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NANOPRECIPITATION WITH SONICATION FOR ENHANCEMENT OF ORAL BIOAVAILABILITY OF FUROSEMIDE

BHANU P. SAHU[1]* and MALAY K. DAS [2]

[1]GIPS, Gauhati University, Azara, Guwahati, India
[2]Department of Pharmaceutical Sciences, Dibrugarh University, Dibrugarh, India

Abstract: Furosemide is a weakly acidic diuretic indicated for treatment of edema and hypertension. It has very poor solubility but high permeability through stomach and upper gastrointestinal tract (GIT). Due to its limited solubility it has poor and variable oral bioavailibility of 10–90%. The aim of this study was to enhance the oral bioavailibilty of furosemide by preparation of nanosuspensions. The nanosuspensions were prepared by nanoprecipitation with sonication using DMSO (dimethyl sulfoxide) as a solvent and water as an antisolvent (NA). The prepared nanosuspensions were sterically stabilized with polyvinyl acetate (PVA).These were characterized for particle size, ζ potential, polydispersity index, scanning electron microscopy (SEM), differential scanning calorimetry (DSC), X-ray diffraction (XRD) pattern and release behavior. The average particle size of furosemide nanoparticles were found to be in the range of 150–300 nm. This was further confirmed by SEM photograph. The particle size varies with an increase in concentration of drug and stabilizer. The preparations showed negative ζ potential and polydispersity index in the range of 0.3 ± 0.1. DSC and XRD studies indicated that the crystalline furosemide drug was converted to amorphous form upon precipitation into nanoparticles. The saturation solubility of prepared furosemide nanoparticles markedly increased compared to the original drug in simulated gastric fluid. The release profiles of nanosuspension formulation showed up to 81.2% release in 4 h. It may be concluded that the nanoprecipitation with ultrasonication have potential to formulate homogenous nanosuspensions with uniform sized amorphous nanoparticles of furosemide. Polyvinyl acetate can be used as a suitable steric stabilizer to prepare stable furosemide nanosuspensions. The enhanced saturation solubility in simulated gastric fluid may lead to enhanced absorption of furosemide.

Keywords: nanosuspension, nanoprecipitation, furosemide, bioavailability

Furosemide is a powerful diuretic used in edemas and chronic hypertension (1). Furosemide is a BCS (Biopharmaceutics Classification System) class IV drug due to its low water solubility (5–20 μg/mL) and low permeability (2). It has a very variable bioavailibity of 10–90% due to its low solubility in the stomach. However, furosemide is preferentially absorbed in the stomach and upper intestine where it has good permeability, but due to its low solubility in this conditions its absorption is very poor and variable (3). Altough it has good solubility in intestinal fluid, being a BCS Class IV drug it has very poor permeability through intestinal region. Hence, improving the solubility in gastric fluid becomes important to increase the systemic absorption of furosemide from stomach region and upper GIT, where it has better permeability and may result in improved bioavailability. The improvement of the bioavailability of poorly water soluble drugs has been of major concern during the last decades. Altough in order to increase the dissolution rate of furosemide several attempts were carried out in the past (4–8), however, most of these techniques require a large amount of additives limiting their use from the safety perspective. So far, no attempts have been reported on enhancement of dissolution by reduction in particle size of furosemide using nanosuspensions.

Recently, the nanosuspension technology has been successfully applied to tackle the formulation issue of several poorly soluble drugs. Nanosuspensions are carrier-free colloidal drug delivery system containing minimum additives (9, 10). These preparations have several advantages and results in considerable increase in drugs saturation solubility. The preparations are more homogenous and have good dispersity and scale up features (11, 12). The methods of preparation of nanosuspenisons are simple and universal in approach (13, 14).

* Corresponding author: GIPS, Hathkhowapara, Azara, Guwahati-17, India; e-mail: pratapsuman2004@yahoo.co.in

Nanosuspensions can be prepared either by top-down or bottom-up processes. The top-down process involves particle size reduction of large drug particles into smaller particles using various techniques such as: media milling, microfluidization and high pressure homogenization. However, all these processes involve high energy input and are highly inefficient. In the bottom-up approach, the drug is dissolved in an organic solvent and is then precipitated on addition of an antisolvent in the presence of a stabilizer. The precipitation method results in smaller size and homogenous particles. Besides, it may lead to amorphous drug nanoparticles which have higher saturation solubility and dissolution rate (15, 16). Various adaptations of this approach include: (i) solvent–anti-solvent method, (ii) supercritical fluid processes, (iii) spray drying, (iv) emulsion–solvent evaporation and (v) ultrasonication (17, 18). Hence, in the present study, the method of precipitation was explored for the preparation of nanosuspensions. The precipitation was combined with sonication to get more homogenous and smaller particles.

The nanosuspensions can be stabilized by electrostatic or steric stabilization or a combination of both. However, steric stabilization is more advantageous than electrostatic stabilization as the latter may be lost in the variable pH condition of the GIT and is effected by electrolytes. The stability of sterically stabilized nanosuspensions depends on the property of the drug like enthalpy and logP as well as the hydrophobicity of the stabilizer (19, 20).

The aim of this study was to enhance the saturation solubility of furosemide in gastric fluid and thereby oral bioavailability of the drug by preparation of nanosuspension. The possibility of producing a stable nanosuspension of furosemide by controlled precipitation with sonication using steric stabilizer has been investigated. The impact of various experimental parameters on particle formation including solvent–antisolvent ratio, diffusing drug concentrations, type and concentration of stabilizer and stir-

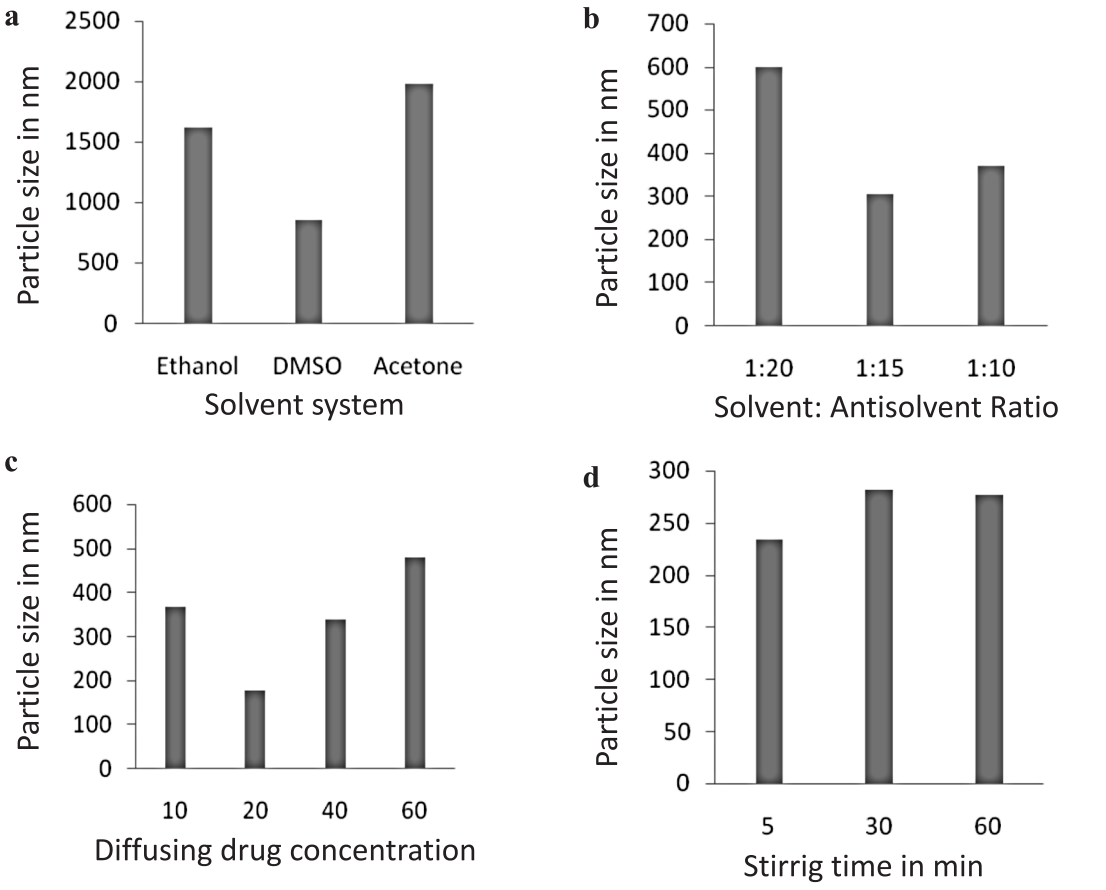

Figure 1. Effect of (**a**) solvent system, (**b**) solvent : antisolvent ratio, (**c**) diffusing drug concentration, (**d**) stirring time on mean particle size

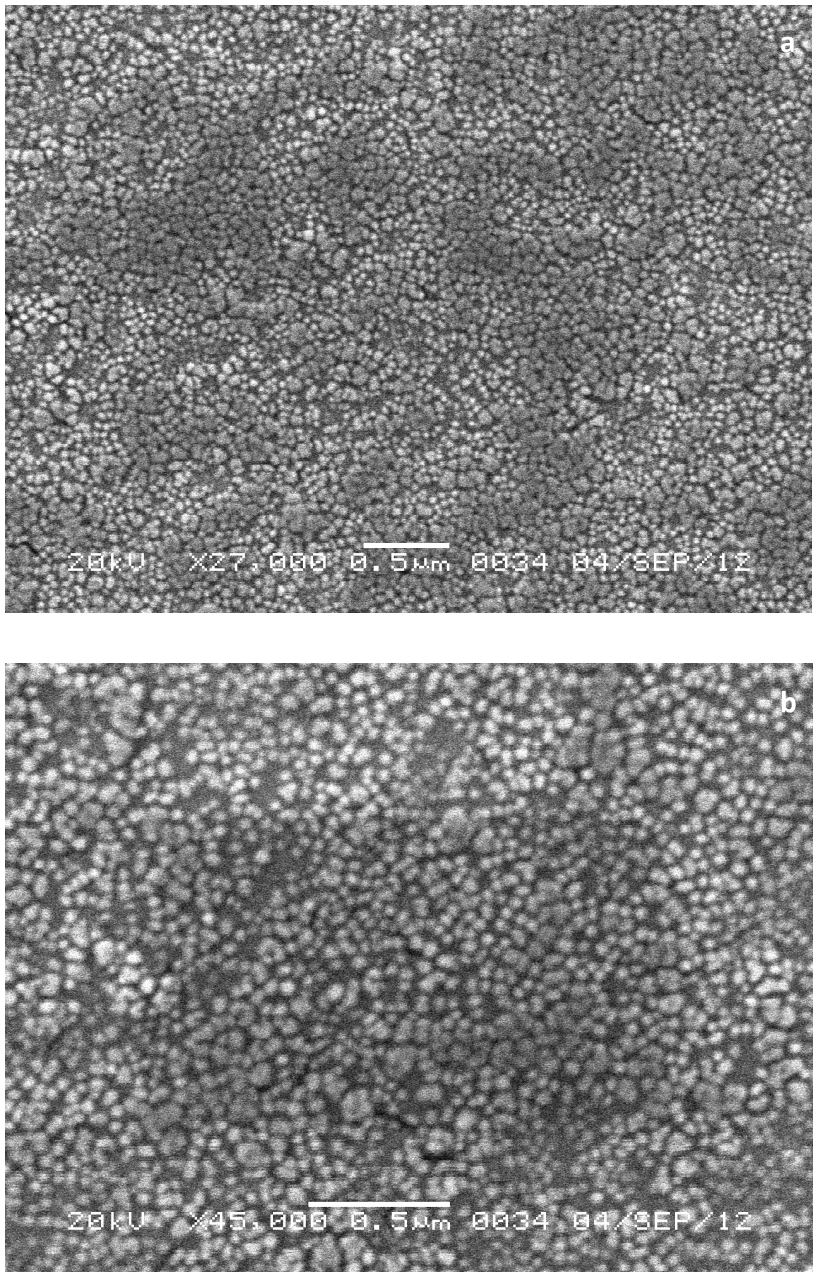

Figure 2. **a**) SEM photomicrograph of furosemide nanoparticles (27,000×); **b**) (45,000×). Scale bar = 0.5 µm

ring time were studied. Characterization and physical stability of the obtained nanosuspension were also carried out.

MATERIALS AND METHODS

Preparation of nanoparticles

Furosemide nanoparticles were produced by precipitation–ultrasonication technique (21). The required amount of drug was dissolved in water-miscible solvent (DMSO). Different concentrations of drug in solvent (5, 10, 25, 40 mg/mL) were used. The obtained drug solution was then injected into the water containing stabilizer (PVA) with stirring. The suspension was then ultrasonicated under cold condition. The preparations were then lyophilized using freeze dryer.

Size measurement and ζ potential analysis

The particle size and the polydispersity index (PI) of the precipitated nanoparticles were measured immediately by dynamic laser light scattering method using (Zetasizer Ver. 6.11 Malvern). The ζ potential of the preparations was also measured using (Zetasizer Malvern).

Scanning electron microscopy (SEM)

The morphology of the dried nanoparticles was observed using scanning electron microscopy (SEM) JSM-6360 (JEOL Inc., Japan). Small drop of the nanosuspension was air dried followed by oven drying and were fixed on an SEM stub using double-sided adhesive tape and coated with Au at 20 mA for 6 min through a sputter-coater (Ion sputter JFC 1100, Japan). A scanning electron microscope with a secondary electron detector was used at an accelerating voltage of 15 kV (22).

Determination of saturation solubility

The saturation solubility of furosemide was evaluated by dispersing lyophilized powder in 20 mL of simulated gastric fluid pH 1.2 to obtain 2 mg/mL of drug suspension. This was placed on a shaking water bath for 48 h to ensure that the solubility equilibrium had been reached. The samples were centrifuged and the resulting supernatant was analyzed by UV spectrophotometer at 274 nm.

X-ray diffraction studies (XRD)

The effect on crystallinity of precipitated furosemide nanopartilces was observed by X-ray diffraction using a XRD-6000 diffractometer (Shimadzu, Japan). The powder was placed in a glass sample holder. CuK radiation was generated at 30 mA and 40 kV and samples were scanned from 5 to 90^{o} with a step size of 0.02^{o}.

Fourier transforms infrared spectroscopy (FT-IR)

Drug excipients interactions were studied by FTIR spectroscopy (23). FTIR spectra were recorded for furosemide, PVA and the dried nanoparticles. Samples were prepared in KBr discs (2 mg drug in 8 mg KBr) with a hydrostatic press at a force of 8 t cm^{-2} for 2 min. The scanning range was 450–4000 cm^{-1} and resolution was 2 cm^{-1}.

Differential scanning calorimetry (DSC)

The DSC analysis of pure drug, PVA and the dried nanoparticles was carried out using Mettler Toledo (Model SW 810) to observe any possible drug-excipients interaction. Samples (5.5–8 mg) were weighed accurately using a single pan elec-

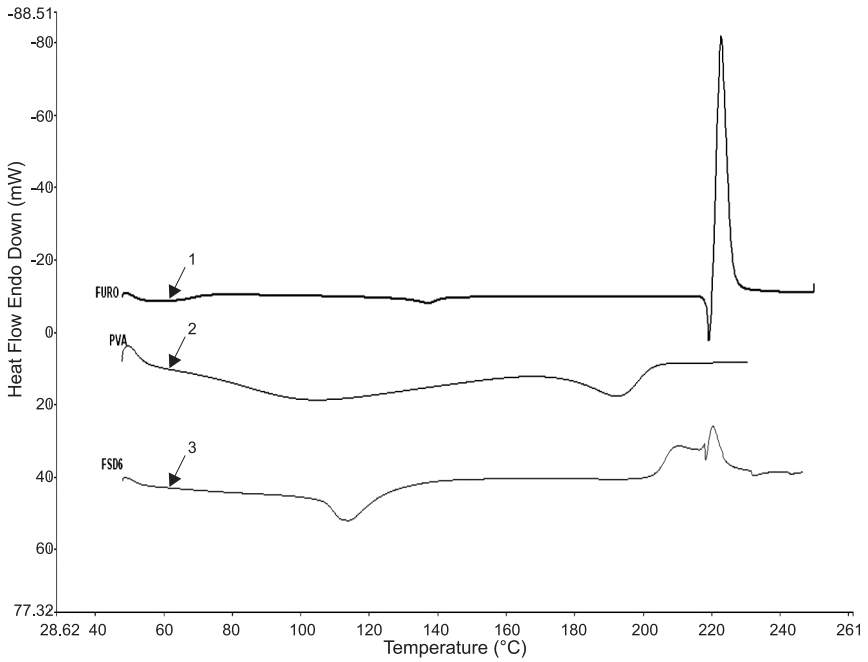

Figure 3. DSC thermogram of (1) pure drug furosemide (Furo), (2) PVA; (3) precipitated furosemide nanoparticles (FSD6)

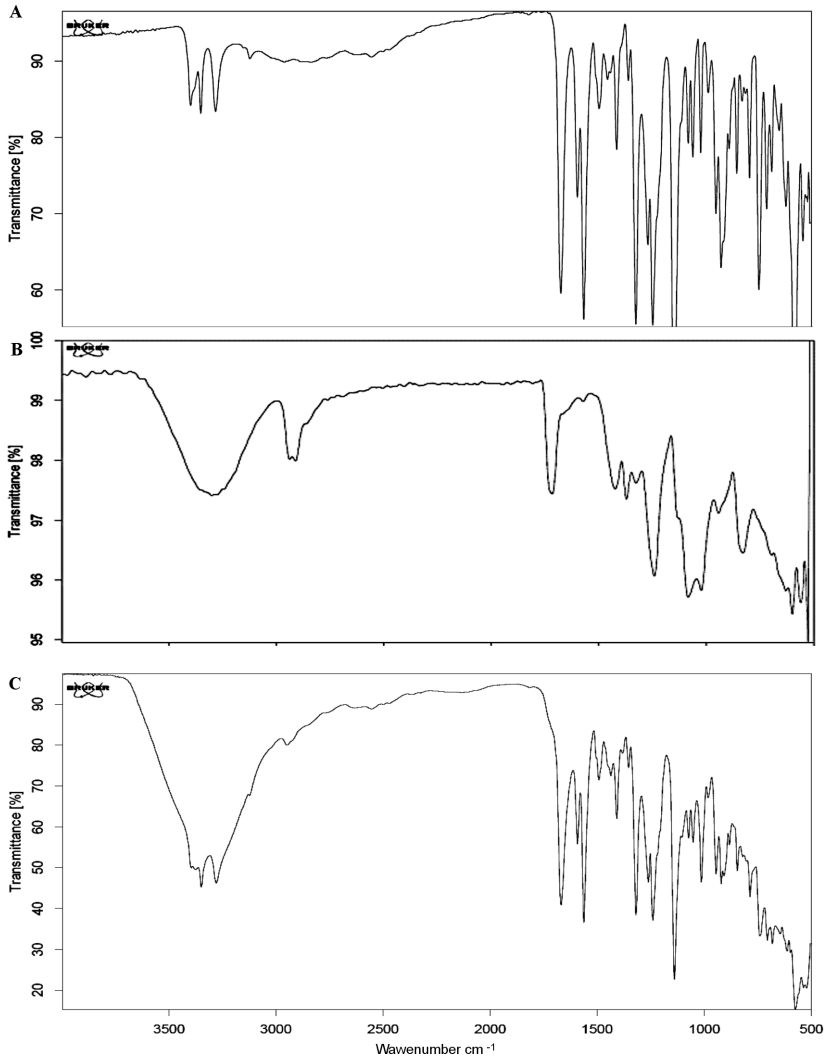

Figure 4. FTIR spectra of (**A**) pure drug furosemide; (**B**) PVA; (**C**) precipitated furosemide nanoparticles

tronic balance and heated in sealed aluminum pan at rate of 5°C/min from 25 to 450°C temperature range under a nitrogen flow of 35 mL/min (24).

In vitro release kinetic experiments

In vitro drug release of the nanosuspensions was determined by the dialysis membrane diffusion technique in phosphate buffer (PB) 6.5 + 0.5% SLS (sodium lauryl sulfate). One milliliter of nanosuspension was placed in the dialysis membrane (M.w. cut off 12000–14000, HiMedia, India), fixed in an apparatus of surface area 1.76 cm^2 and receptor volume of 20 mL. The entire system was kept at 37°C with continuous magnetic stirring. Samples (1 mL) were withdrawn from the receptor compartment at predetermined time intervals and replaced by fresh medium. The amount of drug dissolved was determined UV spectrophotometrically at 277 nm.

Physical stability study

The physical stability of the nanosuspensions on storage was studied at 4°C (refrigerator), room temperature and 40°C (stability chamber) for 6 months. Particle size diameter (PSD) measurements were selected as suitable parameter for evaluation of physical stability (25).

RESULTS AND DISCUSSION

Preparation of nanoparticles

Furosemide nanoparticles were produced by precipitation–ultrasonication technique.The aque-

ous phase containing a suitable stabilizer have been used as the antisolvent and the use of different water miscible solvents (ethanol, acetone, dimethyl sulfoxide DMSO) having good solubility of furosemide has been explored as solvents. The effect of various variables like diffusing drug concentration, solvent : antisolvent ratio, type of stabilizer, concentration of stabilizer, stirring time and ultrasonication have been observed. Ethanol, DMSO and acetone have been tried as solvent for the preparations. These preparations gave particle size of 1617, 856 and 1980 nm, respectively, as shown in Fig 1a. Hence, DMSO was selected as solvent as it produced nanoparticles of smaller size of furosemide on precipitation.

From the preliminary studies, the effect on particle sizes of different solvent : antisolvent ratios (1 : 20, 1 : 15, 1 : 10) was observed, which produced particles of 600, 304, 369 nm, respectively. As such, formulation with S : NS 1 : 15 showing smaller particle size was selected for the preparation as suitable S : NS ratio. The selection of proper S : NS ratio is important for the formulation as it effects the extent of supersaturation and thereby effects the size of the precipitated furosemide particles.

The effect of stirring time (5, 30 and 60 min) on the particle size was studied, which showed particle size of 233.6, 281.2 and 276.9 nm, respectively, when prepared by precipitation with ultrasonication. No sign of aggregation due to stirring have been observed and the particle size doesn't show dependence on stirring time.

In the present study, suitability of steric stabilizer alone for stabilization of the nanosuspension have been investigated. The logP of furosemide is 2.3, hence, moderately hydrophobic stabilizer (HPMC and PVA) have been selected for the preparation. Since the stability of the nanosuspensions depends on the hydrophobicity of the drug and stabilizer, a similar hydrophobicity should result in better surface coverage thereby providing better steric stabilization. HPMC and PVA have been used as stabilizers for the preparations at various concentrations. PVA based formulations at various concentrations 0.15, 0.25 and 0.5% showed particle size 288, 239, and 156 nm, respectively, which were comparatively smaller than HPMC based formulations. Hence, from the preliminary studies, PVA at concentration of 0.5% was found to be optimum stabilizer concentration.

The effect of diffusing drug concentration on the particle size was studied. The nanosuspensions were made with different diffusing drug concentrations 10, 20, 40 and 60 mg/mL. The particle size varies with the change in drug concentration as shown, giving particle size of 366, 179, 339 and 478 nm, respectively. Preparations with 20 mg/mL diffusing drug concentration were found to be optimum for PVA based formulations and were selected for further studies. The study indicates that sufficient supersaturation is required for diffusing drugs to get precipitate in nanoparticulate range due to the enhanced rate of crystal nucleation and growth. But at very high concentration, the particle size increases, as very high supersaturation increases the parti-

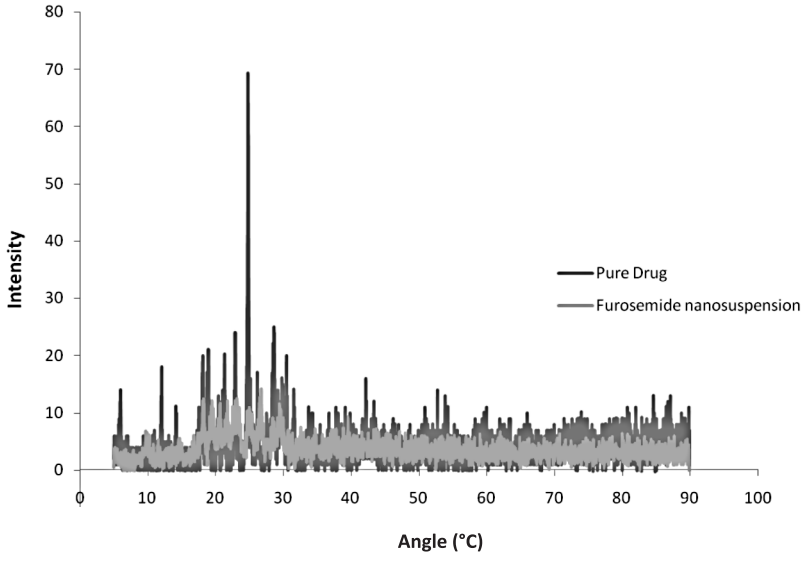

Figure 5. X-ray diffraction patterns of pure furosemide drug precipitated PVA based furosemide nanoparticles

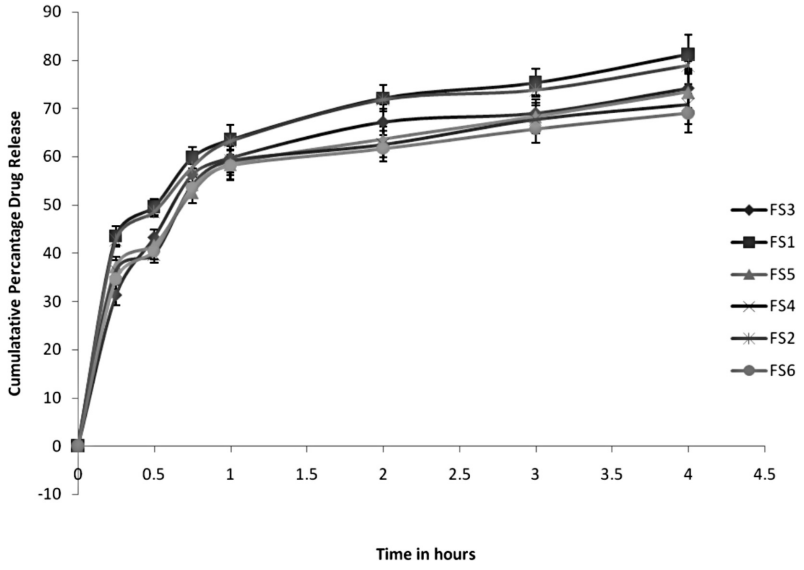

Figure 6. Cumulative percentage of furosemide released from various formulations

cle growth by promoting condensation/coagulation The effect of ultrasonication on the size of the precipitated particles was observed. The particle size was found to be 733 nm in precipitation alone with higher polydispersity index. The particle size was considerably decreased and more uniform in case of precipitation with ultrasonication for 15 min under cold condition showing particle size of 557.5 nm. Application of sonication during precipitation assists in the diffusion of solvent in the antisolvent and results in smaller and more homogenous dispersions. Hence, precipitations with ultrasonication have been used for the further preparations of nanoparticles of furosemide.

After proper selection of the different variables, the furosemide nanosuspensions were prepared and suitably characterized.

Size measurement and ζ potential analysis

The average particle size of furosemide nanoparticles were found to be in the range of 100–300 nm. The particles were homogeneous as indicated by polydispersity index of 0.3 ± 0.1. The ζ potential of the nanoparticles was found to be negative which may be due to the presence of terminal carboxylic groups.

Scanning electron microscopy (SEM)

Morphology of precipitated drug particles in the suspension after air drying followed by oven drying is shown in Figures 2a and 2b. The drug particles precipitated with the PVA as stabilizer are spherical in shape and the size ranges from 100 to 300 nm. The particles are discrete and uniform in size and there is no sign of agglomerations.

Differential scanning calorimetry (DSC)

The DSC analysis of pure drug, PVA and the dried nanoparticles was carried out. The DSC thermogram of furosemide shows a characteristic, sharp exothermic melting point peak at 229.8^{o}C, which indicates the crystalline nature of the drug. The peak corresponding to the melting point of furosemide in the precipitated nanoparticles is broader indicating a decline in the crystallinity of furosemide in nanosuspension. The result is shown in Figure 3. This result was further confirmed by XRD analysis.

X-ray diffraction studies (XRD)

The representative X-ray diffraction patterns of the pure furosemide powder and oven dried nanosuspensions are shown in Figure 4. The figures indicated changes in the drug crystal structure. The X-ray patterns of the pure furosemide displayed the presence of numerous distinct peaks at 6.01^{o}, 12.09^{o}, 18.13^{o}, 18.17^{o}, 24.81^{o}, 24.85^{o}, 24.89^{o} and 28.65^{o}, which suggested that the drug was in crystalline form. The precipitated nanoparticles samples showed diminished peaks suggesting the conversion of crystalline furosemide drug into amourphous form upon precipitation into nanoparticles. The result is shown in Figure 4.

Saturation solubility

The results of saturation solubility of pure drug and lyophilized furosemide nanosuspensions revealed a saturation solubility of 12.0 µg/mL and 438.32 µg/mL in simulated gastric fluid of pH 1.2, respectively. The saturation solubility of furosemide in nanosuspension increased 36-fold than that of pure furosemide. The substantial increase in the saturation solubility may be due to the increased surface area of the small sized nanoparticles. The formation of amorphous particles may also have resulted in this increase in solubility.

Fourier transform infrared spectroscopy (FT-IR)

The IR (infra red) spectra of furosemide in pure drug and in the precipitated nanoparticles were comparable and found to be intact. The spectrum of pure furosemide shows the characteristic peaks at 3647.51 cm^{-1} (O-H stretch), 3286.81 cm^{-1} (N-H stretch), 3147.05 cm^{-1} (C-H stretch), 1568.18 cm^{-1} (C=O stretch), 1672.34 cm^{-1} (N-H bending), and 1263.44 cm^{-1} (S=O asymmetric stretch). FT-IR spectra of precipitated nanoparticles of furosemide showed no substantial shifting of the position of functional groups. The peaks indicated no major interactions between furosemide and PVA in the formulation. The result is shown in Figure 5.

***In vitro* release kinetic experiments**

The release profile of furosemide nanosuspensions in PB 6.5 + 0.5% SLS shows up to 81.2% release in 4 h. The drug release of prepared furosemide nanoparticles markedly increased as compared to the original drug. This enhancement in the drug release may be attributed to the enhanced solubility of drug due to an increase in the surface area. The comparative release results of selected formulations have been shown in Figure 6.

Physical stability study

Recently it has been reported that amorphous particles are more prone to aggregation. Hence, the nanoparticles were stabilized by steric hindrance by sufficient surface coverage using polymeric stabilizer PVA. The physical stability on storage was therefore observed after 6 months. The formulations at 4°C, room temperature and at 40°C remained stable after 6 months. The polymeric stabilizer PVA at 0.5% concentration was found to be sufficient to provide proper steric coverage to keep the amor-

Table 1. Effect of drug concentration and surfactant concentration on mean particle size, polydispersity index and drug release.

Formulation code	Diffusing drug concentration mg/mL	PVA concentration %	Z average (diameter in nm)	Drug release % (PI)	Polydispersity index
FS_1	25	0.5	179.2	81.2	0.395
FS_2	25	1.0	188.6	78.9	0.401
FS_3	40	0.15	339.5	74.2	0.424
FS_4	10	0.15	366.6	70.9	0.385
FS_5	45	0.5	378.4	73.4	0.45
FS_6	5	0.5	382.6	69.1	0.424
FS_7	10	0.75	387.2	71.3	0.412
FS_8	25	0.25	198.4	80.4	0.412
FS_9	25	0.5	179.2	81.2	0.395
FS_{10}	40	0.75	345.6	76.8	0.422

Table 2. Physical stability evaluation of the furosemide nanosuspensions.

Formulations	Storage temperature conditions	Initial particle size	Particle size after 6 months
FS16	4°C	156.7	158.5
	R T		162.2
	40°C		172.5

phous nanoparticles stable. The stability of the nanosuspensions may also be due to the method of precipitation which, in comparision to top down approaches, involves lesser involvement of energy. Moreover, the process results in more homogenous dispersions as indicated by the narrow polydispersity index of 0.3, which may be responsible for the absence of Ostwald ripening on storage generally associated with amorphous particles. The particle size diameter (PSD) data on storage are given in Table 2. Sedimentation was observed in all the conditions but the preparations were easily redispersed on shaking.

CONCLUSION

From the study it may be concluded that stable nanosuspension can be prepared for furosemide by precipitation with ultrasonication. The nanoprecipitation of furosemide results in smaller particles in 150–350 nm range with increased surface area and results in amorphization of the drug. This results in considerable increase in saturation solubility in simulated gastric fluid, which may enhance the oral systemic absorption of furosemide from stomach region where it has better permeability. The polymeric stabilizer PVA was found to be efficient in providing proper steric coverage to the amorphous nanoparticles. The preparation was found to be stable and compatible.

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