixing time of 60 min with 15 min sonication at a high rate of stirring were the key parameters that produced Indomethacin nanocrystals with good particle size. Similarly, the polymeric combination of including Polyvinylpyrrolidone k-30 (1%)- Hydroxypropyl methyl cellulose-15cps (0.5%) and Sodium lauryl sulfate (0.5%) were the appropriate concentrations. The Transmission electron microscopy and Scanning electron microscopy studies demonstrated the homogeneity with a distinct crystalline structure. The stability study displayed stability at 4°C and 25°C. The nanocrystals exhibited a dissolution rate correlated to the marketed and unprocessed drugs.

Keywords: Indomethacin, microchannel fluidic reactor, nanoparticle, experimental conditions, polymers.

INTRODUCTION

The poor aqueous solubility problem for numerous drugs has become a big challenge for researchers and scientists of advanced drug delivery (De Waard *et al*., 2008). According to recent reports, about 70% of the drugs are in the developmental stage and around about 40% of the APIs present in the market as oral dosage forms have poor or no solubility in water (Kawabata *et al*., 2011, Ku and Dulin, 2012). The poor water solubility problem ultimately leads to erratic bioavailability and toxic effects (Plakkot *et al*., 2011). A variety of strategies have been followed by the researchers of drug delivery which consists of solubilization (Aungst, 2000), dispersions (Serajuddin, 1999), micronization (Lawrence and Rees, 2000), émulsions (Floyd, 1999) and micronization (Charoenchaitrakool *et al*., 2000). But these techniques also face various issues related to the stability and production of ionizable groups (Jones and Leroux, 1999, Serajuddin, 1999, Huang and Dai, 2014). Similarly, the increased solubility sometimes leads to potentially toxic and unwanted effects. Therefore it is the need of the day to formulate such a delivery system that may enhance the solubility of APIs and decrease the side effect related to enhanced solubility (Blagden *et al*., 2007, Qiao *et al*., 2011). From the literature review relevant to the current research project it is clear that nanosuspension could be a desirable delivery system that may tackle the problems associated with hydrophobic drugs (Ghosh *et al*., 2011). Nanosuspensions are composed of particles with a size of below 1000 nm and are stabilized by polymers and surfactants (Rabinow, 2004). The formation of nanocrystals can be achieved by top-down and bottom-up methods (Rabinow, 2004). In the top-down procedure, the

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size can be reduced by attrition forces and mechanical forces. Still, there are some problems associated with the top-down methods which include wide distribution of particle size, contamination and long duration of processing time (Kakran *et al*., 2010). Similarly, in the bottom-up method, the only important step for the control of size of particles is the process of nucleation.

The actual principle followed in this procedure is the antisolvent precipitation which takes place by the transfer of drug molecules from organic phases to the water via diffusion with consequent formation of particles by nucleation in the aqueous phase (de Waard *et al*., 2011). The bottom-up method is also associated with some problems such as uncontrolled growth of particles via Ostwald ripening (Kakran *et al*., 2010) and solvent residue removal from the aqueous phase (de Waard *et al*., 2011).

In the same way, one of the issues associated with both procedures is the conversion of crystals particles into an amorphous state which may lead to stability problems and phase transition (Patravale and Kulkarni, 2004). Therefore the nanocrystals are inevitably preferable to be formed for their wide range stability as compared to the amorphous ones (Ghosh *et al*., 2011).

Indomethacin is categorized as NSAID and is classified as a class-II drug (Löbenberg and Amidon, 2000). It is commonly used for conditions like pyrexia, analgesia and inflammation in the management of rheumatic diseases, dysmenorrhea, dermatitis and ductus arteriosus (Heyneman *et al*., 2000). Due to decreased bioavailability, its aqueous solubility and dissolution would be improved (Hirasawa *et al*., 2003).

The current research project has been designed to produce nanocrystals of indomethacin via a procedure of modified microchannel fluidic reactors (MR). MR is a low-energy bottom-up procedure for the formation of stable nanosuspensions with a low-size distribution.

In the microfluidic channel, the liquid don't behave like the conventional flow theory i.e., the flow of the liquid occurs without mixing and turbulence due to diffusion across the interface with the laminar flow pattern (Van Der Woerd *et al*., 2003, Nguyen and Wereley, 2006, Weibel and Whitesides, 2006). In *this* approach, the infusion of the organic phase into the aqueous phase is a key step to produce the high stability nanocrystals with high particle attributes maintenance (Khan, 2013). Moreover, the physicochemical characterizations of the newly produced nanosuspension have been analyzed by PXRD, SEM, DSC, TEM, zeta potential and the *in-vitro* dissolution studies have also been designed to find out the special characteristics of crystals in nanosuspension.

MATERIALS AND METHODS

Materials

Indomethacin (Batch No. BCBP0623V Sigma-Aldrich, UK), Polyvinyl Pyrrolidone K-30 (PVP k-30) (Batch No. 08297052G0, BASF, Germany), Hydroxypropylmethylcellulose (HPMC) having 15cps viscosity and batch No. 8028213 (Shin-etsu-Chemical), SLS having batch No. MKBR3557V (Sigma-Aldrich-UK). The water was distilled in the Bradford University Laboratory.

Methods

Preparation of Indomethacin Nanosuspension

The method followed was a modified form of the MR procedure (Ali *et al*., 2009). Indomethacin was solubilized in ethanol with a concentration of 5mg/ml while the polymers were dissolved in water and subsequently transferred into a microfluidic channel reactor. In this method, the inlet angle of 10°, the flow rate of 0.5:2.0 ml/minute and the internal diameter of 0.5mm were achieved. The prepared nanosuspension was added to the vial having an aqueous solution of polymers, then agitated continuously for sixty minutes. Eventually, the nanosuspension obtained was allowed to sonicate for 15 min to diminish the ethanol content in the dispersion to a minor residual level (Pignatello *et al*., 2006, Rao *et al*., 2008). The microchannel fluidic reactor was used with different inlet angles i.e., 10°, $25^{\circ}$and 50°. Various parameters were assessed to figure out newly produced particles i.e., inlet angle, stirring time, solvent-anti-solvent flow rates and ultra-sonication (Khan *et al*., 2018a).

The nanosuspension particle size was measured using photon correlation spectroscopy with Zetasizer NanoZS, Malvern Instruments-UK. Briefly, the tests were loaded in the cells without any further dilution and analyzed in triplicates for the determination of particle size and PDI. The zeta potential of the Indomethacin loaded nanocrystals was evaluated by adding 700 µl of sample to folded Zeta potential cells, that is placed between the electrodes in Zetasizer and analyzed in triplicate for determination of Zeta potential (Tariq *et al*., 2019).

Morphology

The surface morphology of unprocessed pure Indomethacin was visualized using a scanning electron microscope (SEM, Quanta 400, FEI Company, Cambridge UK). Briefly, the powder sample was added to the double-sided adhesive tape mounted on the surface of an aluminum stub. The stub was gently tapped twice to remove the excess powder from the surface of the aluminum stub leaving a thin film of nanocrystals on the surface of the stub. The sample was gold coated using a sputter coater and representative sections were photographed at different magnifications to get clear images (Khan *et al*., 2020).

Transmission electron microscopy analysis
The structural features of the nanosuspension droplets were visualized by employing transmission electron microscopy (JEM1200EX, Japan). Briefly, the sample was loaded on the surface of the cu grid and dried up at room temperature. The sample was negatively stained with a solution of magnesium uranyl acetate (2%) as mentioned earlier (Khan *et al*., 2018b). The TEM was operated at 100kV, to get clear photographs of nanosuspension droplets.

Thermal analysis
The thermal properties of both processed and unprocessed indomethacin were determined using differential scanning calorimetry (DSC, TA Instrument, UK). The instrument was first calibrated using Zinc having a melting point of 419.5°C and indium 99% (156.6°C). The samples of raw Indomethacin as well as its nanocrystals were separately added to the aluminum pan and scanned under a nitrogen atmosphere. The samples were scanned between 60 to 190°C at a scanning rate of 10°C/min in triplicate (Khan *et al*., 2018e).

X-ray powder diffraction analysis
The crystalline or amorphous nature of the unprocessed and processed Indomethacin was evaluated using X-ray diffractometer (Siemens-D5000, Germany), with Cu Ka radiation *(X* =1.5418 A). The analysis was conducted with an angle range of 5-50°, a step size of 0.05°, a count time of 3 s per step, rotation of 30 rpm at 40kV and 30 mA (Khan *et al*., 2019).

Stability studies
The stability of nanocrystals with respect to particle size, PDI and drug content was evaluated to ensure their integrity throughout their use. The nanosuspension of indomethacin was consistently evaluated for chemical stability concerning drug content at one-week intervals (day-zero, day-first, day-third, day-fourth, day-fifth, day-sixth, day-seventh). The percentage of API was determined spectrophotometrically (V-630 (JAS.C0) at 320 nm. Furthermore, the nanosuspension of Indomethacin was assessed for Physical stability for particle size and PDI at 25°C and 4°C for two months at regular intervals (Khan *et al*., 2018c).

In-vitro dissolution
Indomethacin nanocrystals' dissolution rate was compared to micronized, unprocessed and commercial formulations. The release behavior of the drug from the samples was evaluated by applying the rotating paddle method (USP 2005). Briefly, the test was added to the vessels of dissolution holding 900ml of phosphate buffer as dissolution medium with a pH of 7.2 and stirred at 100 rpm (Shah *et al*., 2016). The samples were collected at regular intervals of 5, 10, 15, 20, 25, 30, 40, 50 and 60 minutes, filtered using a syringe filter (0.22µm pore size, Millex®GP, IRL). The collected samples were replaced with the same volume of fresh phosphate buffer solution to provide sink conditions. The filtered samples analyzed for drug content spectrophotometrically (V-630 (JAS.C0) at 320 nm.

STATISTICAL ANALYSIS

SPSS-18 package was employed for the statistical assessment of the obtained statistics. The data obtained for statistical analysis was in triplicate, which was measured as Mean ± S.E.M. The One-way ANOVA test ($P<0.05$) was used in the SPSS analysis with the least significant difference test.

RESULTS

Influence of procedural conditions on nanocrystals particle size
The stable drug nanocrystals were produced with the low energy method but the optimization of the process is extremely important to be considered. With the change in parameters, a vivid change in particle size has been observed. Following are the parameters and their effect on the size of particles.

Polymers evaluation
An assessment of the effect of concentration and polymer types on the size of indomethacin nanocrystals was carried out. The employed stabilizers blend was PVP-k30 (1% w/v) - HPMC 15cps (0.5% w/v) - SLS (0.5% w/v) and achieved nanocrystals with particle size and PDI were (380 nm ± 5.0) and (0.29 ±0.05). The distribution of particle size has been presented in figs. 1 & 2.

Effect of the flow rate of solvent and Anti-solvent
Solvent volume constant and Anti-solvent volume variable
The flow rate of the solvent and the antisolvent was judged and it has been revealed to have a great influence on the dimension of nanocrystals. By changing the volume of antisolvent and keeping constant the drug solution, the smaller-sized nanocrystals were obtained. The particle sizes of Indomethacin obtained at 2.0/0.5ml/min was 380nm±5.0 with PDI of 0.29±0.05 as shown in table 1.

Solvent volume variable and Anti-solvent volume constant and
By increasing the volume of the drug solution and by keeping the solution of antisolvent constant, larger particles have been observed. The obtained results were maximum at 2.0/2.0 ml/min and hence Indomethacin particle size was observed to be 803 nm ±3.0 with PDI of 0.57±0.19 as shown in table 1.

Equal volume of Solvent and Anti-solvent
The equal distribution of both solutions i.e., antisolvent and drug solutions, a higher stream rate of solutions was

Table 1: Process conditions impact on the particle size of Indomethacin nanocrystals (n=3)

Input variables					Output Variable	
Sample	Antisolvent Flow rate (ml/min)	Solvent Flow rate (ml/min)	Drug Conc. (mg/ml)	Inlet angle	AVG P Size (nm) ± SD	PDI ± SD
1	0.5	0.5	5	10°	826±3.0	0.47 ± 0.14
2	1.0	0.5	5	10°	677±4.0	0.51 ± 0.16
3	1.5	0.5	5	10°	553±3.0	0.44 ± 0.23
4	2.0	0.5	5	10°	380±5.0	0.29 ± 0.05
5	2.0	1.0	5	10°	1037±4.0	0.66 ± 0.16
6	2.0	1.5	5	10°	1639 ± 6.0	0.57 ± 0.23
7	2.0	2.0	5	10°	2446 ± 6.0	0.93 ± 0.31
8	0.5	0.5	5	10°	826 ± 3.0	0.47 ± 0.14
9	1.0	1.0	5	10°	1398 ± 5.0	0.54 ± 0.09
10	1.5	1.5	5	10°	1875 ± 7.0	0.77 ± 0.13
11	2.0	2.0	5	10°	2446 ± 6.0	0.93 ± 0.31
12	2.0	0.5	10	10°	1545 ± 4.0	0.59 ± 0.15
13	2.0	0.5	15	10°	2706 ± 2.0	0.82 ± 0.12
14	2.0	0.5	5	25°	412 ± 5.5	0.31 ± 0.09
15	2.0	0.5	5	50°	437 ± 6.0	0.39 ± 0.10

observed (2.0/2.0 ml/min), which gave rise to large-sized particle-sized i.e., 803 nm ± 3.0 with PDI of 0.57±0.19 as shown in table 1.

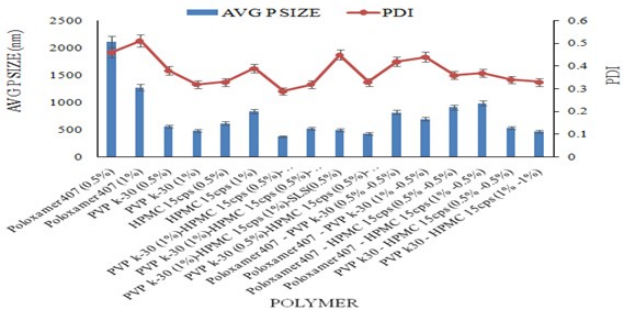


Fig. 1: Polymers impact on nanocrystals of Indomethacin.

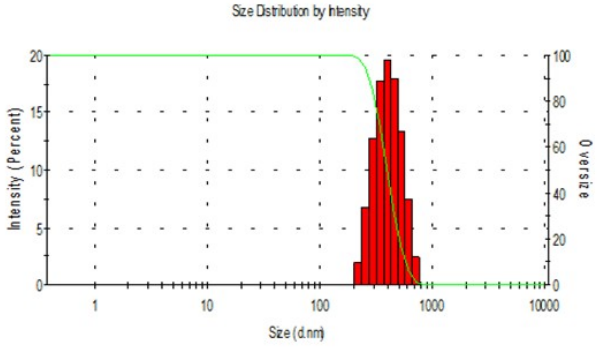


Fig. 2: Particle size distribution of Indomethacin nanoparticle through (PVP-k30 (1 %)- HPMC 15cps (0.5 %)- SLS (0.5 %) polymers.

Drug concentration effect

The evaluation of the concentration of the drug was performed and it was revealed that at higher concentration of the drug large size of particles was obtained. Generally, two kinds of effect were observed at higher concentrations. Firstly, because of the faster nucleation rate, smaller particles were produced. Secondly, because of the large number of nuclei, larger particles were produced due to agglomeration. Table 1 shows that a larger particle size of indomethacin was produced at high concentrations.

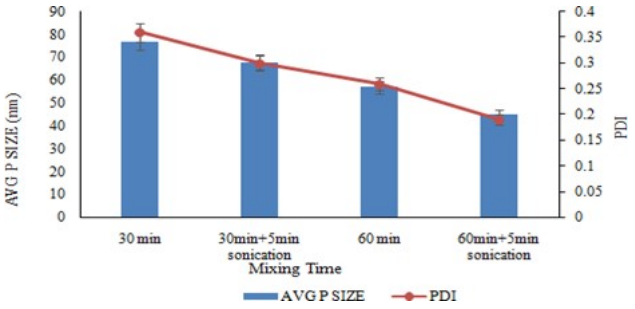


Fig. 3: Effect of Mixing Time on Indomethacin nanoparticle

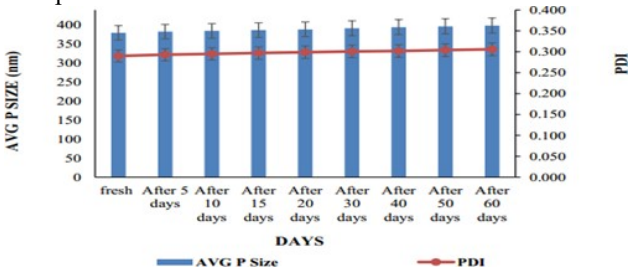


Fig. 4: stability of Indomethacin nanoparticle at 2-8ºC

Inlet angle of Microchannel Fluidic Reactor

To view the effect of the size of particles of indomethacin nanocrystals, three various angles have been investigated. At 50° the particles produced were having a little larger size with broader distribution in comparison with the angle 25° and 10° as shown in table 1.

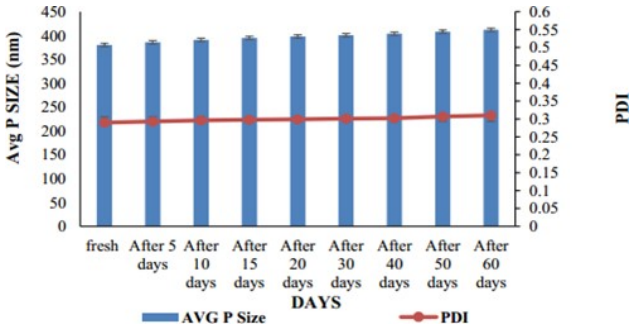


Fig. 5: stability of Indomethacin nanoparticle at 25ºC

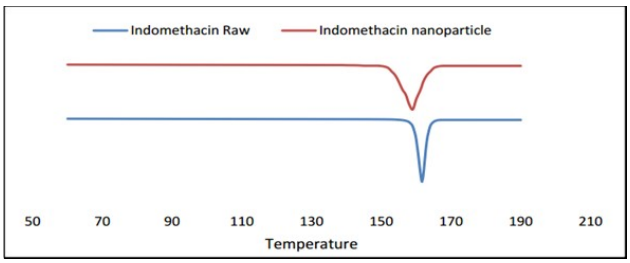


Fig. 6: DSC analysis of processed and unprocessed Indomethacin

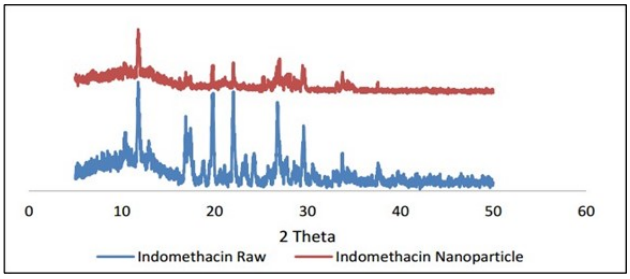
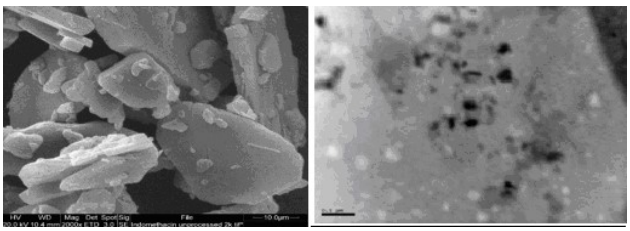


Fig. 7: X-Ray Diffractogram of raw and nanocrystals of Indomethacin



(A) (B)

Fig. 8: SEM and TEM images (A) Raw Indomethacin (B) Indomethacin nanocrystals.

Mixing time effect

The nanosuspension obtained through Micro Fluidic Channel Reactor was streamed into the vial, which contained a polymer mixture and was allowed to mix with the help of a magnetic stirrer. The rpm of the stirrer was allowed to be 1200 as per the reported procedure (Khan *et al*., 2013). The stirrer mixing for 60 minutes created nanocrystals of indomethacin having small particle sizes in comparison to the 30-minute mixing procedure as shown in **fig. 3**. Similarly, the nanosuspension was allowed to sonicate for further 5 minutes, which decreased the particle size and likewise the PDI (Indomethacin particle size 45 nm±3.0 with PDI of 0.19± 0.06).

Stability studies

The drug nanosuspension was processed for One week for the evaluation of the chemical stability. There was no degradation found during this duration. Similarly, the physical stability was studied at 4ºC and also at 25ºC for two months' durations, which showed no degradation with no growth as shown in the figs. 4-5. The 2 months duration study showed a homogenous distribution of the size of particles and PDI value.

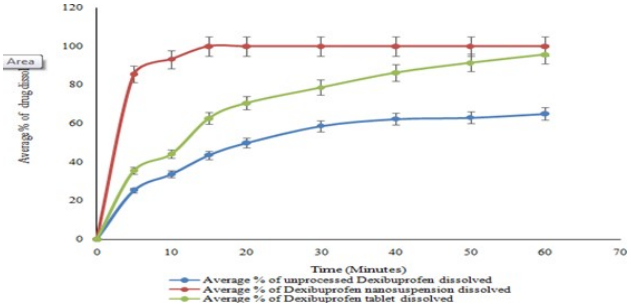


Fig. 9: Dissolution studies of raw, nanoparticle and marketed products of Indomethacin.

Thermal and Powered X-ray Diffraction study

The evaluation of crystalline structure was carried out and was compared with the raw drug PXRD and DSC, which proved the crystalline nature of the nanocrystals. The obtained nanocrystals and raw drug were having sharp melting endotherm. The melting point of the nanocrystals was comparatively low to the raw drugs. The raw and nanocrystals of indomethacin were having 53 and 50ºC melting points respectively as shown in figs. 6-7.

Morphological Studies

For the evaluation of the geomorphology of basic and treated indomethacin, the TEM and SEM were employed. The SEM revealed the raw drug to be crystalline in nature. The particles of indomethacin in raw form were found to be triangular with irregular shapes as shown in fig. 8a. Similarly, the TEM images showed the nanocrystals of indomethacin to have a size of 100 nm having a homogenous distribution of size, with a faster dissolution rate. The TEM showed a defined crystalline morphology of indomethacin as shown in fig. 8b.

In-vitro dissolution

The in vitro dissolution study revealed a high dissolution rate of the nanocrystals in comparison with the raw drug of indomethacin and 100 mg indomethacin tablets. As shown in the fig. 9, 85.4% of nanocrystals of indomethacin have been dissolved in the initial 5 minutes. Similarly, the raw indomethacin demonstrated 25.4% dissolution in the initial 5 minutes, but the tablets showed 35.6% in the first 5 minutes.

DISCUSSION

Fig. 1 describes the impact of all polymers on the nanocrystals of indomethacin. The nanosuspension was not stabilized by single and two polymers but combination of three polymers were effective to create stable nanocrystals of indomethacin. Taking Anti-solvent volume variable demonstrated that the smaller sized nanocrystals were obtained due to two sound reasons. Firstly the appropriate mixing of the solution of the api with the antisolvent, due to high volume of antisolvent in parallel to the drug solution (Su *et al*., 2007). Secondly, the higher initial supersaturation levels are obtained because of less quantity of api solution and strength of antisolvent, which diminishes the concentration of solutes on the surfaces of nuclei (Zhao *et al*., 2007). It has been noted that an increase in the concentration of the drug which provides an opportunity for the solutes to fix at the surface of nuclei and so increase the growth of molecules and give rise to even bigger crystals (Zhao *et al*., 2007).
At equal volume, the large-sized particles were due to the inappropriate blending of antisolvent solution with the drug solution because the in microchannel fluidic reactor the dwelling time was extremely short and so inappropriate infusion of those solutions took place (Zhang *et al*., 2008). This may give rise to irregular region supersaturation and so large-sized particles with extensive dispersion of size may be produced (Ali *et al*., 2009). The table 1 shows that larger particle size of indomethacin was produced at high concentrations, mainly, because of coagulation and condensation phenomena, high concentration growth of crystals is produced (Dong *et al*., 2009). Similarly, high velocities of drugs are there at high concentrations of drugs, which subsequently diminishes the diffusion of solvent and antisolvent solution with the formation of irregular supersaturation, due to which larger particles are produced via crystal growth and agglomeration (Zhang *et al*., 2010). Moreover, prednisolone nanocrystals and BaSO4 nanocrystals in Micro Channel Fluidic Reactor have already been formed following the identical impact (Ali *et al*., 2009, Su *et al*., 2007). it is clear from table 1 that Keener channel ends at angle 25° and 10° possess sharp passage boarder, which allow the two streamlets of liquid for meeting together and then go down the exit with no interruption of the fluid streamlet (Brook, 2006). Similarly, at an angle 50°, the streamlets of the fluid face a more even level to create additional disrupt in the fluid streamlets on the way to the discharge. Mixing effects show sthat at a high rate of stirring and mixing a higher supersaturation, micromixing, quick nucleation and eventually nanoparticle with a comparatively reduced dimension of particles are obtained (Matteucci *et al*., 2006). The stability of nanocrystals gets enhanced due to nanonization, which enhances the exterior space and the exterior available energy as well. Likewise, the solubility of the drug increases at high temperatures, which leads to low saturation levels and growth of nanocrystals following Ostwald ripening (Muller and Peters, 1998). The van der Waals forces between two nanoparticles push to cluster and destabilize the nanosuspension. Likewise, for the stability of nanosuspension, Freitas has recommended being kept at 2-8ºC in the fridge for the maintenance of particle size (Freitas and Müller, 1998). The decrease in melting point reason was the small size of particles and low loading density of the nanocrystals crystal matrix in comparison to the basic drug (Patravale and Kulkarni, 2004). The peak intensity went to a low degree owing to the low-angle reflection of nanocrystals. Moreover, the peak of the nanocrystals was broadened a little as compared to the raw drug due to the sizing effects. The PXRD diffractogram of the basic API was having sharp and elevated peaks as shown in fig 7. While in the case of nanocrystals, some peaks disappeared and the intensity of the peaks also decreased. Due to the effect of the smaller size of particles, the diffraction manner is obtained as different in comparison to the raw indomethacin due to the shifting of peak intensity to a low point as of the slight angle reflection of nanoparticles (Bunjes *et al*., 2000). The enhanced dissolution of the nanocrystals of indomethacin ($P<0.05$) can be connected to the controlled nucleation due to increased surface area (Khan *et al*., 2013). In the same way, from the current study, it may be demonstrated that the smaller particle size enhances the dissolution rate as per Freundlich Ostwald's equation (Muller and Peters, 1998).

CONCLUSION

This research proved that MCFR is an innovative and low-energy bottom-up technology that can create stable nanoparticles. However, the process parameters and experimental conditions should be regulated appropriately. The optimized parameters include antisolvent and solvent flow rates, stirring rate, inlet angles, the concentration of drugs and mixing time. The small inlet angle, high antisolvent flow rate, high stirring rate and mixing time are the significate parameter that controls the particle size.

In addition, low drug concentration and reasonable selection of polymer/surfactant are also crucial parameters to regulate particle size during nucleation. This technique is creating nanocrystals without affecting their characteristics, particularly the crystallinity of the material. This technique can potentially be scaled up in the future. Furthermore, the resulting nanoparticles can be transformed into a different dosage form. Furthermore, the resulting nanoparticles can be transformed into different dosage forms and the techniques can be propagated to industrial scale while employing chemical engineering methods.

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