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Formulation, optimization and characterization of candesartan cilexetil nanosuspension for *in vitro* dissolution enhancement

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According to Biopharmaceutical classification system (BSC), candesartan cilexetil (CC) is a class-II drug which has limited bioavailability mainly due to its low solubility. In order to enhance its solubility, a universal approach of making nanosuspension is been used in the present investigation. High pressure homogenization, controlled precipitation, media milling and high speed homogenization are the various approaches which are most widely used to produce the nanosuspension. Here, nanosuspension is formulated by combination of two approaches; high speed homogenization and media milling to expedite and ease the process. The polymers used to stabilized the nanoparticles were polyvinylpyrrolidone K-30 (PVP K- 30), poloxamer 407, HPMC E 50. To optimize the concentration of the polymer and surfactant the simplex lattice design is used. Various process parameters like homogenization speed, time, media milling cycle, drug to bead ration are optimized by changing one parameter at a time. The nanoparticles produced were of particle size less than 500 nm and were also found to be stable. The saturation solubility was enhaced more than 20 times than the bulk drug. The nanonization of the particles by combination of high speed homogenization and media milling is an effective method of enhancing *in vitro* dissolution of Candesartan cilexetil.

Key word: Media milling, *in vitro* dissolution, solubility enhancement, nanoparticles, formulation optimization.

INTRODUCTION

Poor solubility of a drug is a major concern for the development of new dosage form because about 10% of the present drugs, 40% of the drugs in the pipeline and 60% of drugs coming directly from synthesis have a

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solubility below 0.1 mg/ml (Merisko-Liversidge et al., 2003). There are quite a number of formulation approaches for increasing the solubility of the poorly soluble drug for example, pH shifted aqueous solution, use of solvent mixtures, cyclodextrine and o/w emulsions for intravenous administration (Müller et al., 2001). The principle limitation of all these approaches is that the drug needs to possess certain physicochemical properties (for example, solubility in oils) or having the right molecular size to fit into the cyclodextrine ring (Frömming and Szejtli, 1994). It would be more elegant to have one universal formulation approach which can process any poorly soluble drug. One of such approach of poorly soluble drugs is micronisation which involves transfer of the coarse drug powder to an ultrafine powder with a mean particle size being typically in the range of 25 millimeter (mm) and size distributions normally range from approximately 0.1 to 25 mm (Müller and Peters, 1998). Here, the principle was to increase the dissolution rate by enlarging the surface area of the drug. But nowadays, many of the new drugs exhibit such a low solubility that even micronisation does not provide sufficiently high solubility. Consequently, the next step was taken to move from micronisation to “nanonization" that means producing drug nanocrystal. By definition, “drug nanocrystals” are nano particles being composed of 100% drug without any matrix material. Here, nanocrystal is just a general term which can be used for both crystalline as well as amorphous form. According to the definition “nanosuspension” is the dispersions of nano sized particles in a suitable vehicle. Nanosuspensions of drugs are sub-micron colloidal dispersions of pure drug particles which are stabilized by surfactants (Na et al., 1999).

The nanoparticles can be obtained either by particle size reduction of larger particles up to nano level (top-down approach) or by building up particles by precipitation of dissolved molecules (bottom up approach) (Rabinow, 2004).

The precipitation method involves nucleation and the growth of drug particles from dissolved state to the range of nanometer. Here the primary condition is the solubility of the drug in at least one solvent which should be miscible with another non-solvent. Another important parameter is that it should have been possible to remove the solvent used in this techniques to an acceptable level in the end products (Gassmann and List, 1994; Chen et al., 2010). Due to the complexity of the process, right now there is no product available in the market based on this technology (Shegokar and Müller, 2010).

Second method is the top-down approach by using high-pressure homogenization and media milling. In the formal method, size of the particle is reduced by repeatedly forcing a suspension through a very thin gap (typically about 25 µm) at extremely high velocity, the latter comprises mechanical attrition of suspended drug particles using milling media such as pearls or balls consisting of ceramics (cerium- or yttrium-stabilized zirconium dioxide), stainless steel, glass, or highly cross-linked polystyrene resin-coated beads. (Patravale et al., 2004; Eerdenbrugh et al., 2008). Here, major limitations for media milling is that it usually takes long time (26 to 48 h) in converting drug particles into the nano stage when the speed is mediocre, and high pressure homogenization requires very costly instrumentation.

Another method is to use high speed homogenizer instead to high pressure because it is less costly and easier than high pressure homogenizer but it can hardly give the particle size below 600 nm, which will not be sufficient to take full advantage of nano particles. To overcome this limitation in the present investigation we have used combination method (high speed homogenization and media mill) to prepare the nanosuspension. First, pre nanosuspension was prepared by using high speed homogenizer and then this presuspension was media milled in glass vial to produce final suspension. In this way, time and cost to produce nano suspension was reduced.

Candesartancilexetil (CC) is selective AT1 subtype angiotensin II receptor antagonist. It is a pro-drug which converts to active candesartan moiety after the absorption from the gastrointestinal tract. Aqueous solubility of CC is less than 0.05 µg/L. Its marketed product ATACAND is available in form of tablet. Bioavailability of this tablet formulation is only 15% (FDA Label of CC, ATACAND®) (Michael et al., 2008). Furthermore, micronisation of CC does not enhance its oral bioavailability significantly. So, here nanoparticles of the candesartan were prepared to increase its solubility and dissolution velocity.

MATERIALS AND METHODS

Candesartan cilexetil was obtained as a gift sample from Alembic Research Centre, India. Poloxamer 407 was obtained from BASF, Germany. PolyvinylpyrrolidoneK-30 (PVP K-30), hydroxy propyle methyl cellulose E 50 (HPMC E50), methanol, Tween 20 was purchased from SD Finechem, India. Other ingredients were acetone, isopropyl alcohol (IPA). Here, zirconium oxide beads were obtained as a gift sample from SPARC, India.

Preparation of nanosuspension

Nanosuspension was prepared by combination of high speed homogenization and media milling technique (Van Eerdenbrugh et al., 2008). Initially, the drug was dissolved in acetone:IPA mixture (1:1.1:3 and 3:1). In 10 ml of homogenization cup, stabilizer solution and drug solution was mixed and water was added drop by drop to it which led to precipitation of the drug and formation of pre-suspension. To prepare nanosuspension, the presuspension was added to high speed homoginizer (Remi equipments Pvt. Ltd.,

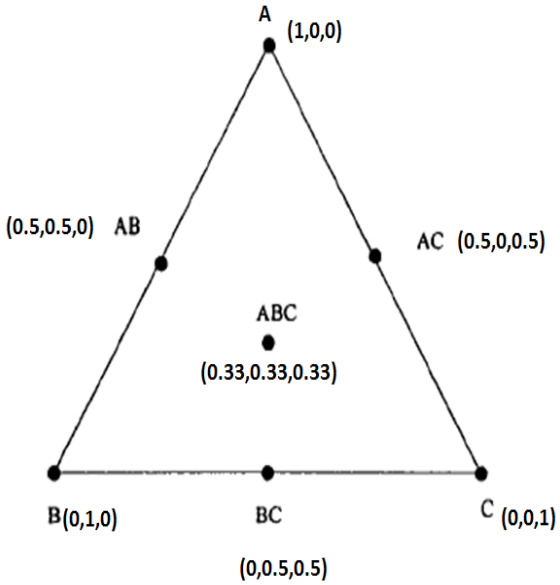

Figure 1. Equilent triangle represents simplex lattice design.

Table 1. Code representation of different formulation prepared.

Formulation code	Code represent	Concentration transformed value		
		Poloxamer 407	PVP K30	HPMC E50
F1	X_1	1	0	0
F2	X_2	0	1	0
F3	X_3	0	0	1
F4	X_1X_2	0.5	0.5	0
F5	X_1X_3	0	0.5	0.5
F6	X_2X_3	0.5	0	0.5
F7	$X_1X_2X_3$	0.33	0.33	0.33

India) for initial size reduction. After the completion of homogenization step, suspension was transferred to 20 ml glass vial containing weighed quantity of zirconium oxide beads and this solution was stirred on magnetic stirrer (Remi equipments Pvt. Ltd., India) for specific time for the preparation of the nanosuspension.

Preliminary parameters like concentration of drug, concentration of beads, stirring time for homogenization and media milling and ratio of beads were optimized by varying one parameter at a time, while keeping others constant, so that the effect of varied parameter could be evaluated (Armstrong and James, 1996). Each batch was repeated thrice (n = 3) for the confirmation of repeatability. The concentration of stabilizers were optimized by simplex lattice design (Figure 1) for which particle size (PS) and saturation solubility (SS) were selected as response parameters. Code representation of formulation with actual and transformed values was shown in (Tables 1 and 2). The responses for seven formulations were used to fit an equation for simplex lattice design which can predict properties of possible formulation. With the aid of Microsoft excel regression analysis was employed to determine the control factors that significantly affect the responses.

Optimization of formulation parameters

Optimization of solvent ratio

Acetone and isopropyl alcohol (IPA) were tried and suitable ratio was selected on the basis of particle size.

Homogenization speed and time

Homogenization speed and time was optimized after selecting the optimized solvent ratio on the basis of particle size.

Table 2. Actual value of X_1, X_2 and X_3.

Stabilizer	Level (0)	Level (1)
X1	0%	0.25%
X2	0%	0.35%
X3	0%	0.50%

Optimization of stirring time

For the optimization of stirring time milling step was performed for different time period and finalized on the basis of particle size.

Ratios of beads

Different ratios of beads ranging from 100:0 to 0:100 of larger: smaller beads were tried. Diameter of smaller beads ranges from 0.4 to 0.7mm and that of larger beads varies from 1.2 to 1.7 mm.

Concentration of drug

Three different concentrations 0.5, 0.75 and 1.0% w/v of drug were tried.

Concentration of beads

Three concentrations of beads were considered for optimization that is, 80, 100, 120% w/v of batch size.

Evaluation of nanoparticles

Particle size and zeta potential

Mean particle size and size distribution of the prepared nanosuspensions were measured by using Malvern Zeta sizer. Here, water was selected as solvent.

Saturation solubility

Prepared nanosuspension was filled in a vial and kept for 24 h with stirring to ensure complete saturation. Samples were then centrifuged, filtered, diluted suitably and analyzed with UV spectrophotometer.

Differential scanning calorimetry (DSC)

The DSC thermograms of bulk CC powder, Poloxamer407, their physical mixture and dried nanosuspension formulation were taken on a Shimadzu DSC-60 differential scanning calorimeter between 40 and 300°C at a heating rate of 10°C/min with nitrogen supplied at 40 ml/min.

Transmission electron microscopy (TEM)

TEM studies were performed in transmission electron microscope (PHILIPS TECHNAI-20). The liquid nano-suspension formulation was dropped on copper-gold carbon coated grid and allowed to dry. This grid was then mounted in instrument and photographs were taken at various magnifications.

Dissolution study

Dissolution experiments were performed using USP type-2paddle instrument (ELECTROLABTDT-06P). Phosphate buffer (pH 6.5) containing 0.7% Tween20 was used as dissolution medium. Dissolution was performed at 37 ± 0.5°C, at 100 rpm. Samples of the plain drug and spray dried nanosuspension was centrifuged for the separation of nanoparticles than equivalent to 15 mg were added to dissolution vessels. Samples of 5ml were taken after suitable time interval of up to 60 min. Samples were filtered immediately through 0.1 µm PTFE syringe filter (Whatman Inc., Clifton, NJ, USA). Subsequently, 5 ml of fresh medium was added to the dissolution vessel. The experiment was performed thrice and mean value was used.

Drug content

The 0.2 ml of drug nanosuspension was dissolved in 50 ml methanol. The stock solution was sufficiently diluted with methanol and absorbance was measured.

Stability study

Stability studies for nanosuspension were conducted at two different storage conditions for a period of forty five days.

1. Room temperature.
2. Refrigerated (2 to 8°C).

Three batches, with optimized batch (F1) of nanosuspension, were used for each condition. The particle size and zeta potential was measured periodically to determine the stability of drug in the formulation at various storage conditions.

RESULTS AND DISCUSSION

Acetone and IPA were tried in different ratio by keeping all other parameters constant and suitable ratio was selected on the basis of particle size (Table 3). Acetone: IPA (1:3) gives satisfactory results, so it was selected as optimized ratio. Batches were taken at different speed and time by keeping the solvent ratio constant and results are displayed in the tables (Tables 4 and 5). Satisfactory results were found at 6000 rpm homogenization speed and 3 h homogenization time, so it was optimized. For the optimization of stirring time of media, milling presuspension obtained from the homogenization cycle was milled for 6, 9 and 12 h and results are displayed in the Table 6, and from the results it was concluded that milling time of 9 h was sufficient to produce the nano suspension. Beyond 9 h the milling cycle have no significant effect of particle size. To optimize the ration of small bead to large beads it was found that as proportion

Table 3. Optimization of solvent ratio.

Batch No.	Acetone:IPA	Homogenization speed (rpm)	Homogenization time (h)	Particle size (µm)
1	1:1			30
2	1:3	6000	3	15
3	3:1			25

Table 4. Optimization of homogenization speed.

Batch No.	Homogenization Speed (rpm)	Homogenization time (h)	Acetone:IPA	Particle size (µm)
1	5000			20
2	6000	3	1:3	15
3	7000			14.5

Table 5. Optimization of homogenization time.

Batch No.	Homogenization time (h)	Homogenization speed	Acetone:IPA	Particle size (µm)
1	3			15
2	4	3	1:3	14.5
3	5			14.1

Table 6. Optimization of milling time.

Batch No.	Milling time (h)	Stabilizer	Particle size
1	6		385.4
2	9	Poloxamer 407	212.3
3	12		209.2

Table 7. Bead ration Optimization.

Batch no.	Conc. of surf. (w/v%)	Conc. Of drug (w/v%)	Larger: smaller beads ratio	Conc. of beads (w/v%)	Milling time (h)	Mean particle size(nm)
1			100 : 0			361.9
2			75:25			350.0
3	0.25	0.5	50:50	100	9	339.6
4			25:75			295.2
5			0:100			243.9

of smaller beads increases, area available for milling also increases and hence surface area increased. So, 100% smaller beads were optimized and used for further study. Batch 5 that is, 100% of smaller beads gave minimum particle size as well as efficient stirring for the size reduction process (Table 7).

0.5% w/v of drug was optimized and this concentration of drug was used in further optimization (Table 8). As

Table 8. Optimization of drug concentration

Batch No.	Conc. of surf. (w/v%)	Conc. of drug (w/v%)	Conc. of beads (w/v%)	Stirring time (h)	Mean particle size (nm)
1		0.5			243.9
2	0.25	0.75	100	14	270.5
3		1.0			320.0

Table 9. Optimization of bead concentration.

Batch No.	Conc. of surf. (w/v%)	Conc. of beads (w/v%)	Stirring time (h)	Mean particle size (nm)
1		80		259.1
2	0.25	100	14	243.9
3		120		223.5

Table 10. Mean particle size (Y1) and saturation solubility (Y2) and of seven different formulations as per simplex lattice design.

Formulation code	Formulation component			MPS	SS	Drug release after 10 min
	Poloxamer 407	PVP K30	HPMC E50			
F1	1	0	0	205.1	2814	96.98
F2	0	1	0	368	1905	84.21
F3	0	0	1	597	1145	74.13
F4	0.5	0.5	0	223.9	2625	93.25
F5	0.5	0	0.5	457	1495	79.28
F6	0	0.5	0.5	386	2315	82.16
F7	0.33	0.33	0.33	264.4	2545	90.12

concentration of drug increases, material required to mill will also increase. So, increase in drug concentration led to decrease in particle size. Increase in concentration is accomplished by increase in surface area available for milling. So, this concentration of beads was optimized and used for further study (Table 9). According to simplex lattice design and the selected concentration ranges of 3 stabilizer, seven different formulations of nanosuspension containing CC were constructed. The results of their mean particle size, saturation solubility and drug release after 10 min.is shown in Table 10. With the help of Microsoft Excel, results of depended variables were fitted in the equation are shown in equations (1), (2) and (3)

$$Y1 = 100.7\ X1 + 221.8X2 + 641.2X3 - 2117.53X1X2X3 + 104.4X12 - 146.2X22 - 44.2X3 \quad (1)$$

Equitation (1) shows that minimum positive effect of stabilizer on mean particle size is of X1. And all other stabilizer has more positive effect than the X1 and shows synergistic effect on mean paticle size.

$$Y2 = 4076X1 + 1705X2 + 1225X3 + 9944.731X1X2X3 – 1262X12 + 200X22 – 80X3 \quad (2)$$

Equitation (2) shows that maximum positive effect of stabilizer on SS is of X1 and all other stabilizer has less positive effect than X1 and X1X2X3 shows synergistic effect on SS.

$$Y3 = 95.28X1 + 96.53X2 + 62.25X3 + 170.937X123 + 1.7X12 – 12.32X22 + 11.88X \quad (3)$$

Equation (3) shows that maximum positive effect of stabilizer on drug release is of X1 and X2 and X3 has less positive effect than X1 and X2 and shows synergistic effect on drug release.Ten minutes from simplex lattice design were almost same as the experiment value (Table 11). Graphics of MPS (Figure 2a), SS (Figure 2b) and drug release (Figure 3) were constructed in form of

Table 11. Comparison of responses between experimental results and calculated values.

Formulation code	MPS		SS		Amount of drug release in 10 min	
	Exp.	Pred.	Exp.	Pred.	Exp.	Pred.
F1	205.1	205.1	2814	2814	96.98	96.85
F2	368	368	1905	1905	84.21	84.21
F3	597	570	1145	1145	74.13	74.13
F4	223.9	223.9	2625	2625	93.25	93.21
F5	457	443.5	1495	1495	79.28	79.28
F6	386	372.5	2315	2315	82.16	82.12
F7	264.4	255.49	2545	2545	90.12	90.0989

Exp. = Experimental. Pred. = Predicted.

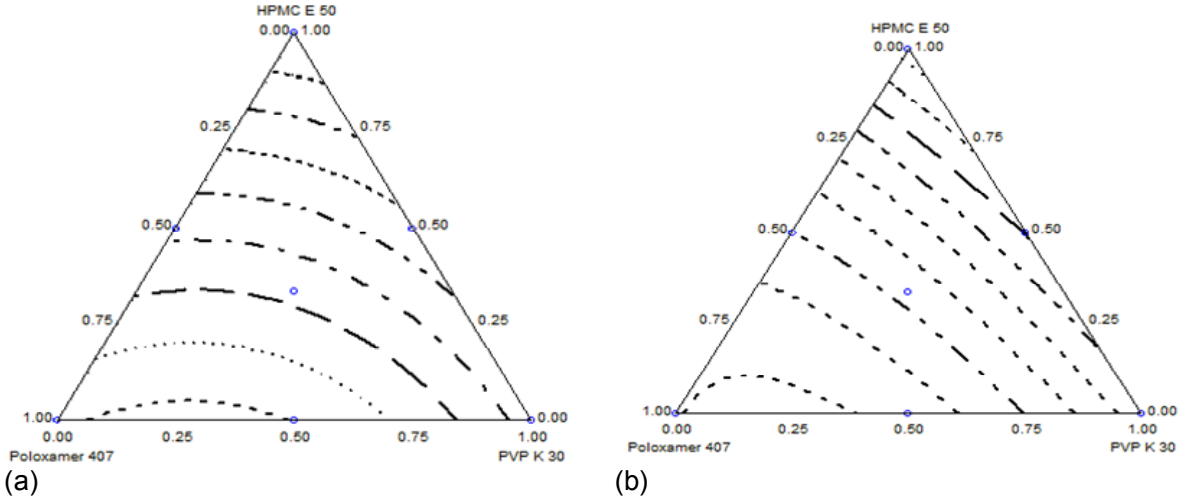

Figure 2. Ternary contour plots for MPS of CC nanosuspension (a) and SS of CC nanosuspension (b).

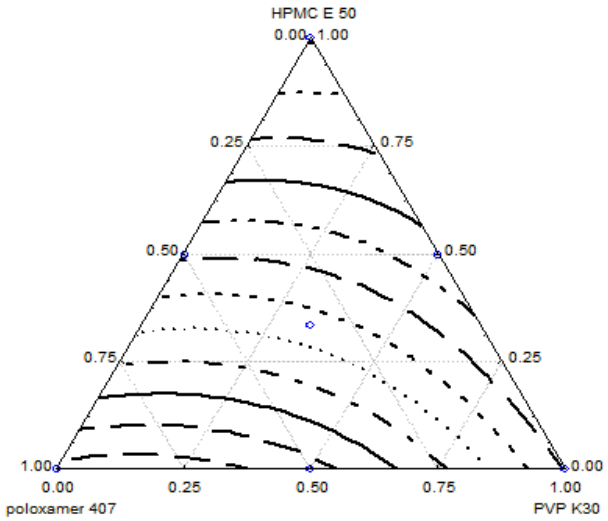

Figure 3. Ternary contour plots for Drug release of CC nanosuspension.

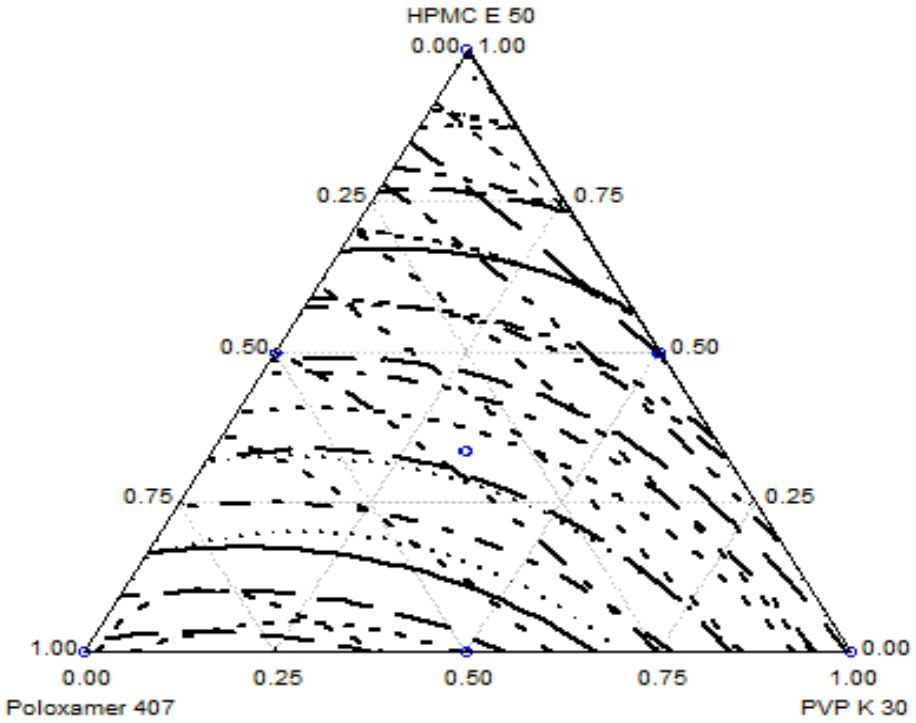

Figure 4. Superimposed ternary contour plots of the three responses.

Table 12. Final formula for further optimization (F1).

Type of surfactant	Poloxamer 407
Homogenization time (h)	3
Homogenization speed (rpm)	6000
Acetone : IPA	1:3
Ratio of beads	100
Concentration of drug (% w/v)	0.5
Concentration of beads (% w/v)	120
Concentration of poloxamer 407 (% w/v)	0.25
Milling time (h)	9

Ternary contour plots (STASTICA 9.0 software), and optimized formulation was chosen by superimposing ternary contour plots of three responses (Figure 4). The process parameter and formulation composition for the optimized batch is given in (Table 12) which was evaluated for further parameter.

Transmission electron microscopy (TEM)

The morphological characteristics of the nanocrystals were observed using TEM (Figure 6). TEM images revealed no aggregation of nanocrystals. It was also observed that nanocrystals are approximately of oval shape.

Zeta potential and particle size analysis

The mean partical size of optimized batch is 205. Zeta potential was found to be -32.47 mV (Figure 7 and Table 13). Zeta potential value of ±30 mV is sufficient for stability of nanosuspension. In our formulation, it is -32.47 mV which means it complies with requirement of zeta

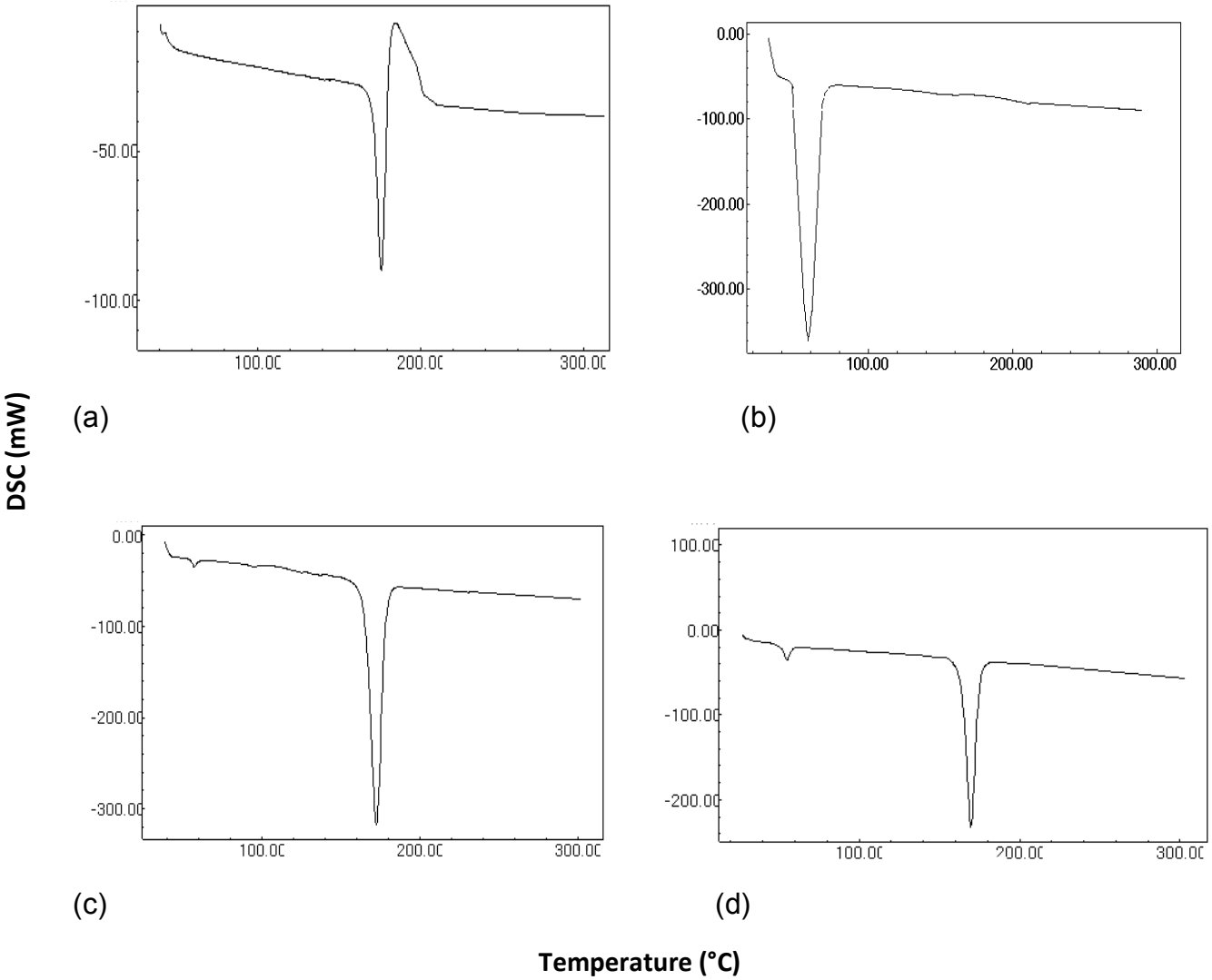

Figure 5. DSC thermogram of CC bulk powder(a) and Poloxamer 407(b) physical mixture (c) and CC nanosuspension (d).

potential for stability.

Saturation solubility

Saturation solubility of optimized batch of nanosuspension and bulk drug is 2814 ± 29.5 and 125 ± 6.9 µg/ml, respectively. In other words, saturation solubility of nanosuspension was 22.51 times that of bulk drug.

Differential scanning calorimetry (DSC)

DSC was performed to investigate the effect of surfacant and milling on the inner structure of CC nano-suspensions. The DSC thermogram of CC bulk powder, physical mixture and poloxamer 407 are shown in Figure 5a and b. The DSC thermogram of CC bulk powder showed a sharp endothermic peak at 169°C followed by an exothermal peak which is due to decomposition of drug (Matsunaga et al., 1999). Poloxamer thermogram showed endothermic peak at 55°C. Physical mixture showed sharp endothermic peak at 168 and 55°C which indicated absence of interaction between poloxamer and drug (Figure 5c). Thermogram of formulation showed peaks at same temperature as physical mixture (Figure 5d). The melting curves of CC nanosuspensions stabilized with poloxamer 407 were not influenced by

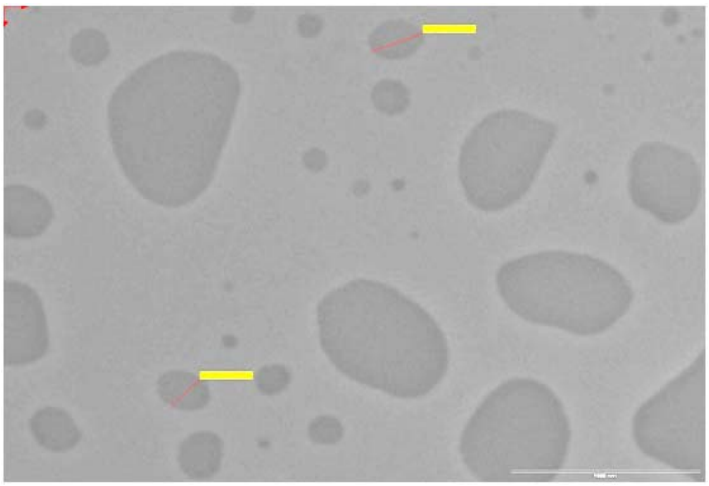

Figure 6. TEM photograph of nanosuspension.

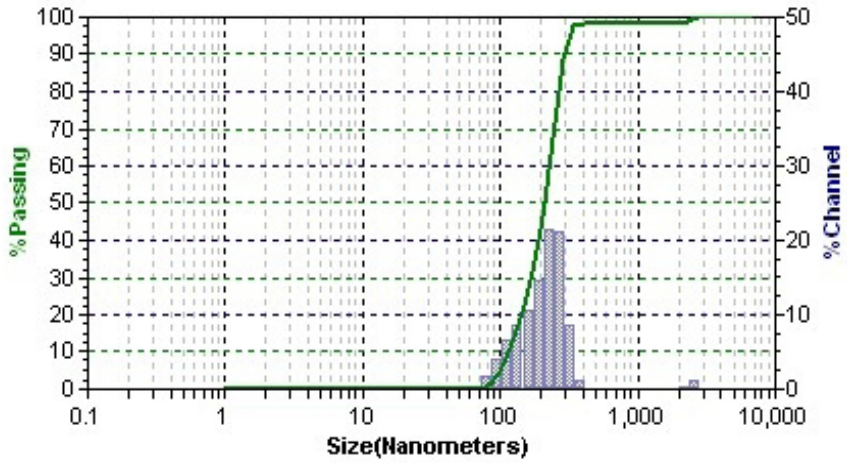

Figure 7. Particle size distribution of CC nanosuspension.

stabilizer and milling process. So, it can be concluded that there is no interaction between polymer and drug.

Drug content

The drug content of optimized batch taken for characterization was found to be 99.64%.

Dissolution study

In spray dried nanosuspension, more than 75% drug dissolved within 5 min and about 100% within 15 min, while plain drug showed only 17% release at the end of 5 min and 92% release in 60 min (Table 14). So, nanosuspension enhanced rate of dissolution of CC to a great extent.

Stability study

Results of stability samples regarding particle size and drug content was found satisfactory for 45 days. Particle size of stability samples after 45 days at room temperature and refrigerator condition was 222.3 and 225.5, respectively. Drug content of stability sample after 45 days at room temperature and refrigerator condition

Table 13. Zeta potential report of CC nanosuspension.

Zeta potential	
Mobility	-2.54 µ/s/v/cm
Zeta potential	-32.47 mv
Charge	-0.04413 fC
Polarity	Negative
Conductivity	6 µS/cm

Table 14. Cumulative % drug release from plain drug and nanosuspension

S/No.	Time (min)	Cumulative % drug release	
		Plain drug	Nano-suspension
1	0	0	0
2	2	8 ± 1.03	65.2 ± 3.65
3	4	17 ± 1.96	76.32 ± 5.69
4	6	26 ± 2.68	86.32 ± 3.65
5	10	37 ± 2.95	96.32 ± 2.36
6	15	53 ± 4.96	99.38 ± 3.65
7	30	69 ± 5.67	98.45 ± 2.65
8	45	81 ± 4.69	99.07 ± 1.65
9	60	92 ± 5.69	99.69 ± 0.95

are 94.25 and 95.02, respectively.

Conclusion

Candesartan cilexetil nanosuspension was prepared by combination of homogenization and media milling technique. This method of manufacturing was found to be simple, did not require specialized equipments and hasscale up feasibility, and process is short in comparism to media milling alone. After preliminary studies, simplex lattice design was applied fruitfully for selection of stabilizer, and poloxamer 407 was selected as stabilizer in the final formulation. Saturation solubility of final formulation was increased 22.44 times that of the bulk drug. DSC thermogram confirmed no interaction between drug and excipients. Dissolution of nanosuspension shows complete dissolution within 15 min, so it can be concluded that *in vitro* dissolution of CC can be enhanced by formulating as nanosuspension by the given method.

Conflict of interest

Authors declare that there are no conflicts of interest.

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