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Fabrication Of Ciprofloxacin Nanocrystals By Probe Sonication Method For Enhancement Of Dissolution Rate.

Md Ali Mujtaba*, Nawaf M Alotaibi, and Lina Eltaib.

Department of Pharmaceutics, Faculty of Pharmacy, Northern Border University, Rafha, Kingdom of Saudi Arabia.

ABSTRACT

Ciprofloxacin (CIP) is a broad-spectrum fluoroquinolone antibiotic used to treat bacterial infections; however, its limited aqueous solubility inhibits its broader clinical uses. One of the major problems associated with poorly soluble drugs is very low bioavailability. Nanosuspension offers the unique advantage of increasing solubility of the drug resulting into faster drug absorption and hence achieving faster maximum plasma concentration. The purpose of work was to develop and evaluate CIP nanocrystals for the enhancement of solubility. Ciprofloxacin nanosuspension was prepared by different surfactants and polymer using probe sonicator, transformed into dry powder using freeze drying and characterized by particle size, polydispersity index (PDI), zeta potential, FTIR, solubility, *in vitro* dissolution and stability studies. Nanosuspension prepared with 2.5% w/v polaxamer 188 and 250 mg CIP, showed the smallest size of 299.1 nm and PDI 0.493. The saturation solubility of formulation NCIP11 and pure drug CIP in water was found to be 45.68 ± 1.86 mg/ml and 298.12± 4.67 mg/ml respectively. Formulation NCIP11 and pure drug exhibited the *in vitro* dissolution about 79.54% and 10.51% within 5 min in 0.5% sodium lauryl sulfate (SLS) media, respectively. Long term stability studies showed that there was no significant change in the mean particle size and PDI at 4 ◦C after 90 days. The prepared nanosuspension showed enhanced dissolution which may lead to enhanced oral bioavailability of CIP.

Keywords: Nanosuspension; Ciprofloxacin; Solubility; Nanocrystal; Lyophilization

Corresponding author

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INTRODUCTION

Low water soluble drugs have serious adverse clinical effects like non-steady absorption due to variability among patients and individual patient dosing. Prior to absorption of a drug, the API should release from the dosage form and it must dissolve in the gastrointestinal fluid. Drugs that have high permeability and low solubility (biopharmaceutical classification system-II) are not easily dissolved hence; they may not be absorbed from the GI tract sufficiently. Recent developments in nanotechnology allow the manipulation of materials at the nanoscale, providing varieties of nanomaterials for diagnosis and therapy. Particle size reduction to nanometer can lead to an increased rate of dissolution and higher oral bioavailability. Nanosuspension has emerged as an important tool in drug delivery to rectify these solubility conflicts. A nanosuspension consists of drug nanocrystals, a stabilizing agent (typically surfactants or polymeric stabilizers) and a liquid dispersion medium [1, 2]. The dispersion medium can be water, mixtures of water and other non-aqueous media (e.g. water–ethanol mixtures) or non-aqueous media (e.g. polyethylene glycol, oils) [3–6]. Drug nanocrystals are pure solid drug particles with a mean particle size below 1000 nm, ideally between 200 nm and 500 nm.

In 2015, ciprofloxacin (CIP) was listed on the WHO Model List of Essential Medicines, which is a list of the minimum medicine needs for a basic health care system containing the most effective, safe and cost-effective medicines for priority conditions [7]. It is approved for a number of acute bacterial infections, including urinary tract infections, bone and joint infections, intra-abdominal infections, infectious diarrhea, respiratory tract infections, skin infections, and typhoid fever [8]. Ciprofloxacin also showed the synergetic antibacterial activity with the combination of some flavonoids against clinical MRSA (methicillin-resistant Staphylococcus aureus) [9, 10]. In nearly all cases, the ciprofloxacin drug must be dispersed well in solution to be absorbed into the bloodstream from the gastrointestinal tract. However, poor aqueous solubility of ciprofloxacin has been a wide problem for its clinical application.

Today, manipulation of drug candidate at nano-scale by the healthcare industries and research laboratories is mainly for desirable solubility, bioavailability and increased patient compliance. Nanosuspension could be an approach to enhance the aqueous solubility, dissolution and oral bioavailability of poorly water soluble drugs. Therefore, the main objective of this research work was to prepare nanocrystals of CIP using probe sonication method to enhance the solubility and dissolution in order to improve the oral bioavailability.

MATERIALS AND METHODS

Materials

Ciprofloxacin (CIP) was procured from Sigma Aldrich, (St. Louis, MO, USA). Tween® 80, PVP K 25 and propylene glycol were purchased from S.D. Fine-Chem. Ltd. (Mumbai, India). Poloxamer 188 was purchased from Sigma–Aldrich, St. Louis, MO, USA. Rest of the chemicals were of analytical grade and were purchased from Merck specialities private limited (Mumbai, India).

Preparation of nanosuspension

The nanosuspensions were prepared with different types and varying concentration (w/v) of surfactants by employing probe sonication method [11]. Aqueous solutions of surfactants like PVP K 25, poloxamer 188, Tween 80 and propylene glycol were prepared in the concentrations as shown in the Table 1 using distilled water. Accurately weigh CIP (250 mg) added to 50 ml of aqueous solution of surfactants. The suspensions mixed by mechanical stirrer to get homogeneous suspensions at 1000 rpm for 10 min. To the resultant homogenous suspension, probe sonication was employed at amplitude of 30%, pulse 30 sec for 10 min to produce nanocrystals (SONICS Vibra cell VC750, USA). During this sonication, the temperature was maintained at 0°C using an ice bath. Those surfactant which showed good stabilizing properties and able to the kept the nanocrystals without any steric barrier were selected. Only poloxamer 188 gave stabilized CIP nanocrystals. After the establishment of process, the three different batches of nanosuspension were prepared (Table 2).

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Table 1: Preparation of nanosuspensions of CIP

Formulation code	CIP (mg)	Formulation Composition			
		Tween 80 (%w/v)	Polaxamer 188 (%w/v)	PG (%w/v)	PVP (%w/v)
NCIP1	250	1.5	-	-	-
NCIP2	250	3	-	-	-
NCIP3	250	-	1.5	-	-
NCIP4	250	-	3	-	-
NCIP5	250	-	-	1.5	-
NCIP6	250	-	-	3	-
NCIP7	250	-	-	-	1.5
NCIP8	250	-	-	-	3

Table 2: Nanosuspension formula for optimization of stabilizing agent

Formulation code	CIP (mg)	Conc. of Polaxamer 188 % (w/v)	Particle size (nm)	PDI	Zeta potential (mV)
NCIP9	250	1.0	474.3	0.767	26.51
NCIP10	250	2	382.9	0.682	31.80
NCIP11	250	2.5	299.1	0.493	22.65

Measurement of particle size, polydispersity index and zeta potential

The particle size (PS), polydispersity index (PDI) and zeta potential (ZP) of nanocrystals were measured using dynamic light scattering zetasizer (PSS NICOMP Z3000, **Port Richey, FL**). Prior to the measurement, the samples were diluted with double distilled water to a suitable scattering intensity and redispersed by hand shaking. Dynamic light scattering measures Brownian motion and relates to the size of the particles. It does this by illuminating the particles with a laser and analyzing the intensity fluctuations in the scattered light. PDI obtained from the DLS measurement purports the width of a hypothetical unimodal particle size. The ZP is considered as one of the highest measured parameters which denotes the overall charges acquired by the particles in a particular medium and it is considered as one of the important factors for the stability of the nanoparticles. The nanosuspension showing the lowest particle size with acceptable zeta potential was selected for further studies.

Lyophilization of nanosuspension

Though the zeta potential indicates the stability of the nanosuspension, however, the freeze drying is good for long term stability of colloidal nanoformulations and thus the optimized nanosuspensions were lyophilized using mannitol as cryoprotectant and subjected to various evaluation [12]. Accurately, 7.5% w/v mannitol as cryoprotectant was added into the nanosuspensions before deep freezing. 50 mL of nanosuspension filled in vials and frozen using deep freezer at −20 ◦C for 24 h. These frozen solids were freeze-dried using lyophilizer (Zirbus technology, Germany) at a vacuum degree of 200 Pascal for 24h to produce free flowing dry powder.

Fourier transform infrared spectrosopy (FTIR) analysis

FTIR spectra of pure drug, poloxamer 188, physical mixture of CIP with poloxamer 188 and freeze dried nanocrystals was obtained using FTIR spectrometer (FTIR 7600, Angstrom Advanced Inc.). Each sample were mixed with potassium bromide of IR grade in the ratio of 1:100 and compressed. The infrared spectrums were obtained between the ranges 400–4000 cm^{-1}.

Solubility studies

The solubility of CIP was determined by addition of excess amount of plain drug (CIP) in distilled water, maintained at 37 ◦C ± 0.5 ◦C in a water bath and continuously shaken into mechanical orbital shaker up to 24 h and used as a control. Samples were taken out and filtered through 0.45 µm pore size filter paper and analyzed using UV-VIS spectrophotometer at 288 nm. In the similar way saturation solubility was determined for lyophilized nanosuspension. Each determination was carried out in triplicate.

Dissolution studies and release kinetics

In vitro dissolution studies were carried out on pure drug (CIP) and freeze dried nanosuspension using USP dissolution assembly Type-II (Erweka Dissolution tester, Germany). The rotation speed of the paddles is set to 100 rpm. About 900 mL of 0.5% SLS at 37±0.5 ◦C was used as the dissolution medium. At predetermine time intervals, 5 mL samples were withdrawn, filtered through 0.45 µm membranes immediately and 5 mL blank dissolution media added for replenishing of the medium, respectively. The amount of dissolved drug is determined using UV-spectrophotometer at 288 nm. The mean results and the standard deviation will be reported. The data obtained from *in vitro* drug release studies was fitted to various release models like zero order, first order, Higuchi, and Korsemeyer Peppas model [13] to understand the mechanism of drug release from the nanosuspension.

Stability studies

The stability studies of optimized lyophilized nanosuspension were done at refrigerated condition at 4 °C and room temperature for a period of 90 days. Optimize formulation batch in dry powder form fill in six different glass vials with rubber stoppers. Three vials were kept in deep freezer maintained at 4 °C for period of 90 days. The remaining three vials were stored at room temperature for same period. After 90 days the samples were redispersed in distilled water and checked for stability with respect to PS and ZP.

RESULTS AND DISCUSSION

CIP nanosuspension formulations were prepared by the probe sonicator using different types of surfactants and polymer (PVP K 25, poloxamer 188, Tween 80 and propylene glycol) with varying concentrations (Table 1). During probe sonication, electrical energy is converted into shock waves through a series of transformation. These shockwaves produce bubbles which violently explode and collide with particles resulting in generation of heat [14]. Stabilizers (surfactants and polymer) are added to nanosuspension formulations to reduce the free energy of the system by decreasing interfacial tension and to prevent nanoparticle aggregation by electrostatic or steric stabilization [15]. The stabilizers Poloxamer 188 resulted in smaller particle size compared to Tween 80, PVP K 25 and propylene glycol. It was due to the difference in the chemical structure of Poloxomer-188 and Tween® 80. It can be easily observed that Poloxomer-188 contains two hydrophilic terminals with central hydrophobic moiety whereas Tween® 80 contains only one hydrophilic terminal. Because of such structural differences Poloxomer-188 is able to show better performance than Tween®80 [16]. Poloxamer 188 showed the good stabilizing properties and it was able to the keep the nanocrystals without any steric barrier. After process optimization of nanosuspension, three different formulation batches NCIP9, NCIP10 and NCIP11 were prepared and characterized by the particle size, PDI and zeta potential.

Particle size, polydispersity index and zeta potential

Particle size, PDI and zeta potential for the final formulation batches (NCIP9, NCIP10 and NCIP11) showed in Table 2. The effect of the concentration of stabilizer on the particle size is shown in Fig. 1. It was also observed that the increase in the concentration of surfactants lead to decrease in the particle size. It can be explain by the poor dispersion of hydrophobic particles of CIP at low concentration of surfactants and formation of agglomerates. On the basis of particle size, PDI and zeta potential, the formulation batch NCIP11 was selected as optimized formulation. Polydispersity of optimized nanosuspensions (NCIP11) was found 0.493 indicating the narrow particle size distributions of particles. The surface charge is an important factor, influencing the stability of colloidal dispersion. The zeta potential of optimized nanosuspensions was observed to be in the acceptable range (+20 to −20 mV) [17].

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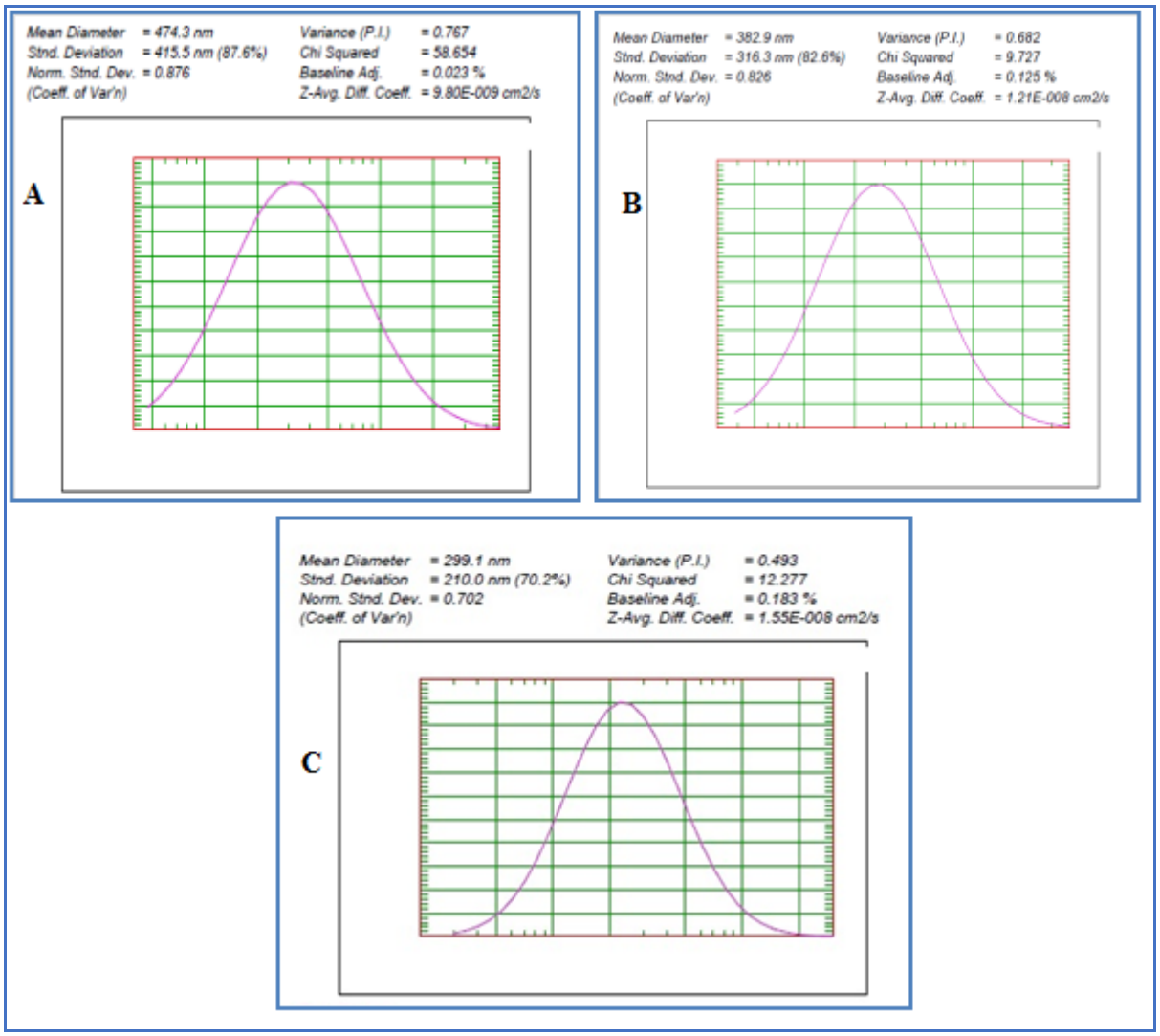

Fig 1: Partcle size of batch (A) NCIP9 (B) NCIP10 (C) NCIP11 nanosuspension formulation.

FTIR spectroscopy

The characteristic peaks at 1495, 1627, 1707 cm^{-1} of CIP were observed from both FTIR spectra of raw CIP and nanocrystals. There was no difference in the absorption bands in chemical bonding of pure drug (CIP) and optimized formulation (NCIP11) containing CIP; this clearly revealed that the drug was compatible with poloxamer 188.

Solubility studies

The Noyes Whitney equation described the dependency of the saturation solubility in comparing the solubility of two particles with different radius. As the particle size decreases, it improves the saturation solubility. The observed solubility for pure drug CIP and nanosuspension (NCIP11) was found to be 45.68 ± 1.86 mg/ml and 298.12± 4.67 mg/ml. Result demonstrated the enhanced solubility of CIP by 6.53 folds in nanocrystal form. It cleared that when particle is in nanosize, saturation solubility of drug has increased. Solubility enhancement of CIP nanocrystal was due to decrease in particle size which leads to providing larger surface area than the pure CIP.

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***In vitro* dissolution study**

The release profiles of the pure CIP powder and CIP nanoparticles were compared in Fig. 2a. The dissolved amount of drug from CIP nanoparticles increased to 79.54% after 5 min, while only 10.51% from pure CIP powder. The increased dissolution rates of the nanoparticles are primarily attributed to the reduced particle size. In addition, the increase in surface wetting by the surfactants in the nanosuspension formulations most likely resulted in further enhanced dissolution rates as compared to pure drug [11]. Therefore, nanonization of CIP using probe sonication can decrease the particle size, enhancing the dissolution rate of CIP in water.

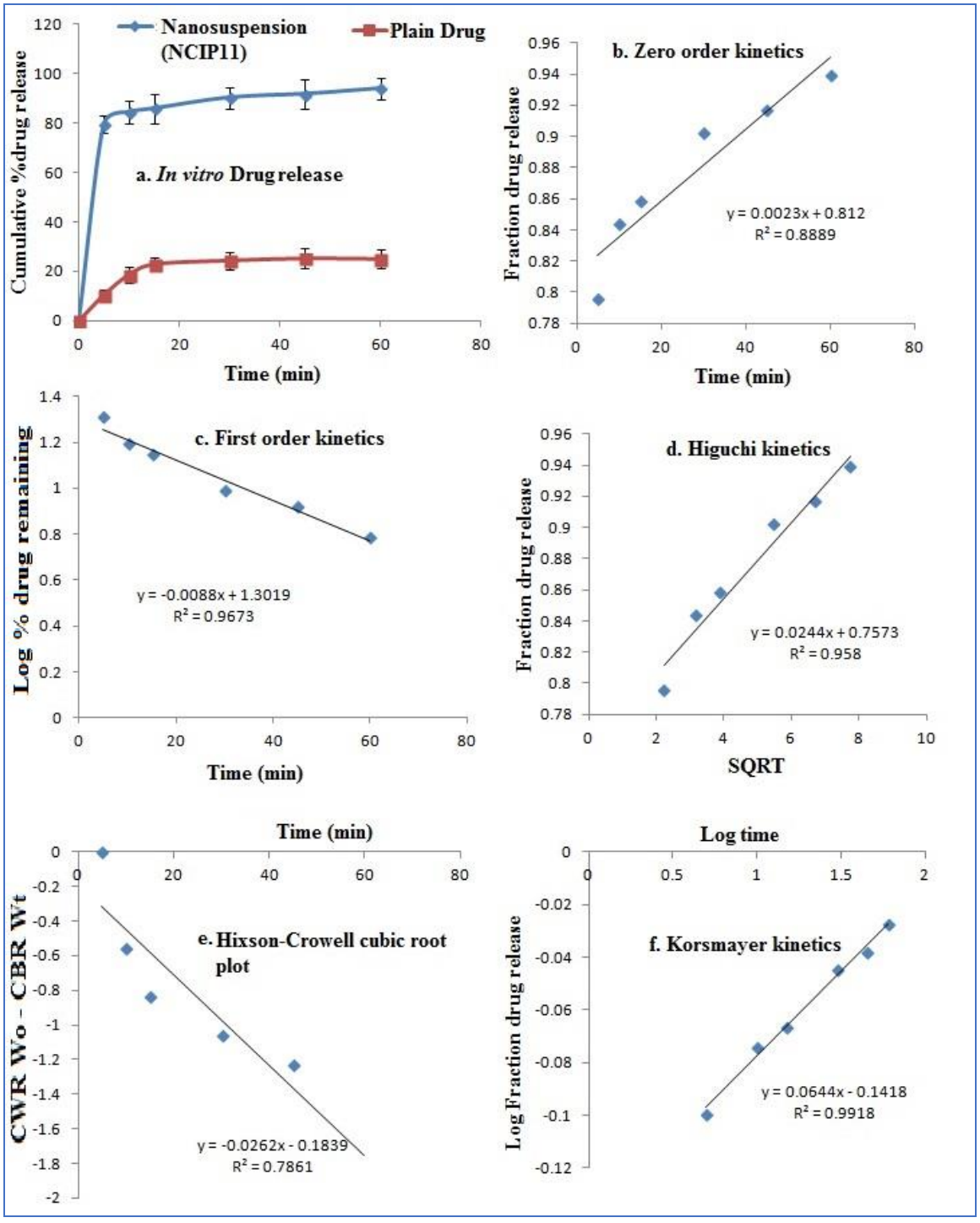

Fig 2: *In vitro* drug release and release kinetics models for CIP nanocrystals (NCIP11)

The drug release data were plotted in various kinetic models including zero order, first order, Higuchi, Korsmeyer-Peppas and Hixson Crowell equation (Fig. 2b-2f). From the analysis of Fig. 2 it was found that the *in vitro* drug release of nanocrystals was best explained by first order, as the plots showed the highest linearity (R^2= 0.958). However, drug release was also found to be close to Higuchi's model (R^2= 0.9393). The release mechanism of CIP-nanocrystals was best fit-ted to Korsmeyer-Peppas model which indicated a good linearity, R^2= 0.9918. The value of exponent constant (n) was 0.03, which suggesting Fickian diffusion process.

Stability studies

Stability studies were conducted for finally optimized formulation (NCIP11) at room temperature and 4 ◦C temperature for 90 days. There was no significant change in PS and ZP of nanocrystal at 4 ◦C but some changes were noticed in PS and ZP values at room temperature and were statistically significant, which indicated the susceptibility for stability problems during storage at room temperature. At room temperature Ostwald ripening was more facilitated due to quicker redispersion on the particles and resulted into the larger particles. Hence, storage of prepared nanosuspension is mandatory at 4 ◦C temperature to stabilize throughout its shelf life.

CONCLUSION

In conclusion, probe sonication method was successfully employed to produce stable ciprofloxacin nanocrystals and the parameters effecting performance of the formulation were optimized. This method was easy to apply, simple, cheap and promising for preparing drug nanocrystals. Nanosuspension prepared with 2.5% w/v polaxamer 188 and 250 mg CIP, showed the smallest size of 299.1 nm and PDI 0.493. Formulation NCIP11 and pure drug exhibited the *in vitro* dissolution about 79.54% and 10.51% within 5 min in 0.5% sodium lauryl sulfate (SLS) media, respectively. Hence, a significant decrease in particle size, enhanced aqueous solubility, improved drug dissolution collectively led to improvement in oral bioavailability of CIP.

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