Combinative Precipitation Ultrasonication Approach for Fabrication for Stable Artemisinin Nanosuspension

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Abstract: The aim of this study was optimization of the processes and experimental conditions for fabrication of stable artemisinin (ART) nanosuspension using precipitation ultrasonication approach with subsequent enhanced dissolution rate compared to raw and micronized ART. The effect of important parameters including concentrations of stabilizer solution using polyvinyl alcohol (PVA), ultrasonication power inputs, length of ultra-sonication and effect of temperature were systematically investigated for fabrication of stable ART nanosuspension. The optimized parameters to produce ART nanocrystals with smaller particle sizes were found to be 0.15% of PVA, at 200 Watt ultrasonic power input over 15 minutes of ultra-sonication at 4°C temperature. The average particle size and polydespersity index for ART nano suspension were found to be 98.77± 1.5 nm and 0.186± 0.01 respectively. Crystallinity of the processed ART particles was confirmed using DSC and PXRD. Physical stability studies conducted for 30 days at different storage temperatures which include 4, 25 and 40°C demonstrated that nanosuspensions stored at 4°C and 25°C were most stable compared to the samples stored at 40°C. The ART nanosuspension showed significantly (P<0.05) increased (87 fold) in dissolution rate of the produced nanocrystals compared to the micronized and raw ART which include 97%, 70% and 10.5% respectively after 60 minutes.

Key words: Artemisinin • Optimization • Experimental conditions • Ultrasonic inputs • Polyvinyl alcohol

INTRODUCTION

The recent literature has shown that more than 40% of active pharmaceutical ingredients (APIs) coming from high throughput screening have tendency to suffer from poor aqueous solubility with subsequent erratic dissolution rate and bioavailability [1]. These drug compounds are very challenging to formulate through conventional approaches [2]. To address the issue of poor water solubility multiple approaches are used which include liposomes, salt, micells, neosomes [3], solid dispersion [4], micronisation [5], complexation [6], hot melt extrusion [7] and nano suspensions [8]. Nanosuspensions of pharmaceutical active ingredients where the particles have increased surface area, high diffusion pressure and dissolution pressure have been reported the most promising approach [9].

Solvent anti-solvent precipitation is an effective way for fabrication of nano or micro-size drug crystals. Briefly, in this technique the drug is dissolved in a suitable solvent followed by rapid injection into anti-solvent phase resulting immediate precipitation of drug particles [10]. Aqueous solution containing stabilizers/growth inhibitors is commonly used as anti-solvent phase. For a number of stable nano suspensions methylcellulose, hydroxypropylmethylcellulose, sodium lauryl sulphate, sodium deoxycholate, pluronics, tween-80 and polyvinyl alcohol have been reported the most suitable stabilizers [11-12].

Nanoparticles could be produced by simple anti-solvent nanoprecipitation method, which is a low energy method and does not need highly sophisticated technology. This approach has now become the focus of pharmaceutical scientists, however some limitations and issues associated with this method are still need to be addressed [13]. The technical challenge exploiting simple precipitation approach is fabrication and stabilization and to maintain particle size and particle size distribution (PSD). Nanoparticles are more prone to aggregation and Ostwald ripening with more potential for sedimentation.
and caking. The stability of suspensions relies on the method of generating particle and associated formulation components [8]. Currently static mixer and impinging reactors have been reported to control particle sizes and particle size distribution [14]. Also S. Khan et al. [8] has reported the controlled crystallization method while using different APIs (active pharmaceutical ingredients) which demonstrated that the stable nanosuspensions could be produced if the process and experimental conditions are properly controlled. How do the nucleation and seed crystal control the particle growth in crystallisation process? has recently been investigated by combined experimental and simulation studies [15] and using impinging reactor as well [16].

In nanoprecipitation nucleation is very paramount to be controlled for tailoring particle sizes and their distribution [17] numbers of factors are responsible for controlling nucleation including supersaturation level, mixing time, micromixing and mass transfer etc [18]. Insufficient micromixing in simple stirring process during mixings of solvent anti-solvent phases could potentially be one of the major causes for instability of the produced nano suspensions[8]. Insufficient micromixing can reduce the supersaturation level with subsequent slower nucleation rate and broader size distribution [14]. To address the issue of micromixing in particular combined precipitation-ultra-sonication has been very effective [19]. Irradiation has been known to be effective for mixing, to accelerate molecular diffusion and have the immense intensity for mass transfer [18].

In Western Medicine Malaria was one of the challenging diseases until the middle of the 20th century. At that time, malaria was still endemic in different parts of Europe and North America [20].

Malaria is caused by the genus Plasmodium. There are four different species of plasmodium which cause malaria in human beings including P. ovale (malaria quartana), P. falciparum (malaria tropica), P. malariae (malaria tertiana) and P. vivax (malaria tertiana) [21]. The first rationale attempt to treat malaria by Cinchona bark was made in early 18th century which has already been used in early 17th century in the treatment of fever [20]. Pyremethamine, chloroquine, halofantrine and mefloquine are the well established drugs which have been used to treat malaria. These drugs are however offered strong resistance by the malarial parasites. A new class of anti malarial group of drugs was derived from traditional Chinese medicine. The use of Artemisia annua has been reported for the treatment of haemorrhoids and fever in China for at least 2000 years [22]. Currently artemisinin and its derivatives which include dihydroartemisinin, artesunate, arteether and lumezantrine in combination with artemether [23], have been reported strong antimalarial drugs which are effectively used for 20 years [24]. Contrary to conventional antimalarial drugs which are required 2-3 days to treat the malaria, the artemisinin act faster with an approximate parasite- and fever-clearance time of 32 h [25]. Nonetheless artemisinin is poorly water soluble which causes the erratic absorption and poor bioavailability with consequent less antimalarial effect. It is therefore still an important issue to address the poor water solubility issue of this drug which can potentially boost up the dissolution and bioavailability. There has been a reported nanoparticles of the artemisinin using stirring method and evaporation precipitation [26]. However, the particle size was not effectively reduced which caused stability issue as well. Moreover due to ductile nature of this drug which is more prone to deform rather than fragmentation at nano size [27], the wet milling methods can blunt the effectiveness of this drug.

The aims of the current study were to optimize both experimental (concentration of polymer) and process conditions (temperature and ultrasonic inputs) for fabrication of stable artemisinin (ART) nanosuspensions using modified and less expensive combined precipitation-ultrasonication approach. Additionally the comparative dissolution study was conducted to compare dissolution rate of the produced nanocrystals with micronized and raw artemisinin to demonstrate the effectiveness of crystallized artemisinin.

**MATERIALS AND METHODS**

Artemisinin (ART) was gifted by institute of Material Medica, China, supplier Chengduwagoot, Pharmaceutical Co, Ltd having Batch No: (110916), polyvinyl alcohol (PVA, 87-90% hydrolyzed and M. Wt 30, 000-70, 000) was purchased from Sigma Aldrich, (USA) having batch No: (MKBR5960V), dimethylformamide (DMF) Batch No 1373199, were purchase from Sigma Aldrich, (USA).

Artemisinin nanosuspension was fabricated using combined precipitation ultrasonication method. Briefly, on the basis of artemisinin solubility 10mg/ml was dissolved in dimethylformamide (DMF) and a series of anti-solvent (aqueous) solutions containing different concentration of PVA(0.1%, 0.15%, 0.25%, 0.5% and 1%) were prepared. Both organic (Solvent) and aqueous (Anti-solvent) solutions were filtered through 0.4 µm filter to ensure maximum clarity. Afterwards, 02 ml of the solvent
containing drug was injected with stirring at 1200rpm into pre cooled(4°C) anti-solvent solution (20 ml). The resulted precipitated artemisinin suspension was followed by ultra-sonication at different ultrasonic input (50, 100, 150, 200, 250 and 300 Watt) at different time of interval (5, 10, 15 and 20 min) using anti-solvent phase. A number of Nano suspensions were prepared at millilitre scale (20ml) in order to identify the most suitable concentration of the stabilizer solution. The time of ultra-sonication burst was set to 3 sec with a pause of 3sec between two ultra-sonication burst. During fabrication the temperature of the vial was controlled with ice cold water. After ultra-sonication at above mentioned parameters, the particle size measurements were carried out using Zetasizer Nano-ZS instrument (Malvern instrument Ltd, UK).

Once the most appropriate concentration of stabilizer and ultrasonic power were identified, the effect of anti-solvent phase temperature (04, 25 and 40°C) was also evaluated for particle sizes. The physical stability study on the produced nano suspensions was conducted for a period of one month at three different temperatures which include 4, 25 and 40°C.

The artemisinin nano suspension was subjected to centrifugation at 16000 rpm for 01hr, the supernatant was decanted and the sediment nano crystals were collected and characterized using differential techniques. Finally the comparative in-vitro dissolution study of artemisinin nano suspension was compared with raw and micronized artemisinin powder.

**Characterization**

**Particle Size Measurement:** The particle size measurement of the produced nanocrystals was carried out using Malvern Zetasizer Nano-Zs dynamic light scattering instrument (Malvern Instruments, UK).

**Morphological Studies**

**Scanning Electron Microscopy (SEM):** The morphology of unprocessed (raw) artemisinin drug substance was determined at different ranges of magnification power by using Scanning electron microscope (Quanta 400 SEM, FEI company Cambridge U.K). Preparation of samples was involved fixing of artemisinin into a metal stub with the help of double sided adhesive tape.

**Transmission Electron Microscopy (TEM):** The particle size of artemisinin nanocrystals was confirmed by TEM using transmission electron microscope (JEM-1200EX, Electron optics Lab, corp, Japan). The sample was prepared by depositing a single drop of artemisinin nano suspension onto a copper grid of 200 mesh coated with carbon which was dried at room temperature. The grid was then negatively stained with 2% magnesium uranyl acetate solution followed by drying at ambient temperature. The images were taken at 120KV.

**Differential Scanning Calorimetry (DSC):** The melting point and heat of fusion of processed and unprocessed artemisinin was determined using DSC (TA Instrument, Q500 and Q200, West Sussex, U.K) at heating range from 25°C to 200°C at the heating rate 5°C/minute. The experiment was performed under atmosphere of dry nitrogen having flow rate of 25 ml/min. All samples were analysed in triplicate.

**Powder X-Ray Diffraction Studies (PXRD):** The crystallinity of raw and processed artemisinin particles previously recovered by centrifugation 16000 rpm was determined by PXRD with powder diffractometer (Bruker-Germany). Silicon-well sample holder was employed for nanocrystals, while plastic sample holder was used for raw drug material. In triplicate the sample and the raw drug was scanned in the range of 5-50° at 2θ/min by using copper Kα as a radiation source with 1mm slit at 1.542 Å wavelength.

**Stability Studies:** To assess the rate of crystals growth by Ostwald ripening and aggregation the stability was monitored for one month at different temperature i.e. (4, 25 and 40°C) at regular time interval (0, 10, 15, 20, 25 and 30 days).

**Dissolution Study:** Dissolution rate of the produced nanocrystals was compared with raw and micronized artemisinin by using USP, dissolution type II apparatus at 200 rpm using reported method of kakran et al. [26]. All the drug sample were added separately to the dissolution apparatus, maintaining the temperature of dissolution medium at 37± 3°C and 5ml of the aliquots was withdrawn at specific interval of time (0, 2, 6, 10, 15, 30, 45 and 60 min). All the samples were analyzed with reverse phase high performance liquid chromatography (RP-HPLC).

**Statistical Analysis:** Statistically data were interpreted using Statistics 8.1 software. All the data were run in triplicate and results were given as mean± standard mean error (SEM). Mean values were compared using Anova-test and difference were considered statistically significant at level (p < 0.05).
RESULTS

**Results**

**Effect of Stabilizer Concentrations on Drug Particle Size and Distribution:** The average particle sizes and polydispersity index (PI) values of the artemisinin nano suspensions produced by simple stirring and combined approach (Simple stirring and ultra-sonication) using different concentrations of PVA solution (Anti-solvent) (0.1%, 0.15%, 0.25%, 0.5% and 1%) pre-cold at 4°C, are shown in Table 1. The results demonstrated a substantial decrease in the particle sizes (98.77 nm) and PI (0.18) values using the combined approach compared to the simple stirring method which resulted the particle size and PI values 580.5 and 0.52 respectively at 0.15% PVA concentration (Table 1).

**Effect of Ultrasonication Power Inputs and Length of Time:** When the stabilizer concentration was fixed at 0.15%, different Ultrasonication inputs were applied (50, 100, 150, 200, 250 and 300 Watt) for (05, 10, 15 and 20 min). The initial decrease in particle size and distribution was observed by increasing the power input. The most stable nano suspension with minimum particle size was achieved at ultrasonic input at 200 watt, with processing time of 15 minutes (Fig. 1A). The increase in particle size and distribution with rapid crystal size growth was however observed for ART by increasing the ultrasonication inputs >200 watt. Whereas at 200 Watt with increasing processing time (0-15 minutes) there was observed decrease in the particle size and distribution. Both the parameters were however increased at > 15 minutes processing time (Fig. 1B).

**Effect of Anti-Solvent Phase Temperature on Particle Size and Distributions:** At optimum formulation conditions previously identified for fabrication of ART nano suspensions, the anti-solvent solution (PVA solution) which was previously stored at different ranges of temperature (04, 25 and 40°C) showed marked effect on the precipitation process with subsequent impact on particle size and PI values (Fig. 2). There was observed that both particle size and PI are temperature dependent. The minimum particle size (98.0) and PI (0.18) values were obtained at 4°C (Fig. 2).

**Physical Stability:** The physical stability of ART nanosuspensions prepared at optimised process and formulation conditions was monitored at different storage temperature (04, 25 and 40°C) for one month. The particle size measurements and optical observations were carried out at 0, 10, 15, 20, 25 and 30th day. This study showed that ART nanosuspension was most stable at 4°C with negligible particle growth (20 nm) and slight growth observed at 25°C (50 nm) (Fig. 3A and B). Similarly at 4°C and 25°C the increase in PI values was not substantial (0.005 and 0.088). However a noticeable increase in particle size (300 nm) and PI (0.45) occurred in the nano-suspensions at 40°C as shown in Fig. 3C.

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**Table 1:** Mean particle size of artemisinin (PS) in nanometre (nm) scale and distribution at different concentration of PVA (4°C) by simple precipitation and ultra-sonication (Values are expressed as mean ± SEM)

<table>
<thead>
<tr>
<th>Method</th>
<th>Parameter</th>
<th>PVA 0.1%</th>
<th>PVA 0.15%</th>
<th>PVA 0.25%</th>
<th>PVA 0.5%</th>
<th>PVA 1%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple Precipitation</td>
<td>PS (nm)</td>
<td>912 ± 2.05</td>
<td>580.5 ± 0.13</td>
<td>1213 ± 2.17</td>
<td>1636 ± 1.70</td>
<td>2781 ± 2.46</td>
</tr>
<tr>
<td></td>
<td>PDI</td>
<td>0.764 ± 0.01</td>
<td>0.526 ± 0.02</td>
<td>0.899 ± 0.007513</td>
<td>0.926 ± 0.02031</td>
<td>0.01 ± 0.0</td>
</tr>
<tr>
<td>Precipitation Ultra-sonication</td>
<td>PS (nm)</td>
<td>386.5 ± 2.01</td>
<td>98.77 ± 1.05</td>
<td>211 ± 1.26</td>
<td>1264.3 ± 2.37</td>
<td>1994.7 ± 1.86</td>
</tr>
<tr>
<td></td>
<td>PDI</td>
<td>0.251 ± 0.02</td>
<td>0.186 ± 0.01</td>
<td>0.447 ± 0.04007</td>
<td>0.744 ± 0.0653</td>
<td>0.886 ± 0.03032</td>
</tr>
</tbody>
</table>

**Table 2:** Dissolution rate of raw, micronized and artemisinin nanosuspensions as a function of different time intervals. Values in each column are significantly different (P ≤ 0.05)

<table>
<thead>
<tr>
<th>Dissolution time (minutes)</th>
<th>Raw ART</th>
<th>Micronized ART</th>
<th>Nanosuspension ART</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM (LC 95% upper/ lower limits)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>2.0±0.057 (2.24/1.75)</td>
<td>15.3±0.10 (15.43/14.57)</td>
<td>70.8±0.321 (71.38/68.61)</td>
</tr>
<tr>
<td>5</td>
<td>3.066±0.176 (3.82/2.30)</td>
<td>22.06±0.145 (22.69/21.44)</td>
<td>70.26±0.384 (71.92/68.61)</td>
</tr>
<tr>
<td>10</td>
<td>5.25±0.011 (5.29/5.20)</td>
<td>35.5±0.251 (36.08/33.91)</td>
<td>85.03±0.176 (85.79/84.27)</td>
</tr>
<tr>
<td>15</td>
<td>7.50±0.015 (7.56/7.43)</td>
<td>55.5±0.230 (55.99/54.00)</td>
<td>90.0±0.100 (90.43/89.57)</td>
</tr>
<tr>
<td>30</td>
<td>8.25±0.011 (8.28/8.20)</td>
<td>68.0±0.300 (69.29/66.70)</td>
<td>92.03±0.176 (92.79/91.27)</td>
</tr>
<tr>
<td>45</td>
<td>9.03±0.145 (9.65/8.40)</td>
<td>70.0±0.404 (71.73/68.26)</td>
<td>92.66±0.260 (93.78/91.54)</td>
</tr>
<tr>
<td>60</td>
<td>10.250±0.005 (10.27/10.22)</td>
<td>72.0±0.208 (72.89/71.10)</td>
<td>97.0±0.115 (97.49/96.50)</td>
</tr>
</tbody>
</table>
Fig. 1: Effect of ultrasonication power input (A) and length of time (B) on mean particle size and distribution of artemisinin nano suspension.

Fig. 2: Effect of stabilizer (PVA) temperature on mean particle size and distribution (PDI) of artemisinin nano suspension.
Fig. 3: Effect of storage temperatures including; (A) 4°C, (B) 25°C and (C) 40°C, on average Particle size and polydispersity index values of artemisinin nanosuspensions for 30 days.

Fig. 4: SEM image of raw artemisinin.
SEM Studies: Samples of raw Artemisinin were analysed by scanning electron microscope at 200X magnification power, as shown in (Fig. 4) the artemisinin crystals were found to be triclinic with average size of 200 micron.

TEM Studies: Transmission electron microscope (TEM) images of raw ART were taken at 80K and the average particle size was found to be bellow 100nm. As illustrated in (Fig. 5).

Fig. 5: TEM image of artemisinin nanocrystals

Fig. 6: DSC profile of processed and unprocessed artemisinin particles

Fig. 7: PXRD patterns of raw and artemisinin nanocrystals
DSC AND P-XRD Studies: Artemisinin nano crystals were characterized by using DCS and P-XRD, which were identified to be crystalline in nature. A single sharp endotherm was observed for both the nanocrystals and raw ART having slight reduction in the melting onset temperature (T\textsubscript{onset}) and in the melting peak maximum, of the nanocrystals compared to raw ART. Also dropped in heat of fusion for nanocrystals was observed (Fig. 6). In PXRD studies both nanocrystals and raw artemisinin produced sharp x-ray chromatograms which confirmed the crystalline nature of the produced nanocrystals (Fig. 7).

Dissolution Studies: The dissolution rate of artemisinin nanosuspension was compared with unprocessed and micronized (0.2 µm) artemisinin. The dissolution rate of artemisinin nano suspension was significantly faster as compared to its raw and micronized drug (Fig. 8). Artemisinin nanosuspension showed enhanced dissolution release rate 70% while micronized and unprocessed artemisinin could only release 15 and 2% drug respectively within 2 min. For artemisinin nanosuspension statistics showed (Table 2) significance (p< 0.05) difference in the in-vitro studies as compared to raw and micronized drugs.

DISCUSSION

Effect of Stabilizer Concentrations on Drug Particle Size and Distribution: The approach utilized here for fabrication of artemisinin nanosuspension was found effective and simple. The appropriate selection and concentration of the growth stabilizer/inhibitor is an important parameter for crystal size growth and distribution. The growth of the crystals was inhibited by adsorbed stabilizer and for full coverage the concentration of the stabilizers should be enough for complete coverage on the crystals surface to provide sufficient sterric force of repulsion between the nanocrystals [13]. Owing to different crystal faces of nanocrystals the molecular interaction of drug nanocrystals and polymers is worth mentioning, because it does not necessary that the same type of polymers works for different drugs [28]. In this connection, our findings showed that < 0.15% PVA concentration the growth in nanocrystals occurred rapidly. However at optimised PVA concentration (0.15%) the nanosuspension was found stable with no particle growth which demonstrated the sufficient adsorption of polymer onto the produced nanocrystals surfaces (Table 1). The effect of stabiliser concentration was also validated by both simple stirring and combined approaches where in both methods we observed the stable nano suspensions at 0.15% PVA concentration. Owing to high level micro mixing and mass transfer, the combined approach was found more effective in terms of particle size reduction and PI [29]. Agglomeration and crystals growth could result due to insufficient coverage of the crystals by stabilizer, while high concentration of the stabilizer hinder transmission of ultrasonic vibration, due to high viscosity of solution, which adversely affect the diffusion between solvent and anti-solvent [19].
Effect of Ultra-Sonication Power Inputs and Length of Time: In optimisation of ultrasonication input power and length of processing time a range of input powers (50-300 watt) and processing time including 5 to 20 minutes were evaluated for production of stable nanosuspensions (Fig. 1). Our findings suggested that stable nanosuspension was obtained at ultra-sonication input (200 Watt) with processing time of 15 minutes. Nonetheless increase in ultra-sonication input >200 Watt and processing time >15 minutes resulted increase in particle size and PI with rapid crystal growth. This increase in particle size can probably be caused by increase in temperature due to high energy input [19]. Similarly the longer time was not effectively helped to reduce the particle size which might be due to the micro mixing level which has already been achieved at 15 minutes. The stability was interestingly increased after finding the most appropriate concentration of stabilizer solution (0.15%) and ultrasonic inputs 200 Watt for (15minutes) (Fig. 1B), which is due high power of micro mixing [18]. The energy addition step (ultrasonication) was found to be a kind annealing (conversion of thermodynamically unstable matter to stable one) step for stable nano suspension by lowering its energy. The lowering of energy can be achieved by converting less ordered solid state to more order form i.e., from amorphous form to crystalline state and vice versa or by reordering the stabilizer/growth inhibitor at the solid-liquid, or increasing adsorption of surfactant on the surface of crystal, which interns will reduce the surface free energy [30].

Effect of Anti-Solvent Phase Temperature on Particle Size and Distributions: Figure 2 illustrated that increasing the temperature of stabilizer (PVA) solution from (04-40°C) resulted in nanocrystals growth with subsequent unstabilisation of the produced nanosuspensions. Lower the temperature of the solution (04°C), smaller the particle size and distribution was observed. While increased in mean particle size and its distribution was observed with sudden growth of crystals at high temperature resulting in unstable nano suspension. It has been reported that in solvent and anti solvent precipitation method the temperature of stabiliser solution (anti solvent) have greater impact on produced nanocrystals [31]. At high temperature the solubility of the ART increased, therefore upon mixing decreasing the level of supersaturation, which resulting the low rate of nucleation having less availability for nuclei for the growth of crystals, ultimately resulting large crystals [32].

Physical Stability: The physical stability study of ART nano suspensions at different temperature was considered very paramount to investigate the impact of temperature on nanocrystals growth. This study revealed that ART nano suspension was very stable at 04 and 25°C with little increased in size after end of day 10⁶, with no marked change in particle size and distribution over one month. Although, at 40°C increased in particle size (300.0 nm) and its distribution was observed (Fig. 3 C).

Probably, at high temperature the growth of the particle size is due to Ostwald ripening [33]. At low temperature the kinetic energy and diffusion are likely to be decreased, which is expected to increase the level of supersaturation and rapid nucleation, since the number of nuclei increased with decreased of solute on each nuclei [32].

Morphology Studies: Morphology of the produced nanoparticles has a direct impact on the dissolution studies and other physicochemical attributes. It is therefore very imperative to find whether the produced particles are amorphous or crystalline in nature. In contrast to SEM images of the raw ART which showed the particle size around 200 micron and shape was triclinic (Fig. 4), the TEM analysis demonstrated that the particle size substantially reduced to nano level (Fig. 5). The nanocrystals were found separate from each other with mix spherical and crystalline shapes. The surfaces were found very rough as compared to the raw ART. TEM images showed that particle size of artemisinin was in the nanometre scale that is being consistent with dynamic light scattering (DLS) determination. However the average size was bit smaller compared to the DLS which is because of the difference in measuring principles of the techniques. In DLS the hydrodynamic layer around the particles is also measured, while in TEM the particles are directly measured [34].

DSC AND P-XRD Studies: DSC and PXRD studies have been reported very important to identify the changes in packing density and crystallinity of the produced nanoparticles [35]. During nanoparticles formation processes including wet milling and anti-solvent crystallisation either the raw crystals are broken down due to attrition forces or new particles are formed from solution. The newly produced particles should behave differently from the unprocessed particles. In this connection DSC and PXRD studies are very helpful to compare and contrast the melting point, heat of fusion and crystallinity of unprocessed and processed particles.
DSC analysis exhibited that melting point and heat of fusion of produced ART nanoparticles were slightly reduced (Fig. 6). This decreased in heat of fusion is due to the adsorbed stabilizer(s) on the nanocrystals surface in trace amount [36, 37]. Also this could be because of the low packing density in case of nanoparticles [35].

In PXRD studies there was observed that intensity of the produced nanoparticles reduced, broadened and disappearance of some peaks also occurred (Fig. 7). Due to particle size broadening effect, the peak for ART was slightly broadened, which is the key to attain low particle size. There was observed disappearance and broadening of some of the peaks for ART nanocrystals which is considered to be due to preferred effects of orientation.

**Dissolution Studies:** The comparative dissolution study of the processed, micronized and unprocessed artemisinin was carried out to reassure the importance of size reduced artemisinin. Interestingly dissolution rate of the ART nanocrystals was markedly increased compared to raw and micronized artemisinin (Fig. 8). The enhance dissolution rate is encouraging owing to the fact that smaller particle size provide immense surface area resulting high rate of dissolution, faster rate of absorption and ultimately resulting maximum drug bioavailability [38].

Despite the fact, the nanoparticles benefits primarily occurred because of increased in surface area. It has also been reported that increase in saturation solubility particularly at a particle size >100 nm, the rate of dissolution become faster as described by the equation of Freundlich Ostwald [39].

**CONCLUSION**

The combined approach (precipitation-ultra-sonication) with controlled process and experimental conditions was found to be successful for fabrication of artemisinin nanosuspension. The mean particle size, particle size distribution and stability of artemisinin nanosuspension were found to be greatly dependent on the process parameters for production of nanosuspension. There was observed a substantial increase in dissolution for the produced artemisinin nano crystals compared to the raw and micronized counterparts. This showed that the nanocrystals were very stable and maintained their increased surface area during dissolution studies. The cost effective simple precipitation followed by ultra-sonication approach needs simple equipment which is easy to be employed for preparation of stable nanocrystals with smaller particle sizes. This study also concludes that pertaining to chemical engineering aspects if both process and formulation conditions are properly controlled then this economic technology could be scaled up for nano formulation of this challenging API (ART).

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