Research Article

Formulation and Evaluation of Nanosuspension Drug Delivery System of Furosemide Produced by Nanoprecipitation Method

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ABSTRACT

Formulation of poorly water-soluble drug has always been a challenging problem confronted by the formulation scientist since more than the 40% of the new chemical entities being generated through the drug discovery programme are poorly water soluble. A poorly water-soluble drug indicates insufficient bioavailability. The purpose of this study was to formulate and evaluate the furosemide nanosuspension by nanoprecipitation method with the different stabilizer like Poloxamer 188, PVP K30 and tween 80. The mixture of the drug and methanol as an organic phase and distilled water containing carrier as an aqueous phase. Nanosuspension containing Poloxamer 188 (30mg) and tween 80 (10mg) were selected to be the best based on their drug content, entrapment efficiency and particle size. The result showed that the prepared nanosuspension have particle size in the range of 0.0035 to 0.017µm. The nanosuspension of furosemide was successfully prepared using nanoprecipitation method.

Keywords: Furosemide, Nanosuspension, Nanoprecipitation, Poloxamer 188.

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INTRODUCTION

Oral route of drug delivery is the most commonly used route because of its convenience, painless administration and patient compliance than other routes of drug administration. [1] More than 40% of the NCEs generated through the drug discovery programme are poorly water soluble. Therefore the pharmaceutical industries seeking new approach in order to obtain an adequate oral bioavailability of this type of new chemical entities. Formulation of poorly water soluble or practically insoluble (i.e. BCS class II and BCS class IV drug) drug has always been a challenging problem confronted by the pharmaceutical scientist.[2] There are various approaches have been used to solve the problems of poor solubility and bioavailability such as prodrug, salt formation, complexation (inclusion complexation with β-Cyclodextrin), Co-solvency, use of surfactant, liposomes, microemulsion etc., but these methods have the limitations such as requirement of the large amount of the additives induce the stability and toxicity issues.[3] These methods lack the universal applicability to all drug.[3,4] Nanotechnology can be used to solve the problems associated with the earlier approaches to enhance solubility and bioavailability.[5,6] Reduction of the particle size to nanoscale can be applicable to both BCS class II and IV to increase the solubility, dissolution and hence their absorption into the gastrointestinal tract.[7] Nanotechnology have various advantages over other conventional technology such as it can enhanced solubility, dissolvability, extends the oral bioavailability, lessens the amount of dose. There are various type of drug delivery system using the nanotechnology such as nanosuspension, solid lipid nanocrystals, nanoemulsion, etc.[8,9] Nanosuspension preparation is technically easy, simple and less costly than other nanosizing method.[10]

Nanosuspension is the colloidal dispersion of the drug particle in an aqueous vehicle mainly water which is stabilized by the surfactant, polymer or mixture of the both, for either oral, topical use or the parenteral and the pulmonary administration, with the reduced particle size which leads to increase the dissolution rate and improved bioavailability.[11,12] The particle size distribution of the solid drug particle in nanosuspension is usually less than 1 micron with an average particle size range in between 200-600nm.[13]

Nanosuspension technology is most suitable for the compound with high log P value, high melting point and high dose.[14-16] There are mainly two types of stabilizer used for the stabilization of nanosuspension – steric and electrostatic stabilizer.[16] No single one stabilizer suitable for all drug nanosuspension.[17] In the nanosuspension drug particle size reduction leads to an increase into the particle surface area and consequently the rate of dissolution as described by the Nernst – Brunner and Levich modification of the Noyes- Whitney equation. Increase in saturation solubility postulated by the particle size reduction due to increase into the dissolution pressure explained by Ostwald – Freundlich equation.[11, 18] The Nanosuspension can be prepared by bottom- up technology, top- down and third one is the combination technology. The 'Bottom up

Technology' includes the method of precipitation. The 'Top Down Technologies' are the disintegration method. The top down technologies which include media milling (Nanocrystals), High Pressure homogenization in Water (Dissocubes), High Pressure Homogenization in Non-aqueous Media (Nanopure), and the combination of precipitation and High-Pressure Homogenization (Nanoedge).[15,19]

The aim of this present study was to develop furosemide nanosuspension using a precipitation method. The prepared nanosuspension was then characterized for the drug content, Entrapment efficiency, particle size and the final batch for saturation solubility study.

MATERIALS AND METHODS

Materials

Furosemide was obtained as a gift sample from Aquatic remedies, Mumbai. Polyvinylpyrrolidone K30 and Poloxamer 188 were procured from Balaji drugs, whereas Methanol was purchased from Fischer Scientific, Mumbai. All the ingredients used in the research work were of analytical grade.

Method

Preformulation characterization

In the preformulation characterization the physicochemical parameter of the drug substance are characterized with the goal of designing a drug delivery system.

Organoleptic properties

The pure drug substance was studied for the organoleptic properties such as color, odor, taste and appearance.

Determination of melting point

The melting point of the drug was determined by a capillary glass method. The melting point of the drug was determined by taking small amount of drug in a capillary tube that was closed at one end. The capillary tube was placed in thermionic melting point apparatus and the temperature at which the drug melt was noted. Observed values of the melting point compared with the reported values.[20, 21]

Determination of λ_{max} by UV - Spectrophotometer

The wavelength at which the drug absorbs to its maximum is called as λmax. Drug has a characteristics λ_{max}, and that cannot be changed easily. The λ_{max} of the substance can be find out by scanning the substance in the range of 200-400nm.

Construction of Calibration curve in Methanol

A stock solution of a pure drug furosemide of concentration 1000µg/ml was prepared by dissolving accurately weighed a 10mg of furosemide in 10ml of Methanol. From this solution take 1ml and diluted upto 10ml with corresponding solvent (100µg/ml). Appropriate volumes of the above solution (0.2, 0.4, 0.8, and 1ml) were further diluted to obtained final concentration in range of 2 to 10µg/ml. The spectrum of this solution was recorded using UV; Visible Spectrophotometer against the blank (Methanol) in the range of 200-400nm.

Solubility determination of Furosemide

Solubility of the furosemide was determined qualitatively. 10mg of drug in 10ml of solvent (aqueous / Non-aqueous) taken in a conical flask. Different solvent were used for the solubility determination to determine the solubility of drug. After shaking the sample was examined for the presence of any undissolved suspended particles and clarity.[22]

Preparation of Furosemide Nanosuspension

Furosemide nanosuspension was prepared according to nanoprecipitation method. Pure drug furosemide was dissolved in a (2ml) methanol to form uniform organic solution. The stabilizer was dissolved in water (50ml) to form aqueous phase. The organic solution was injected slowly drop wise with the help of syringe into an aqueous phase with the mechanical agitation at 1000 rpm for 30 minutes. After the mechanical agitation the formulation homogenized at 1500 rpm for 120 minutes with the help of lab homogenizer.

Table 1: Formulation of Furosemide nanosuspension

Formulation Code	Drug	Type of Stabilizer			Methanol (ml)	Water (ml)	Stirring Speed (R.P.M.)
		Poloxamer 188	PVP K30	Tween 80			
FNS1	20	5	-	5	2	50	1500
FNS2	20	10	-	10	2	50	1500
FNS3	20	15	-	10	2	50	1500
FNS4	20	20	-	10	2	50	1500
FNS5	20	25	-	10	2	50	1500
FNS6	20	30	-	10	2	50	1500
FNS7	20	-	5	5	2	50	1500
FNS8	20	-	10	10	2	50	1500
FNS9	20	-	15	10	2	50	1500
FNS10	20	-	20	10	2	50	1500
FNS11	20	-	25	10	2	50	1500
FNS12	20	-	30	10	2	50	1500

Characterization of Nanosuspension

Particle Size analysis: The particle size of the formulated nanosuspension batches was determined by using the Motic digital microscope. The particle size of the batches was recorded in micrometer.

Entrapment Efficiency: Determination of entrapment efficiency is suitable for determining the free concentration of drug present in the supernatant after centrifugation. For the determination of entrapment efficiency 10ml of the freshly prepared nanosuspension was taken and centrifuged at 1000 rpm for 10 minute. The supernatant was removed and the amount of drug unincorporated was measured by taking the absorbance of supernatant solution at 275nm by using UV- Visible spectrophometer.[9]

Total drug content: An accurately measured nanosuspension equivalent to 10mg of drug was taken in 100ml volumetric flask and diluted to 100ml with methanol. (To prepare the stock solution of 100µg/ml). The amount of drug determined spectrophotometrically at 275nm. (UV Spectrophotometer LABINDIA 3000+).[23, 24]

Saturation Solubility: Furosemide loaded nanosuspension (5ml) was subjected to centrifugation at 10000 rpm for 30 minutes. After that the supernatant was examined for drug content by using UV- Visible spectrophotometer at 275nm.[25]

RESULTS AND DISCUSSION

Furosemide is a BCS class IV drug which exhibits a low solubility and low permeability characteristics. The current investigation aimed to increase the solubility of furosemide by preparation of its nanosuspension by using nanoprecipitation technique using Poloxamer 188, PVP K30 and the Tween 80 as a stabilizer. According to the result the batch FNS21 shows better particle size than other. Therefore, this batch can be further evaluated for the saturation solubility. The results of saturation solubility indicate the improvement in saturation solubility than pure drug.

1. Preformulation Characterization

Table 2: Preformulation studies of Furosemide

Sr. No.	Parameter	Observed value	Reported value
1	Organoleptic properties	White	White
I.	Color	Odorless	Odorless
II.	Odor	Tasteless	Tasteless
III.	Taste	Smooth	Crystalline
IV.	Melting point	204 -208°C	206°C
2	Determination of λ_{max} by UV-Spectrophotometer	275nm in methanol	275nm
3	Preparation of calibration curve of Furosemide	Linear standard curve of Furosemide	-
4	Solubility Studies	Soluble in methanol, acetone; insoluble in Water	-

Determination of Melting point:

The melting point of the drug was found to be in the range of 204 - 208°c. it complies with standards thus indicating the purity of the drug sample.

Determination of λ_{max} by UV - Spectrophotometer:

From the number of dilutions of stock solution, the middle dilution i.e. 8 can be used for the determination of the maximum absorbance using UV-visible spectrophotometer in the range of 200-400nm. The absorption maximum and same was used as a λmax for the estimation of furosemide. The λ_{max} of the substance was found to be a 275nm.

Construction of Calibration curve in Methanol:

Furosemide in methanol showed absorption maxima at 275nm and it was chosen as an analytical wavelength. The plot of concentration vs. the absorbance was plotted. Beer's law was obeyed in range 2 to 10µg/ml. Regression analysis was performed on the experimental data. Regression equation for experimental curve was y=0.094x+0.500. The correlation coefficient was found to be 0.999, which indicates the linear relationship between the concentration of drug and its absorbance. The calibration plot of drug in methanol was depicted in figure 1.

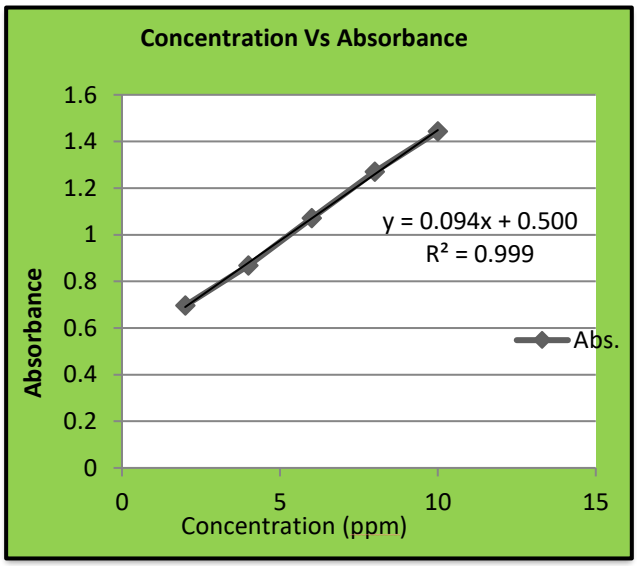

Figure 1: Calibration plot of furosemide in methanol.

Solubility determination of furosemide:

Solubility of the furosemide determined qualitatively. Furosemide is insoluble in water. Soluble in acetone and methanol.

Particle size analysis:

The particle size of the formulated nanosuspension batches was carried out by using Motic digital microscope. The particle size of the nanosuspension batches (FNS1 to FNS12) were shown in table. The average particle size of the FNS1 –FNS12 are in between the0.0035 to 0.017µm. According to result the batch FNS6 showed better particle size reduction than others. (Figure 2)

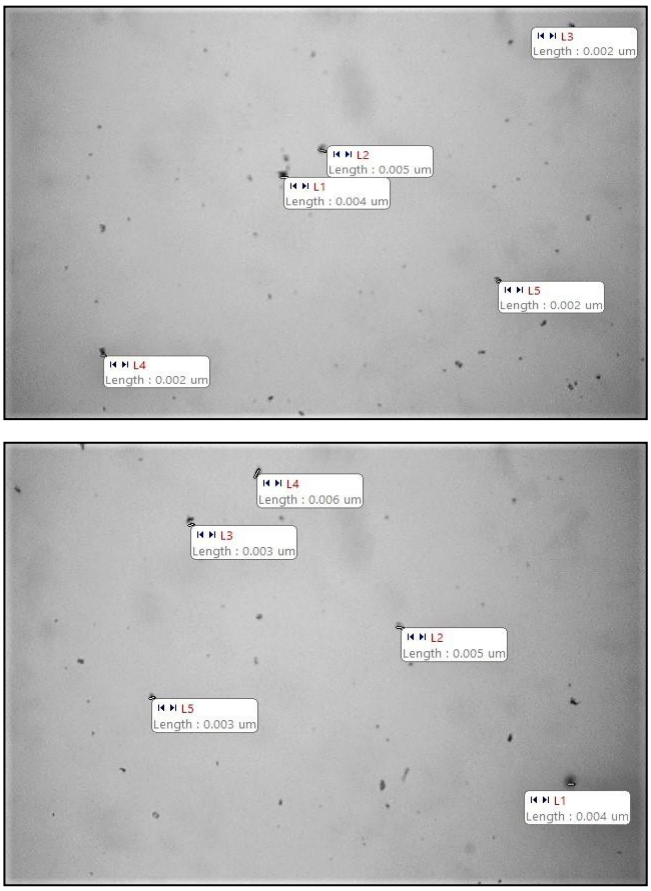

Figure 2: particle size analysis by Motic microscope of FNS21 and FNS20 batch.

Table 3: Particle size of the nanosuspension batches.

Formulation Batch	Particle size (µm)		
	Max	Min	Average
FNS1	0.011	0.004	0.0075
FNS2	0.01	0.002	0.006
FNS3	0.012	0.005	0.017
FNS4	0.007	0.003	0.005
FNS5	0.006	0.003	0.0045
FNS6	0.005	0.002	0.0035
FNS7	0.009	0.002	0.0055
FNS8	0.007	0.003	0.005
FNS9	0.012	0.009	0.0105
FNS10	0.012	0.003	0.0075
FNS11	0.013	0.004	0.0085
FNS12	0.013	0.001	0.007

Entrapment efficiency: The entrapment efficiency of the nanosuspension batches is highlighted in figure 3. It is observed that the formulation batch FNS7 shows minimum entrapment efficiency of 77% and formulation batch FNS6 shows maximum entrapment efficiency of 97.75%. The entrapment efficiency of the nanosuspension was found to be in the range of 77% - 97.75% respectively.

Total Drug Content: The drug content of the nanosuspension was in the range of 76.59 to 99.46% respectively, which indicates that loss of drug was lower during preparation process. The % drug content was shown in table 4. The total drug content of the entire nanosuspension batch was found to be greater than 75%. The batch FNS6 shows the maximum total drug content of 99.46% and batch FNS7 shows minimum drug content, this can be shown in figure 4.

Table 4: Drug content and Entrapment efficiency of the formulation batches.

Formulation Code	% Drug Content	% Entrapment Efficiency
FNS1	86.38	82.75
FNS2	96.80	84.25
FNS3	97	87.77
FNS4	97.87	97.5
FNS5	98.13	97.35
FNS6	99.46	97.75
FNS7	76.59	77
FNS8	78.1	78.75
FNS9	78.72	79
FNS10	81.06	81.38
FNS11	81.31	84.25
FNS12	84.04	87.78

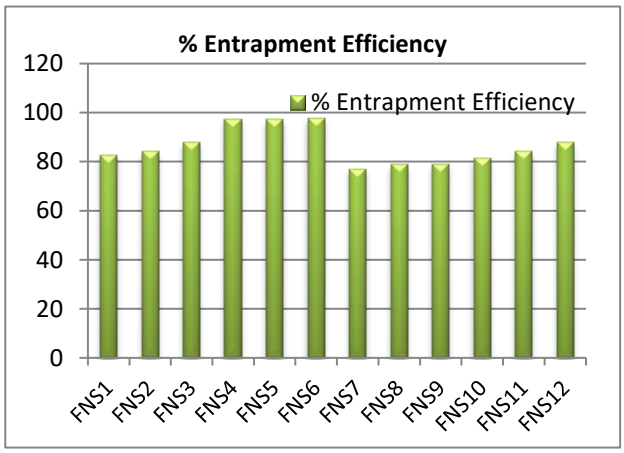

Figure 3: Entrapment efficiency of the formulated nanosuspension.

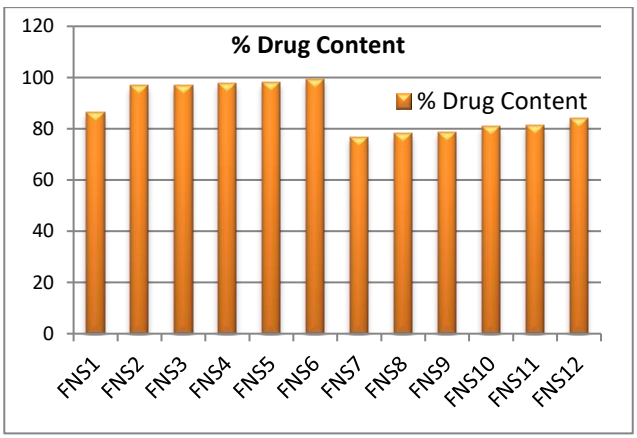

Figure 4: Drug content of the formulated nanosuspension

Saturation solubility: The saturation solubility of the batch (FNS6) was 12.73/ml which was higher than that of the bulk drug.

CONCLUSION

Nanosuspension is the submicron colloidal dispersion of the drug particle. This drug delivery system is important in recent years. It offers the potential advantages like improve bioavailability and patient compliance over the other drug delivery system. In the present study the nanosuspension of furosemide was prepared by using Poloxamer 188, PVP K30 and tween 80 as stabilizers. Furosemide nanosuspension was successfully prepared by using nanoprecipitation technique. The evaluations result confirmed that prepared formulation exhibit satisfactory result. In this process the particle size of the furosemide can be obtained in nano-size ranges by adjusting the concentration of the stabilizer, organic phase volume, and the stirring speed. The best nanosuspension of the furosemide can be obtained by using 20mg of furosemide, 30mg of Poloxamer and 10mg of tween 80 as a stabilizer using nanoprecipitation at laboratory scale. This batch is evaluated for the saturation solubility indicates the improvement in the solubility of furosemide nanosuspension as compared to pure drug.

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