ORIGINAL ARTICLE

Influence of Different Stabilizers on Dissolution of Cilnidipine Nanosuspension

K. Kuralarasan, S. Subramanian

Department of Pharmaceutics, PSG College of Pharmacy, Coimbatore, Tamil Nadu, India

Abstract

Aim: Cilnidipine is a new antihypertensive agent and it is categorized under the biopharmaceutical Class-II drug. Hence, our perspective is to improve dissolution through *in vitro* rate of Cilnidipine. **Materials and Methods:** Nanosuspensions are a good choice for enhancing the solubility of poorly water-soluble drugs and improve bioavailability. Cilnidipine nanosuspension was formulated by pure drug with poloxamer-188, polyvinylpyrrolidone K-30 as stabilizer, polyethylene glycol-4000 as polymer, and ethanol as solvent. Nanosuspension technique such as combination technique of antisolvent precipitation and high-speed homogenization is involved in the formulation. **Results and Discussion:** Cilnidipine nanosuspension is characterized by particle size, polydispersity index, zeta potential, atomic force microscopy, and scanning electron microscopy. Cilnidipine nanosuspension converted into powder by lyophilization process and 1% mannitol was used as lyoprotectant. Lyophilized powder was analyzed for drug content at 240 nm by ultraviolet spectroscopy. After that, lyophilized powder was formulated to tablet. Formulated tablet was analyzed for weight variation, disintegration, friability, hardness, thickness, and *in vitro* drug release. *In vitro* drug release was studied by distinguishing between formulated and marketed tablet. **Conclusion:** Cilnidipine nanoformulation was proved with 18.59% increase in *in vitro* release when compared to Cilnidipine marketed tablets.

Key words: Cilnidipine, nanosuspension, bioavailability, stabilizer, lyoprotectant

INTRODUCTION

At present, 40% of drug shows low solubility in aqueous medium and approximately 70% of drugs coming from synthesis or poorly soluble in an aqueous medium.[1-3] Solubilization methods using cosolvent, use of permeation enhancement, oily solution and surfactant dispersions, and solid dispersion reduce the problem.[4] The recent development step involved the use of micronized drug powder converted to drug nanoparticles.[1,5]

In submicron colloidal particle, dispersions of a stabilized drug by surfactant or mixture of surfactant are called as nanosuspension.[3] It is not necessary that nanosuspension drug particles have to be rendered into nanosize a domain.[6]

The top-down techniques basically depend on mechanically attrition to render large crystalline particles into nanoparticles by various means such as wet milling, ball milling, and bottom-up techniques, which were dissolved and precipitated by adding the solvent to a non-solvent.[3,7]

Cilnidipine is a choice of new antihypertensive agent and Biopharmaceutical Classification System Class-II drug. Hence, it has low solubility and permeability. Nanosuspension technique is suitable for Cilnidipine.[8-10]

MATERIALS AND METHODS

Nanosuspension formulation (NSF)

Cilnidipine (Free sample), poloxamer-188 (Yarrow Chemie products), polyvinylpyrrolidone K-30 (HiMedia Laboratories), ethanol and polyethylene glycol-4000 (PEG-4000) (Loba Chemie Pvt. Ltd.), and mannitol (Loba Chemie Pvt. Ltd.) were used.

Address for correspondence:
Dr. S. Subramanian, Department of Pharmaceutics,
PSG College of Pharmacy, Coimbatore - 641 004,
Tamil Nadu, India. Phone: +91-9486220270.
E-mail: subbu3j@gmail.com

Received: 28-09-2019
Revised: 11-11-2019
Accepted: 19-11-2019

Excipients of tablets

Cross carmellose (Yarrow Chemie products), magnesium stearate (HiMedia Laboratories), povidone (Yarrow Chemie products), and talc (Loba Chemie Pvt. Ltd.) were used.

Preparation of cilnidipine nanosuspension

Nanosuspension was prepared by the combination method of antisolvent precipitation and high-speed homogenization. Cilnidipine was dissolved in ethanol at room temperature. The solvent containing drug solution was poured into fixed amount of water containing suitable amount of stabilizer at room temperature using syringe simultaneously kept for stirring on magnetic stirrer to allow volatile solvent evaporation. Organic solvent was left to evaporate under stirring room temperature for 2 h.[11,12] After this, solution was homogenized by high-speed homogenization for 20 min at room temperature.[13]

Characterization of cilnidipine nanosuspension

Particle size and polydispersity index (PDI) measurement

The average particle size was determined using dynamic light scattering principle (Malvern Instrument, Malvern, UK). Malvern Zetasizer used for measuring 0.1–10,000 nm and was performed on the well-diluted suspension in cuvette at 25°C.[2,14]

Zeta potential

The zeta potential of the nanosuspension was measured using an additional electrode used for particle size analysis (Malvern Instrument, Malvern, UK). Zeta potential was found on samples well diluted with water and studied on electrophoretic cell at 25°C.[2,12,15]

Morphology study

Scanning electron microscopy (SEM)

A minute amount of lyophilized nanosuspension powder of the best formulation was fixed on an SEM stub using double side adhesive tape and coated with aluminum at 20 mA for 6 min through a sputter coater. SEM is a secondary electron detector was used to obtain digital images of the sample at an accelerating voltage of 15 kV.[16,17]

Atomic force microscopy (AFM)

The best formulation liquid sample was adsorbed on the surface of silicon wafer and allowed to dry at room temperature. The sample was examined using multimode scanning probe microscope (NTMOT, NTEGRA Prima, Russia) in semi-conduct mode with a force constant range of 0.35–6.06 N/m and a resonating range of 47–150 kHz. The phase image was used to determine the morphology of Cilnidipine nanosuspension.[18]

Lyophilization of cilnidipine nanosuspension

Cilnidipine nanosuspension was lyophilized using lyophilizer (Lyodel freeze dryer). Our study included 1% mannitol as a lyoprotectant and other one is without mannitol. Many lyoprotectant are available such as sucrose, lactose, glucose, sorbitol, maltose, fructose, dextran, and avicii.[17]

Drug content

In 100 ml volumetric flask, 75 mg of lyophilized nanoparticles was dissolved in 10 ml ethanol and make up with distilled water. The volume was adjusted to 100 ml by the same solvent mixture and the drug content was measured by ultraviolet (UV) spectra and the drug content was calculated by standard absorbance values. The observed λ max was 240 nm.[4,5]

Preparation of tablet

Tablet was prepared by direct compression method. Different excipients were used like cross carmellose as superdisintegrant, magnesium stearate as lubricant, povidone as binder, and talc as glidant. Prepared tablets were characterized by different parameters such as weight variation, thickness, hardness, disintegration, and *in vitro* drug release. The percentage composition of formulation is given in Table 1.

Characterization of tablets

Weight variation

Weight variation test is determined by mean weight of individual's tablets. Weight was measured to analytical digital balance. The mean value was recorded. Average weight should not more than 5% for individual tablets.[19,20]

Hardness

Tablet hardness was determined using a Pfizer hardness tester, tablets place on the holder and set to "0" on Monsanto tester scale. Then, the range of Monsanto tester is "0"–"20." The time at less than or equal to the tablet breaks, the pressure applied is noted and finally reading value is recorded.[20,21]

Table 1: Percentage of excipients composition

Excipients	Weight (mg)
Cross carmellose	8.24
Magnesium stearate	2.1
Povidone	2.1
Talc	4.2

Thickness

Tablet thickness was determined by Vernier caliper. Vernier caliper is set to zero. Each five tablets placed in between the jaws of Vernier caliper. Make sure jaws just touch object to be measured and values were recorded.[20,22]

Friability test

Tablet friability was determined by friability test apparatus. Connect the main socket and weigh the tablet before placing in friability apparatus. Tablets were placed in the friability apparatus. After 100 revolutions were completed, samples were taken out and reduction and then weighed.[20,21]

Disintegration test

Disintegration test was determined using Labindia Disintegration apparatus. One tablet was introduced into each tube and disc was added. The purified water was filled in the beaker. Then, the apparatus is operated until the tablet completely disintegrated. The time was noted down until the tablets completely disintegrate without any granules.[20,23]

In vitro drug release profile

In vitro drug release study was carried out in the United States Pharmacopeia Apparatus II – Paddle. The dissolution medium was water containing 0.4% sodium lauryl sulfate (SLS) maintained at 37 ± 0.5°C. The paddle rotating speed was 75 rpm and within the interval of 1 h, samples are collected. The drug release at different time intervals was measured by UV spectroscopy by 240 nm. The release studies were conducted in triplicate and the mean values of the percentage of drug released were plotted drug release versus time. The *in vitro* drug release comparison study was carried out between the formulated tablet and marketed tablet.[24,25]

RESULTS AND DISCUSSION

Different batches of liquid nanosuspension were prepared. The ingredients and stabilizers used in formulation of Cilnidipine nanosuspension are poloxamer-188, PVP K-30, and polymer as PEG-4000. The effect of each varies with the ratio of surfactant involved in their preparation. The combination of poloxamer-188 and PEG-4000 was observed to be effective

Table 2: Formulation of Cilnidipine nanosuspension

Formulation code	Drug (mg)	PEG-4000 (mg)	Poloxamer-188 (mg)	PVP K-30 (mg)
NSF-1	2	2	-	0.5
NSF-2	2	2	-	1.0
NSF-3	2	2	-	1.5
NSF-4	2	2	-	2.0
NSF-5	1	2	-	1.5
NSF-6	1	2	0.5	-
NSF-7	1	2	1.0	-
NSF-8	1	2	1.5	-
NSF-9	1	2	2.0	-
NSF-10	1	2	-	2.5
NSF-11	1	2	2.5	-

NSF: Nanosuspension formulation

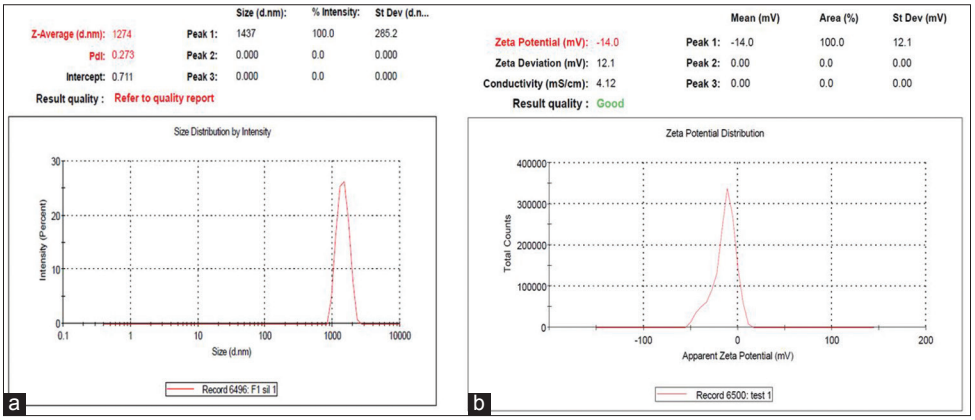

Figure 1: (a) Nanosuspension formulation (NSF)-7 particle size (b) NSF-7 zeta potential

in stabilization of prepared nanosuspension. The particle size in this study was reduced by magnetic stirrer and high-speed homogenizer. Cilnidipine NSF-7 was selected as the best formulation. The different batches of formulation of Cilnidipine nanosuspension shown in [Table 2]. The particle size distribution has the most important characteristics affecting the *in vivo* fate of nanosuspension. The particle size and PDI are shown in Table 3. The NSF-7 formulation contains poloxamer-188 particle size of 1274 nm and PDI of 0.273, indicating narrow size distribution. The PDI is the measure of size distribution of the nanoparticles, where it <0.5 indicates monodisperse size distribution. NSF-7 was used further studies [Table 3 and Figure 1a]. The zeta potential of formulation NSF-7 was found to be -14 mV and it has better stability when compared to other formulations. Zeta potential value can be related to the stability of colloidal dispersion, which indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. Poloxamer-188 used as stabilizer for this formulation is a non-ionic compound providing steric stabilization. Zeta potential value is shown in Table 4 [Figure 1b].

Table 3: Particle size and PDI of nanoformulations

Formulation code	Size (nm)	PDI
NSF-1	3417	1
NSF-2	3067	1
NSF-3	2155	0.458
NSF-4	1254	0.968
NSF-5	1823	0.336
NSF-6	1088	0.834
NSF-7	1274	0.273
NSF-8	1625	0.837
NSF-9	3686	1
NSF-10	3112	0.382
NSF-11	2120	1

PDI: Polydispersity index, NSF: Nanosuspension formulation

Table 4: Zeta potential of NSF-7

Formulation code	Zeta potential (mV)
NSF-7	−14

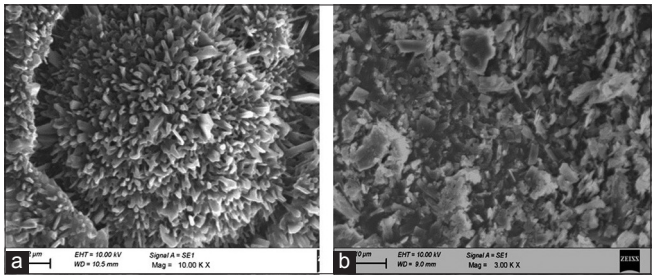

Figure 2: Scanning electron microscopy images (a) with mannitol image (b) without mannitol image

The particles of Cilnidipine nanosuspension were rod in shape. F-7 formulation was lyophilized using batches with mannitol and without mannitol. Mannitol interferes with the image of drug particles. Mannitol acts as a cryoprotectant in the formulation [Figure 2]. AFM described the morphology of given sample. NSF-7 formulation showed [Figure 3] spherical-shaped particles. For AFM, liquid sample represented spherical-shaped particle and for powder sample, it represented rod-shaped particles. Cilnidipine nanosuspension has lyophilized using 1% mannitol as a lyoprotectant and the other one as a without mannitol. Liquid nanosuspension was first kept for deep freezer at −80°C and then the sample was introduced to lyophilizer for 74 h. The drug content of the NSF-7 formulation was found to be 90.23%. Therefore, 83 mg of lyophilized powder contains almost 100% of drug.

Tablet evaluations were done within the limit [Table 5]. Tablets were individually weighed and the weight was calculated for the variation test. It should be present within the limit. Tablets have been evaluated. Weight of individual tablets has been noted and mean weight of tablets also calculated. Tablets were used in the hardness test and values are reported. It was expressed in kg/cm^2 and shows good in hardness for all the tablets. It indicates that tablets are able to withstand structure during transported and handling. Tablets thickness represents

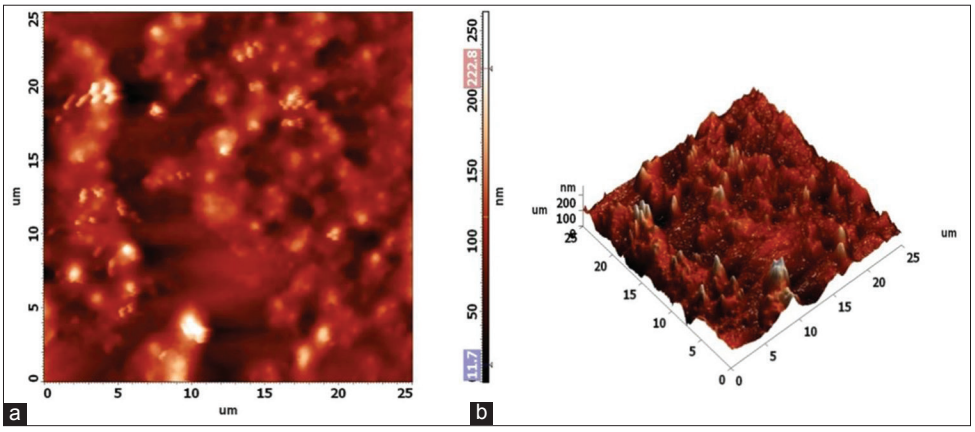

Figure 3: Atomic force microscopy image of Formulation NSF-7

Table 5: Evaluation report of nanosuspension particles loaded tablets

Weight variation±SD (mg)	Hardness (kg/cm²)±SD	Thickness±SD (mm)	Friability±SD	Disintegration time±SD
99.533±0.833	6±0.141	0.38±0.044	0.474±0.471	6.86±0.215

SD: Standard deviation

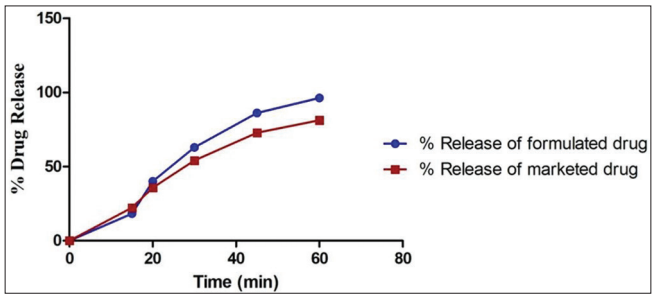

Figure 4: Comparison of *in vitro* drug release of NSF-7 formulation with marketed formulation

uniformity in production of tablets with this method. Friability test for five formulated tablets was carried out and average friability values are recorded. Friability was below 1% and proves the quality of tablet. The formulated tablets were tested individually for disintegration. Tablets disintegrate within the time of 15 min and passed the test. *In vitro* drug release study carried for 1 h and the sample was withdrawn of 15, 20, 30, 45, and 60 min. Formulated and marketed tablets were used for both the media 0.4 SLS in distilled water. Formulated tablet drug release [Figure 4] was found to be 96.30% and marketed tablet drug was found to be 81.20%. Hence, formulated tablet is better than the marketed tablet.

CONCLUSION

Cilnidipine nanosuspension was prepared by the combination method of antisolvent precipitation and high-speed homogenizer. Using as stabilizer of poloxamer-188, PVP K-30, and polymer PEG-4000 and the formulation NSF-7 showed better particle size and zeta potential with good PDI. NSF-7 has been selected best formulation. Hence, it was converted into solid particles using lyophilizer. The best formulation was analyzed for SEM, AFM, and drug content. The nanoparticles were made into tablets by direct compression method with excipients such as cross carmellose, magnesium stearate, povidone, and talc. Formulated tablet drug release was more than marketed tablet within an interval of 1 h. About 18.59% increase in *in vitro* release was found for the formulated tablet as compared to marketed one. Hence, formulated tablet may be further studied for increase in bioavailability of Cilnidipine nanosuspension using animal model.

REFERENCES

1. Bhalekar MR, Upadhaya PG, Reddy S, Kshirsagar SJ, Madgulkar AR. Formulation and evaluation of acyclovir nanosuspension for enhancement of oral bioavailability. Asian J Pharm 2014;8:110-8.
2. Agarwal V, Bajpai M. Preparation and optimization of esomeprazole nanosuspension using evaporative precipitation-ultrasonication. Trop J Pharm Res 2014;13:497-503.
3. Matteucci ME, Hotze MA, Johnston KP, Williams RO. Drug nanoparticles by antisolvent precipitation: Mixing energy versus surfactant stabilization. Langmuir 2006;22:8951-9.
4. Patel HM, Patel UB. Shah C, Akbari B. Formuation and development of nanosuspension as an alternative approach for solubility and dissolution enhancement of aceclofenac. Int J Adv Pharm 2018;7:30-47.
5. Taneja S, Shilpi S, Khatri K. Formulation and optimization of efavirenz nanosuspensions using the precipitation-ultrasonication technique for solubility enhancement. Artif Cells Nanomed Biotechnol 2016;44:978-84.
6. Zhang J, Xie Z, Zhang N, Zhong J. Nanosuspension drug delivery system: Preparation, characterization, postproduction processing, dosage form, and application. In: Nanostructures Drug Delivery. New York: Elsevier; 2017. p. 413-43.
7. Rao GC, Kumar MS, Mathivanan N, Rao ME. Nanosuspensions as the most promising approach in nanoparticulate drug delivery systems. Pharmazie 2004;59:5-9.
8. Arunkumar N, Deecaraman M, Rani C. Nanosuspension technology and its applications in drug delivery. Asian J Pharm 2014;3:168-73.
9. Chandra KS, Ramesh G. The fourth-generation calcium channel blocker: Cilnidipine. Indian Heart J 2013;65:691-5.
10. Pansuriya V, Bhandari A. Formulation and optimization of press coated pulsatile tablet of cilnidipine for chronopharmaceutical approach for treatment of hypertension. Int J Pharm Pharm Res 2015;3:169-82.
11. Yuan H, Li X, Zhang C, Pan W, Liang Y, Chen Y, *et al*. Nanosuspensions as delivery system for gambogenic acid: Characterization and *in vitro/in vivo* evaluation. Drug deliv 2016;23:2772-9.
12. Shariare MH, Sharmin S, Jahan I, Reza HM, Mohsin K. The impact of process parameters on carrier free paracetamol nanosuspension prepared using different stabilizers by antisolvent precipitation method. J Drug Deliv Sci Tech 2018;43:122-8.
13. Liu D, Xu H, Tian B, Yuan K, Pan H, Ma S, *et al*. Fabrication of carvedilol nanosuspensions through the anti-solvent precipitation-ultrasonication method for the improvement of dissolution rate and oral bioavailability. AAPS PharmSciTech 2012;13:295-304.

14. Kathpalia H, Juvekar S, Shidhaye S. Design and *in vitro* evaluation of atovaquone nanosuspension prepared by pH based and anti-solvent based precipitation method. Colloid Interface Sci Commun 2019;29:26-32.
15. Sunita SS, Avinash HH. Preparation and evaluation of nanosuspensions for enhancing the dissolution of lornoxicam by antisolvent precipitation technique. Indo Am J Pharm Res 2014;4:398-405.
16. Kalvakuntla S, Deshpande M, Attari Z, Kunnatur K. Preparation and characterization of nanosuspension of aprepitant by H96 process. Adv Pharma Bull 2016;6:83.
17. Papdiwal A, Pande V, Sagar K. Design and characterization of zaltoprofen nanosuspension by precipitation method. Pharm Chem 2014;6:161.
18. Lamprou DA, Venkatpurwar V, Kumar MR. Atomic force microscopy images label-free, drug encapsulated nanoparticles *in vivo* and detects difference in tissue mechanical properties of treated and untreated: A tip for nanotoxicology. PLoS One 2013;8:e64490.
19. Sem S, Sharma A, Gupta A, Darwhekar GN. Formulation and evaluation of sublingual tablet containg antiemetic drug. J Drug Deliv Ther 2018;8:107-11.
20. Chatuvedi H, Garg A, Rathore US. Post compression evaluation parameters for tablets-an overview. Eur J Pharm Med Res 2017;4:526-30.
21. Gupta A, Kataria MK, Bilandi A. Formulation and evaluation of solid dispersion of glipizide for solubility and dissolution rate enhancement. Int J Pharm Drug Anal 2014;2:74-87.
22. Basu B, Bagadiya A, Makwana S, Vipul V, Batt D, Dharamsi A. Formulation and evaluation of fast dissolving tablets of cinnarizine using superdisintegrant blends and subliming material. J Adv Pharm Tech Res 2011;2:266.
23. Munde AV, Bharti VP, Attal VR, Bais SK. Formulation and *in vitro* evaluation of orodispersible tablets of lansoprazole. J Innov Pharm Biol Sci 2015;2:469-77.
24. Nagar SK, Soniwala MM. Optimization of cilnidipine nanosuspension using a center composite design. Int J Pharm Sci Drug Res 2017;9:149-59.
25. Attari Z, Kalvakuntla S, Reddy MS, Deshpande M, Rao CM, Koteshwara KB. Formulation and characterisation of nanosuspensions of BCS class II and IV drugs by combinative method. J Exp Nanosci 2016;11:276-88.

Source of Support: Nil. **Conflict of Interest:** None declared.