Pharmaceutical Sciences, November 2015, 21(Suppl 1), 136-144
doi: 10.15171/PS.2015.28
http://journals.tbzmed.ac.ir/PHARM

Research Article

Optimization of Cefixime Nanosuspension to Improve Drug Dissolution

Elham Ghasemian[1], Behnaz Rezaeian[1], Samaneh Alaei[1], Alireza Vatanara[1*], Vahid Ramezani[1,2]
[1]*Pharmaceutics Department, Faculty of Pharmacy, Tehran University of Medical sciences, Tehran, Iran.*
[2]*Department of Pharmaceutics, Faculty of Pharmacy, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.*

Article Info

Article History:
Received: 28 April 2015
Accepted: 27 July 2015
ePublished: 18 November 2015

Keywords:
Cefixime
Nanosuspension
Dissolution Rate
Solubility
Optimization
Factorial Design

ABSTRACT

Background: The aim of this study was to prepare and characterize cefixime nanosuspensions in order to enhance the dissolution rate and solubility of this drug. ***Methods:*** Nanosuspensions were prepared using sonoprecipitation method and the effects of surfactant type, surfactant and solid content, sonication power input and interval of acid addition on the yield and particle size of nanosuspensions were investigated. ***Results:*** Particle size and yield of the optimal nanosuspension formulation were 266±10 nm and 35±2%, respectively. Scanning electron microscopy (SEM) results showed a nearly spherical morphology for cefixime nanoparticles. Thermal analysis indicated that there was a partial crystalline structure in the nanoparticles and *in vitro* dissolution rate of the drug was significantly increased by the reduction in particles size. ***Conclusions:*** Sonoprecipitation was shown to be a successful method to produce cefixime nanosuspensions and the optimum conditions of the process were introduced.

Introduction

Cefixime is the only oral third generation cephalosporin with a broad spectrum of antimicrobial effect on Haemophilus influenzae, Moraxella catarrhalis, Neisseria gonorrheae, Escherichia coli and Klebsiella resistant to ampicillin, other oral cephalosporins and trimethoprim-sulfamethoxazole. This characteristic of cefixime permits its use in urinary and respiratory tract infections.[1] But, one of the major problems with this drug is its low water-solubility that leads to poor bioavailability (about 40-50%) after oral administration (classified as class II of BCS).[2] Also, some evidence has shown that cefixime is absorbed in a limited region in gastrointestinal tract, stomach and the upper part of the intestine.[3] For this reason the absorption rate and extent of its bioavailability are controlled by its dissolution rate in gastrointestinal fluids.[4] The most reported methods that have been used in order to enhance the solubility and dissolution rate of cefixime are solid dispersion and the use of some hydrophilic compound and polymers such as croscarmellose and β-cyclodextrins in formulation of solid dosage forms.[5,6]

Formation of nanoparticles could provide some benefits such as increased saturation solubility and drug dissolution rate, improved bioavailability and dose proportionality, reduced fed/fasted and inter-subject variability in comparison with the coarse or micronized drug powder.[7] In this manner, nanopercipitation technique involves the formation of crystalline or semicrystalline drug nanoparticles by nucleation and growth of drug crystals.[8] In this method, nanoparticles could be formed by different techniques such as microfluidic reactors,[9] pH controlled precipitation,[10] anti-solvent precipitation with or without surfactant [11] and sonopercipitation.[12]

Nanosuspensions, as colloidal dispersion of drug particles, are stabilized by surfactants and have an average particle size in the range of 200 to 600 nm.[13] In this technology, nano-sized drug particles maintain a crystalline state with increased dissolution rate due to the increase in surface area and saturation solubility.[14]

The aim of this study was preparation, characterization and optimization of cefixime nanosuspension by ultrasonication-antisolvent precipitation method to enhance solubility and dissolution rate of the drug.

Materials and Methods

Materials

Cefixime trihydrate was a gift from Jaber Ebne Hayyan Pharmaceutical Co. (Iran). Polyvinylpyrrolidone (PVP) K-30 was purchased from Fluka (Germany). Sodium lauryl sulfate (SLS), Hydroxypropyl methylcellulose (HPMC) E50 and E15, sodium deoxycholate (NaDC) and sodium carboxymethylcellulose (Na-CMC) were supplied by Sigma-Aldrich (Germany). Phosphoric acid of analytical grade and sodium hydroxide were obtained from Merck (Germany).

***Corresponding Author:** Alireza Vatanara, Tel: (+98) 21 66959057, Fax: (+98) 21 66461178, E-mail: vatanara@tums.ac.ir

Preliminary study on nanosuspension formulation

Cefixime nanosuspensions were prepared by the sonoprecipitation method in the presence of different amounts and type of surfactants as presented in Table1.

Table 1. Preliminary screening of different types and ratios of stabilizers in the formulation of cefixime nanosuspension.

Stabilizer type	Amount of stabilizer (mg)			
	25	50	100	150
NaCMC	R_1	R_2	R_3	R_4
NaDC	R_5	R_6	R_7	R_8
PVP	R_9	R_{10}	R_{11}	R_{12}
SLS	R_{13}	R_{14}	R_{15}	R_{16}
HPMC E_{15}	R_{17}	R_{18}	R_{19}	R_{20}
HPMC E_{50}	R_{21}	R_{22}	R_{23}	R_{24}

NaCMC: sodium carboxymethylcellulose, NaDC: sodium deoxycholate, PVP: polyvinylpyrrolydone, SLS: solium lauryl sulfate, HPMC: hydroxypropyl methylcellulose

At first, 150 mg of cefixime with the proper amount of surfactant and 20 mL of distilled water were placed in a 40 mL beaker and pH of the mixture was raised by NaOH (1M) to a final value of 11. At this pH, cefixime was completely dissolved in the solvent. Then, the solution was placed in an ice-water bath and treated with an ultrasonic probe (Hielscher, Germany) at power input of 280 W and a cycle of 1.0 per second. In the next step, precipitation initiated by the addition of phosphoric acid 85% drop-wise (one drop per 20 s). As the nanosuspension emerged, size and polydispersity index (PDI) were evaluated. Samples were freeze dried for 48 hours through lyophilization procedure (Christ, Germany) and stored at 2-8° C for further experiments.

Experimental Design

The design and statistical analysis were performed using Design-Expert® V6 (DX6) Software for design of experiments (DOE). Tests were performed as a factorial study to assess the effects of formulation and process variables on particle characteristics. The tested variables and their levels are described in Table 2. Experimental factors and factor levels were determined in preliminary studies. The evaluated responses were size (nm) and yield (%). Measurements were carried out in triplicate. The relationships linking the main factors and their interactions to the responses were determined and presented as a general form in the following equation:

$$Y = \text{intercepts} + \sum \text{main effects} + \sum \text{interactions} \qquad \text{Eq.(1)}$$

Table 2. Numeric variables and levels in the experimental design for preparation of cefixime nanosuspension

factors	Variables		
	Low (-1)	Medium (0)	High (+1)
Surfactant content (mg) (B)	25	87.5	150
Solid content (mg) (C)	300	600	900
Power input (W) (D)	120	200	280
Interval of acid addition (s) (E)	15	45	60

Equation coefficients were calculated using coded values; hence the various terms were compared directly, regardless of magnitude. Coding throughout the statistical analysis denotes that -1 was taken as the actual value of the factor at its lower level and +1 for the upper level. This coding was taken to the surfactant type, too: -1 for PVP and +1 for SLS. Thus, a positive parameter coefficient indicates that output increases at a higher level evaluation for the variable and vice versa. Values are given as mean±SD. Statistical significance of the results was determined using one-way analysis of variance (ANOVA), employing a confidence interval of 95%. The numerical output of ANOVA includes the F-value, stating magnitude of the impact of each factor and P-value as representative of the statistical significance with smaller figures signifying greater importance.

Physicochemical characteristics

Particle size analysis

The mean hydrodynamic size (z-average) of nanoparticles was measured by photon correlation spectroscopy (Zetasizer®, Malvern Instruments, UK) at 25˚C. Particle sizes were analyzed right after preparing nanosuspensions.

Yield

Nanosuspensions were centrifuged (sigma, Germany) at 20000 rpm for 30 minutes. Dissolved drug in supernatant was quantified using a UV spectrophotometer (Spekol, Germany) at a wavelength of 288 nm. The yield of the process was calculated using equation 2.

$$\text{Yield} = \frac{Total\ drug - Dissolved\ drug}{Total\ drug} \times 100 \qquad \text{Eq.(2)}$$

Scanning electron microscopy

The surface morphology of unprocessed cefixime and optimal nanoparticles (NPs) were evaluated using a scanning electron microscope (S-4160, Hitachi, Japan) at a voltage of 20 kV. Lyophilized samples of nanosuspensions were spread on stubs using double sided carbon tape and then sputtered with gold using a sputter coater (BAL-TEC, Switzerland).

Thermal analysis

Thermal behavior of all materials used in the optimal nanosuspension was studied using a differential scanning calorimeter (Mettler Toledo, Switzerland). The equipment was calibrated using Indium and Zinc. About 5-10 mg of samples was heated to temperatures

within the range of 20-330° C at a scanning rate of 20° C/min in aluminum pans under nitrogen gas.

Dissolution studies

Drug release of the lyophilized nanoparticles, coarse cefixime and the physical mixture of coarse cefixime and PVP were studied in phosphate buffer (PBS) at a pH of 7.4 as the dissolution medium. Amounts of the powders, equal to 250 mg of cefixime, were dispersed in screw-capped glass vials (100 mL), containing 50 mL of the medium, by shaking at 50 rpm at 37±0.5°C in a shaker incubator (LABOTEC, Germany). At predetermined time intervals (1, 5, 10 and 15 min) 1 mL of the dispersion were taken away and replaced with 1 mL of the fresh PBS. Then, samples were filtered through 0.22µ syringe filters and the amounts of dissolved cefixime were determined using a UV spectrophotometer (Spekol, Germany) at a wavelength of 288 nm. All tests were carried out in triplicate.

Results

Cefixime nanosuspension was successfully prepared using the ultrasonication-precipitation method. In the first step, various types and concentration of stabilizers were screened according to Table 1 to achieve the most favorable particle size. Data demonstrated that application of HPMC (E_{15}) and HPMC (E_{50}) in 4 levels of concentration resulted in the formation of agglomerated particles in the size range of 926-1423 nm and 1002-2105 nm, respectively. In the same way, particle sizes were in the range of 369-400 nm for NaDC and 2005-2670 nm when NaCMC was used. On the other hand, addition of 25-150 mg of PVP and SLS to the formulations showed a better effect on size reduction and produced particles in the range of 208-514 nm and 190-685 nm, respectively (Figure 1). The polydispersity index (PDI) was <0.5 in all formulations which indicated narrow size distribution. In addition to the size, the other important factor evaluated for selecting stabilizer type was the yield of the process. The nanosuspensions formulated by PVP and SLS showed higher yields in comparison with other excipients. So, SLS and PVP were selected for further investigations in an experimental design approach.

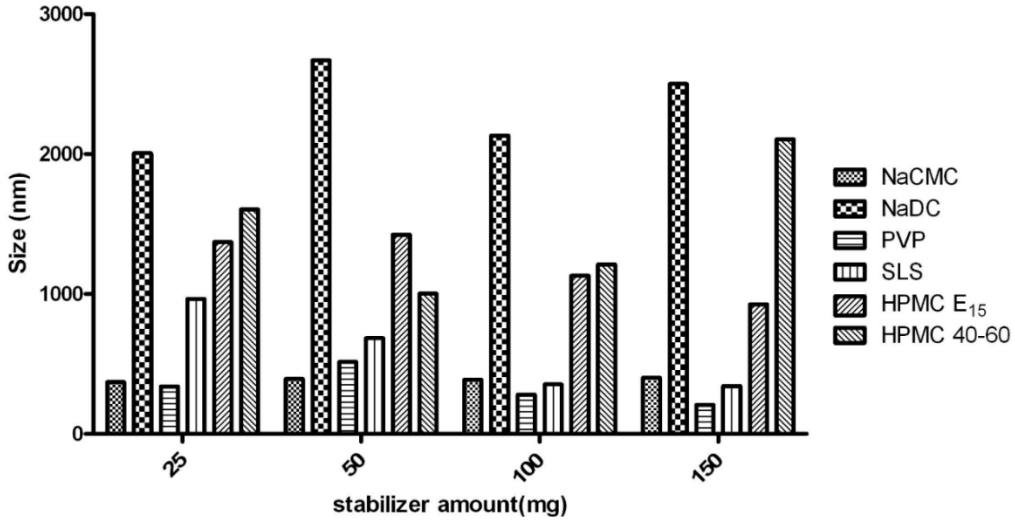

Figure 1. The effect of type and concentration of stabilizers on the size of nanoparticles.

Physicochemical characteristics

Particle size

As presented in Table 3, particle size of nanosuspensions varied in the range of 216.5-252.6 nm. Formulations displayed PDI of <0.5 which showed narrow particle size distribution. To predict the particle size, the best fitted model on the data was based on 2FI model that is presented in equation 3 (R-squared: 0.8):

Size= +679.73 + 251.38A - 143.57B + 43.76C + 4.53D + 60.48E + 267.33BD - 147.17CD + 297.89CE Eq.(3)

Table 3. Formulation parameters and responses for full factorial experimental design.

Run Number	Surfactant type (A)	Surfactant content (B)	Solid content (C)	Power input (D)	Interval of acid addition (E)	Size (nm)	Yield (%)
R_1	SLS	-1	-1	-1	-1	1080	56.3
R_2	PVP	-1	-1	-1	+1	537.3	48.3
R_3	SLS	+1	+1	-1	-1	375.8	29.1
R_4	PVP	0	0	0	0	285.8	25.8
R_5	PVP	+1	+1	-1	+1	361.6	41.1
R_6	SLS	-1	+1	+1	-1	374.7	42
R_7	SLS	+1	-1	-1	+1	216.5	38.3
R_8	SLS	-1	+1	-1	+1	2526	43.1

Table Continued.

R_9	SLS	+1	+1	+1	+1	1336	32.7
R_{10}	PVP	+1	+1	+1	-1	436.7	63.4
R_{11}	SLS	+1	-1	+1	-1	1395	38.3
R_{12}	PVP	+1	-1	+1	+1	280.9	15
R_{13}	SLS	-1	-1	+1	+1	848.2	65.7
R_{14}	PVP	-1	-1	+1	-1	843.1	50.3
R_{15}	PVP	+1	-1	-1	-1	319.8	11
R_{16}	PVP	-1	+1	+1	+1	392.5	31.3
R_{17}	PVP	-1	+1	-1	-1	417.6	30.1
R_{18}	SLS	0	0	0	0	247.9	40.8

Table 4. F-value and associated cut offs for p-value determining statistical significance of the main variables and their interactions affecting the responses, (*) *p<0.05*, (**) *p<0.01.*

Parameters	F value of size	F value of yield
A	7.01*	6.17*
B	2.03	13.71**
C	0.19	0.15
D	2.024E-003	2.43
E	0.36	0.11
BD	7.04*	-
CD	2.13	-
CE	8.75*	-
AC	-	12.28**
BC	-	26.95**
DE	-	12.48**

As presented in Table 4, the parameter of stabilizer type had a significant effect on the particle size by F-value of 7.01 ($p<0.05$); but the interaction between solid content and interval time of acid addition was the most effective parameter with F-value of 8.75 ($p<0.05$). Also, the interaction between stabilizer content and power input showed significant effects. The parameter of stabilizer type had a positive value in the size equation which means SLS produced larger particles.

Figure 2 shows that by an increase in the stabilizer content, particle size could be increased or decreased depending on the applied power input. In other words, at higher stabilizer content, the produced particles were larger when power input was higher in comparison with lower power input. Furthermore, at maximum interval of acid addition, increase of the solid content caused a rise in size and when the acid was added rapidly, the produced particles were smaller.

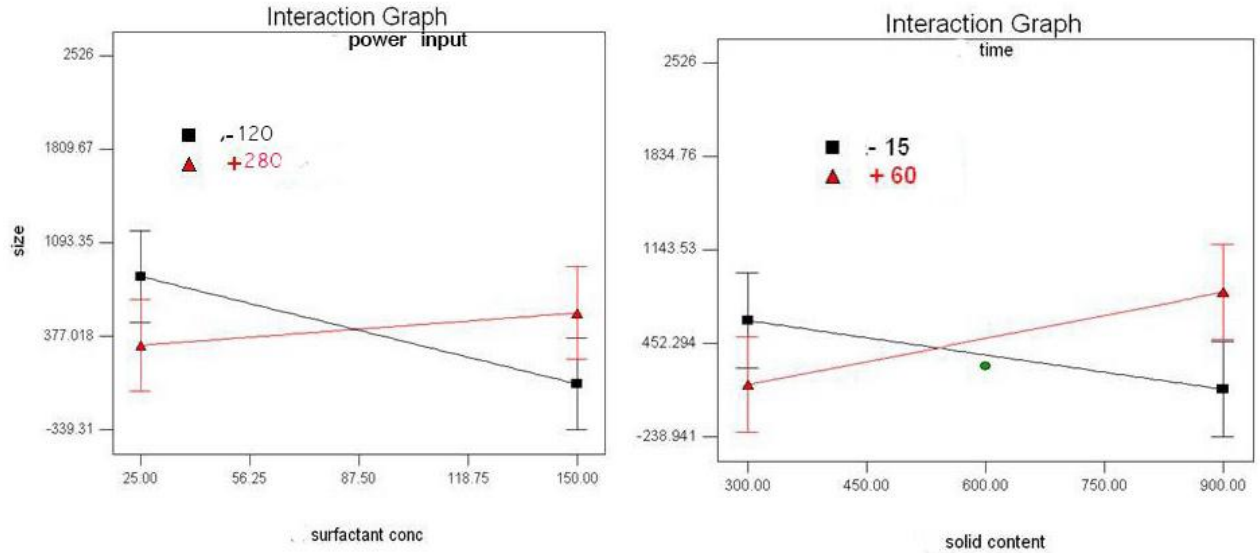

Figure 2. Interaction plot for the effect of main interactions of particle size: between surfactant content and power input and between time and solid content.

Yield

Yields of the nanoprecipitation varied between 11% and 65.7%, as presented in Table 3. ANOVA tests showed that stabilizer type ($p<0.05$) and stabilizer content ($p<0.01$) had a significant effect on the yield of the process (Table 4). Moreover, there were three significant interactions affecting the yield response, i.e. the interactions between: 1) stabilizer type and solid content, 2) stabilizer content and solid content and 3) power input and time interval of acid addition. The most significant parameter was the interaction of stabilizer content and solid content with F-value of 26.95 ($p<0.01$). As shown in Figure 3, in the lower amounts of solid content, the yield was decreased by

increasing the stabilizer content and in higher amounts of solid content; the yield was increased by increasing the stabilizer content. In addition, when the time interval of acid addition was at a minimum level, increasing the power input of the device caused improvement of yield.

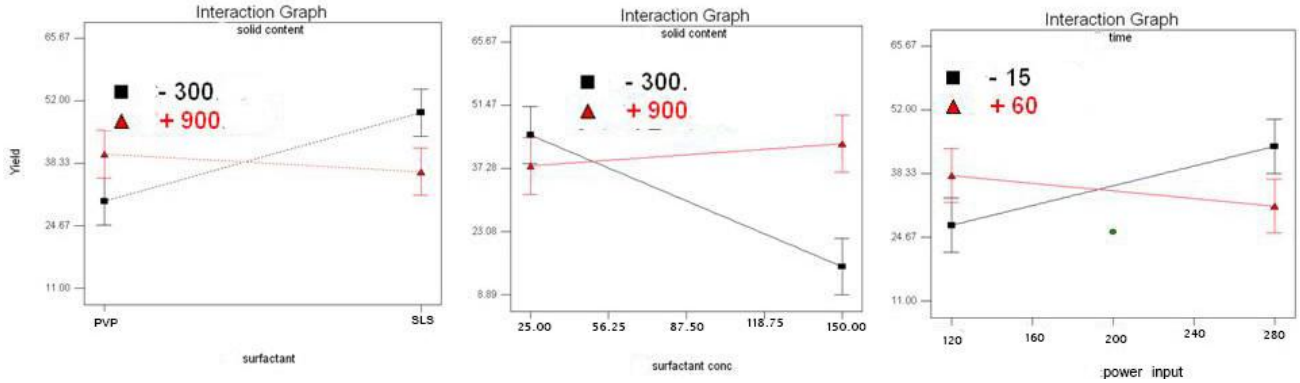

Figure 3. Interaction plot for the effect of main interactions on the yield of process: between surfactant type and solid content, between solid content and surfactant content and also between time intervals of acid addition and power input of the device.

The yield of formulation was predicted (R-squared:0.9) by equation 4:

Yield = +39.07+3.88A–6.14B–0.65C+2.59D–0.55E–5.81AC+8.61BC – 5.85DE Eq.(4)

Optimization

The optimization equation, relating the response and independent factors, was constructed based on a 2FI model. The desirability function was applied in order to obtain the maximized level of yield and minimized particle size. Coefficients with p-value <0.05 had a significant effect on the predictive efficacy of the model for measuring responses.

In this manner, the formulation containing PVP as stabilizer in content of 89.02 mg, solid content of 900 mg, 120W power input and acid addition with time interval of 15 s confirmed the highest desirability. This formulation was prepared and evaluated. Predicted and actual amounts of responses were compared and shown in Table 5. It can be observed that the difference between predicted and actual amounts of the responses were less than 10%.

Table 5. Comparison of actual and predicted properties of optimized nanosuspension (the optimized formulation containing PVP as stabilizer in content of 89.02 mg, solid content of 900 mg, 120W power input and acid addition with time interval of 15 s).

	Size (nm)	Yield (%)
Predicted amount	245	32.56
Actual amount	266±10	35±2
Error (%)	8.5	7.5

Characterization of optimum nanoparticles

SEM images of unprocessed drug and lyophilized samples of optimal nanoparticles are shown in Figure 4. Coarse cefixime particles were needle-shaped crystals with rough surfaces, whereas nanoparticles were relatively spherical in shape with some degree of agglomeration that could be related to the drying process.

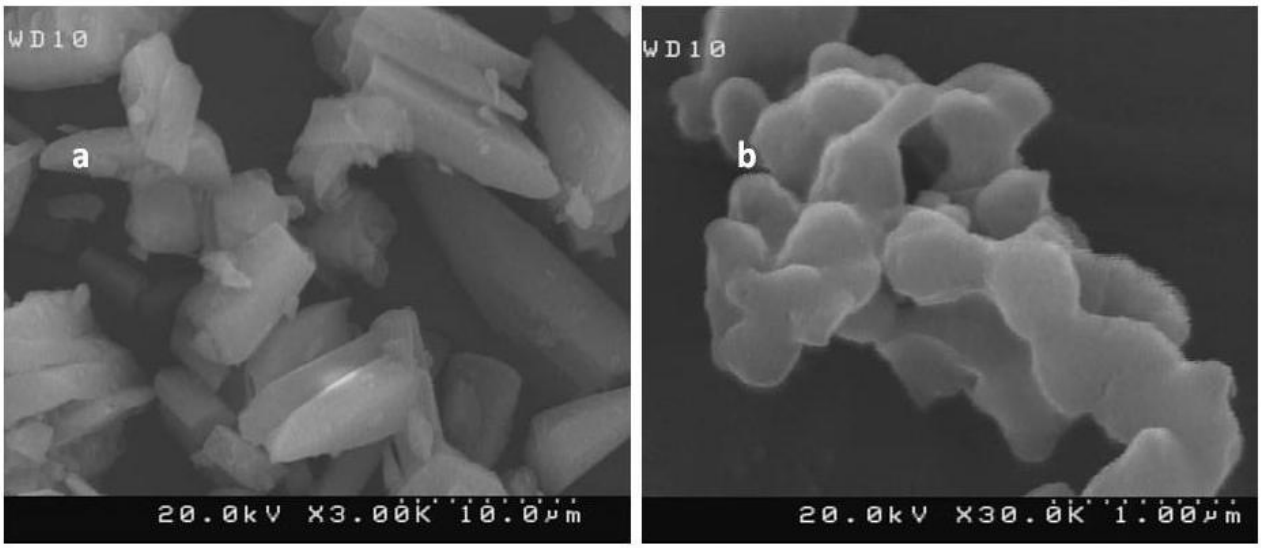

Figure 4. SEM images of a) coarse cefixime and b) lyophilized optimal nanosuspension.

Thermograms of unprocessed drug, PVP and nanoparticles are presented in Figure 5. Coarse cefixime trihydrate shows an endothermic peak around 104° C and two exothermic peaks at 211° C and about 250° C. The endothermic peak around 100-110° C is related to the loss of water molecules from its crystal lattice,[15] exothermic peak at 211° C shows the crystalline state transition due to the drug decomposition [16] and the second exothermic peak is the result of cefixime melting.[17] For PVP, a wide endothermic peak is observed at 80-120° C which could indicate the evaporation of the residual moisture in the structure of PVP.[18,19] In the thermogram of nanoparticles, the exothermic peak of cefixime melting is still visible at 250° C.

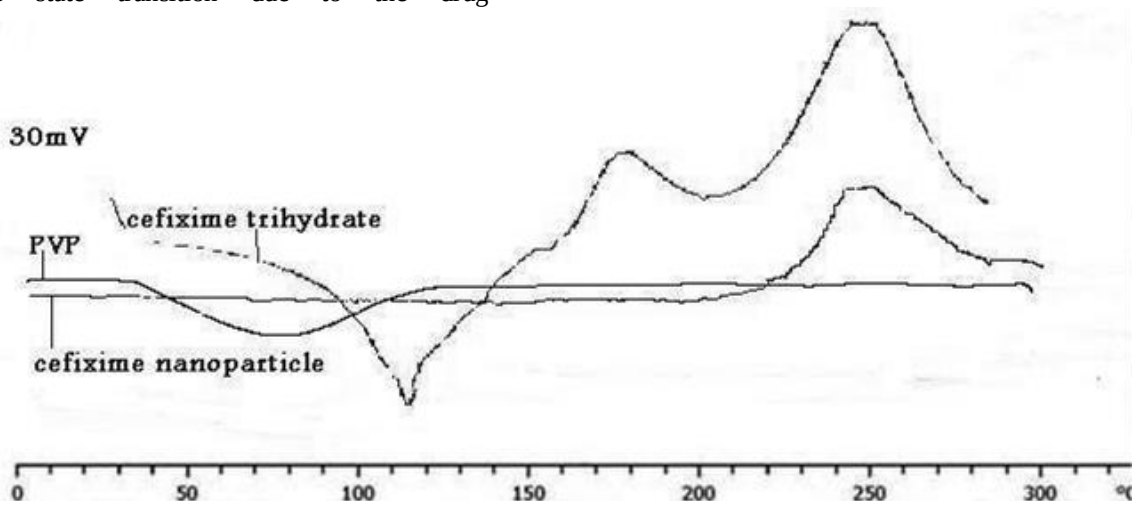

Figure 5. DSC thermograms of coarse cefixime, PVP and optimal nanosuspension.

The dissolution profiles of lyophilized nanoparticles in comparison with unprocessed cefixime and the physical mixture of the drug and PVP are illustrated in Figure 6. As seen, more than 85% of nanoparticles were dissolved in the medium after 1 min and drug content completely released after 15 min. However, the maximum dissolution of unprocessed cefixime and the physical mixture reached up to only 20% of drug content after 15 min. The maximum apparent solubility of nanoparticles of cefixime after 24 h was 5 times more than coarse cefixime and reach to 275 mg/L.

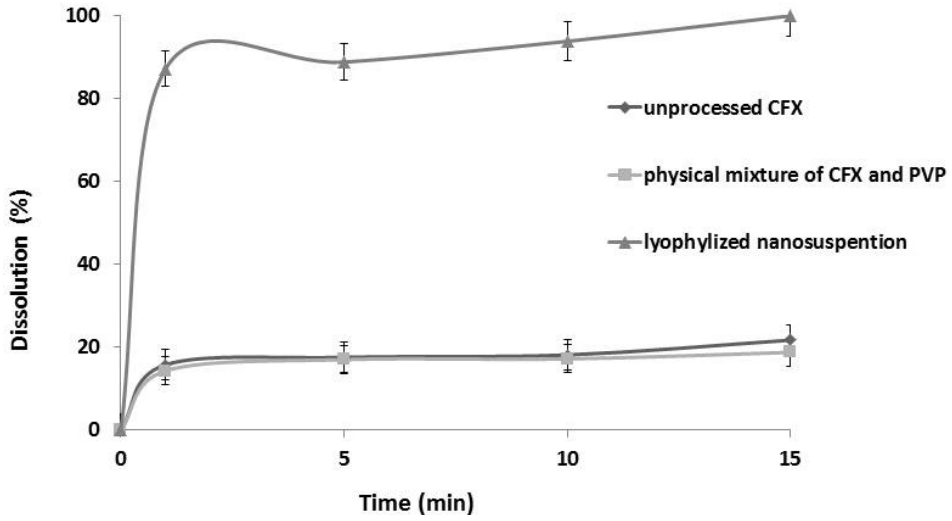

Figure 6. Dissolution profiles of unprocessed cefixime, physical mixture of cefixime and PVP, lyophilized optimal nanosuspension.

Discussion

The results showed that the nanoparticles were successfully prepared by ultrasonication- precipitation method and optimal condition could be predicted by an experimental design.

As presented in Figure 2, increase of the stabilizer ratio, depending on the applied power input of sonication, resulted in a decrease or increase in particle size. Generally, the concentration of stabilizer is an important factor to produce nanosuspensions, which

means that sufficient amounts of stabilizer must be adsorbed onto the particles surface to provide a barrier against agglomeration. Moreover, the precipitated nanoparticles tend to grow to micro-scale particles, whereas an "annealing" process such as homogenization or sonication could fix the size of nanoparticles during the precipitation. In this procedure, the particle size is controlled by the energy input and the processing time.[20] Higher levels of the ultrasound energy enhance the cavitation phenomenon and subsequently, the collapsed cavity in the solution creates a shockwave and hence, the particle sizes are decreased. Moreover, the shockwaves can create high velocity inter-particle collisions.[21] In this way, when the surfactant content is increased, the low level of power input is enough for inhibition of the particles size growth, but the continuous increase in the power input and cycles would probably cause damages to the stabilizer layer and might induce agglomeration and particles size increase.[20]

The particle size of nanosuspensions was also affected by the interaction of solid content and the time interval of acid addition. The ratio of the drug to the stabilizer depends on the stabilizer content and could decrease or increase; so, the coverage of the drug by surfactant is variable which could affect the size of particles. In addition, ultrasonication method involves nuclei formation and crystal growth. In order to achieve optimum values of these factors, to produce stable suspension with minimum particle size, high nucleation rate and low crystal growth are primarily required.[22] Rapid addition of acidic solution, as the anti-solvent, leads to rapid super saturation of drug in the solution, and thus formation of the ultrafine amorphous or crystalline drug. Lower rates of acid addition, provides sufficient opportunity for particles growth by promoting condensation/ coagulation.[23]

In the plot representing interaction between stabilizer type and solid content (Figure 3), it is noticeable that in lower solid content level, formulations containing SLS as the stabilizer had higher yields. This phenomenon could be justified regarding critical micelle concentration of the stabilizers. PVP is a synthetically produced homopolymer of N-vinyl-2-pyrrolidone and SLS bears an alkyl chain of C_{12} unit. The hydrophobic influence of PVP is much higher than SLS and it enhances micellisation. Therefore, PVP exhibits higher solubilization rather than SLS which causes lower yields of nanosuspension.[24]

The effect of the interaction between surfactant and solid content on the yield could be explained by this fact that in higher surfactant content and lower solid content, the surfactant not only could stabilize the nanosuspension, but also dissolve a notable portion of the drug which results in lower yields.

The aim of optimization was to find conditions to produce nanosuspensions with lower particle size and acceptable yield. The optimal formulation consisted of PVP in content of 89.02 mg and solid content of 900 mg was prepared by application of 120 W power input and acid addition with time interval of 15 s.

Nearly spherical shape of optimized nanoparticles has been reported for other nanosuspensions.[9,25] Moreover, thermal analysis confirmed partial crystalline state of these particles. Difference in the morphology and crystalline structure of unprocessed cefixime and nanoparticles can affect both pharmaceutical and biopharmaceutical properties such as solubility and dissolution profile of the drug.[26] Because, drugs in partial crystalline state tend to have higher dissolution rates since lower energy is needed to break the crystal lattice during the dissolution process.[27]

As shown in Figure 6, dissolution rate and the amount of the dissolved drug from optimal nanosuspension were significantly higher than unprocessed cefixime and physical mixture of the drug with PVP. This phenomenon could be related to the size of the drug in nanoparticles which provides extended surface area and creation of high energy surfaces resulting from the disruption of the drug microcrystals to nanoparticles.[13] In addition, particle size reduction to the nanoscale decreases the diffusional distance on the surface of the drug nanoparticles, thus leading to an increased concentration gradient. The increases in surface area and concentration gradient lead to an increase in dissolution velocity and saturation solubility. Moreover, wetting properties of particles are greatly increased because of the properties of PVP, resulting in reduced interfacial tension between the medium and drug and therefore, higher dissolution rates. Consequently, these factors may enhance cefixime bioavailability.[28-30]

Conclusion

The data confirm that ultrasonication-precipitation is a feasible method for preparation of cefixime nanosuspension. Besides, the experimental design software successfully could determine the optimal conditions to achieve the desired responses. This optimum condition could be proposed as a beginning for scale up and industrialization of cefixime nanoparticle formation. As compared to unprocessed cefixime, the formed nanocrystalline structures can significantly enhance solubility properties and dissolution rate of the drug.

Acknowledgement

This work was supported by Tehran University of Medical Sciences

Conflict of Interest

The authors report no conflicts of interest.

References

1. Williams J, Naber K, Bryskier A, Høby N, Gould I, Periti P, et al. Classification of oral cephalosporins. A matter for debate. *Int J Antimicrob Agents*

2001;17:443-4450. doi:10.1016/s0924-8579(01)00309-0
2. Ali T, Mohiuddin OA. In vitro interaction study of cefixime with diclofenac sodium, flurbiprofen, mefenamic acid and tiaprofenic acid. *J Chem Pharm Res* 2012;4.
3. Rao NR, Ram P, Kulkarni U. Formulation and evaluation of gas powered system of cefixime tablets for controlled release using hydrophillic polymers. *Int J Res Pharm Biomed Sci* 2010;1:82-91.
4. Ramirez E, Laosa O, Guerra P, Duque B, Mosquera B, Borobia AM, et al. Acceptability and characteristics of 124 human bioequivalence studies with active substances classified according to the biopharmaceutic classification system. *Br J Clin Pharm* 2010;70:694-702. doi: 10.1111/j.1365-2125.2010.03757.x
5. Prasanna S, Koppula SB. Enhancement of dissolution rate of cefixime trihydrate by using various solid dispersion techniques. *Int J Biopharm Res* 2013;2:104-107.
6. Mehta P, More A, Kulkarni A. Effect of hydrophilic polymers on cefixime complexation with β-cyclodextrin. *Int J Curr Pharm Res* 2013;5:66-70.
7. Kesisoglou F, Panmai S, Wu Y. Nanosizing — oral formulation development and biopharmaceutical evaluation. *Adv Drug Deliv Rev* 2007;59:631-644. doi: http://dx.doi.org/10.1016/j.addr.2007.05.003
8. Chen H, Khemtong C, Yang X, Chang X, Gao J. Nanonization strategies for poorly water-soluble drugs. *Drug Discov Today* 2011;16:354-360. doi:10.1016/j.drudis.2010.02.009
9. Ali HSM, York P, Blagden N. Preparation of hydrocortisone nanosuspension through a bottom-up nanoprecipitation technique using microfluidic reactors. *Int J Pharm* 2009;375:107-113. doi: http://dx.doi.org/10.1016/j.ijpharm.2009.03.029
10. Podaralla S, Perumal O. Preparation of zein nanoparticles by ph controlled nanoprecipitation. *J Biomed Nanotechnol* 2010;6:312-317. doi:10.1166/jbn.2010.1137
11. Zhang J-Y, Shen Z-G, Zhong J, Hu T-T, Chen J-F, Ma Z-Q, et al. Preparation of amorphous cefuroxime axetil nanoparticles by controlled nanoprecipitation method without surfactants. *Int J Pharm* 2006;323:153-160. doi:10.1016/j.ijpharm.2006.05.048
12. Dhumal RS, Biradar SV, Yamamura S, Paradkar AR, York P. Preparation of amorphous cefuroxime axetil nanoparticles by sonoprecipitation for enhancement of bioavailability. *Eur J Pharm Biopharm* 2008;70:109-115. doi:10.1016/j.ejpb.2008.04.001
13. Kocbek P, Baumgartner S, Kristl J. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs. *Int J Pharm* 2006;312:179-186. doi: http://dx.doi.org/10.1016/j.ijpharm.2006.01.008
14. Chingunpituk J. Nanosuspension technology for drug delivery. *Walailak J Sci Tech* 2007;4:139-153.
15. Satoshi K, Akira M, Shigetaka K, Yukiyoshi M. Effect of grinding on the solid-state stability of cefixime trihydrate. *Int J Pharm* 1989;56:125-134. doi:10.1016/0378-5173(89)90005-7
16. Singh BK, Parwate DV, Das Sarma IB, Shukla SK. Study on gamma and electron beam sterilization of third generation cephalosporins cefdinir and cefixime in solid state. *Radiat Phys Chem* 2010;79:1079-1087. doi:10.1016/j.radphyschem.2010.04.014
17. Kalavathy D, Panduranga K, Prakash Rao B. Preparation and evaluation of dispersible tablets of a model antibiotic drug. *Int J Pharm Sci Rev Res* 2013;18.
18. Sethia S, Squillante E. Solid dispersion of carbamazepine in pvp k30 by conventional solvent evaporation and supercritical methods. *Int J Pharm* 2004;272:1-10. doi:10.1016/j.ijpharm.2003.11.025
19. Yadav PS, Kumar V, Singh UP, Bhat HR, Mazumder B. Physicochemical characterization and in vitro dissolution studies of solid dispersions of ketoprofen with pvp k30 and d-mannitol. *Saudi Pharm J* 2013;21:77-84. doi:10.1016/j.jsps.2011.12.007
20. Xu Y, Liu X, Lian R, Zheng S, Yin Z, Lu Y, et al. Enhanced dissolution and oral bioavailability of aripiprazole nanosuspensions prepared by nanoprecipitation/homogenization based on acid-base neutralization. *Int J Pharm* 2012;438:287-295. doi:10.1016/j.ijpharm.2012.09.020
21. Siddiqui A, Alayoubi A, El-Malah Y, Nazzal S. Modeling the effect of sonication parameters on size and dispersion temperature of solid lipid nanoparticles (slns) by response surface methodology (rsm). *Pharm Dev Technol* 2014;19:342-346. doi:10.3109/10837450.2013.784336
22. Patel VR, Agrawal Y. Nanosuspension: An approach to enhance solubility of drugs. *J Adv Pharm Technol Res* 2011;2:81. doi:10.4103/2231-4040.82950
23. Sahu BP, Das MK. Nanoprecipitation with sonication for enhancement of oral bioavailability of furosemide. *Acta Poloniae Pharmaceutica* 2014;71:129.
24. Rahaman T, Fardous J, Perveen FF, Sultana S. Capacity of non ionic and ionic surfactants for solubilisation of paracetamol. *Bangladesh Pharm J* 2013;16:77-80. doi:10.3329/bpj.v16i1.14498
25. Juliana N. Schianti NNPC, Adriano M. de Oliveira, Silas Derenzo, Antônio C. Seabra and Mário R. Góngora-Rubio. Rifampicin nanoprecipitation using flow focusing microfluidic device. *Nanomedicine Nanot* 2013;4. doi:10.4172/2157-7439.1000172
26. Manish M, Harshal J, Anant P. Melt sonocrystallization of ibuprofen: Effect on crystal

properties. *Eur J Pharm Sci* 2005;25:41-48. doi:10.1016/j.ejps.2005.01.013
27. Vasconcelos T, Sarmento B, Costa P. Solid dispersions as strategy to improve oral bioavailability of poor water soluble drugs. *Drug Discov Today* 2007;12:1068-1075. doi:10.1016/j.drudis.2007.09.005
28. Jinno J-i, Kamada N, Miyake M, Yamada K, Mukai T, Odomi M, et al. Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs. *J Control Release* 2006;111:56-64. doi:10.1016/j.jconrel.2005.11.013
29. Hecq J, Deleers M, Fanara D, Vranckx H, Amighi K. Preparation and characterization of nanocrystals for solubility and dissolution rate enhancement of nifedipine. *Int J Pharm* 2005;299:167-177. doi:10.1016/j.ijpharm.2005.05.014
30. Rasenack N, Müller B. Dissolution rate enhancement by in situ micronization of poorly water-soluble drugs. *Pharm Res* 2002;19:1894-1900. doi: 10.1023/A:1021410028371