Saudi Journal of Medical and Pharmaceutical Sciences **ISSN 2413-4929 (Print)**
Scholars Middle East Publishers **ISSN 2413-4910 (Online)**
Dubai, United Arab Emirates
Website: http://saudijournals.com/

Development, Characterization & Comparative Evaluation of Nanostructured Lipid Carriers and Solid Lipid Nanoparticles for Potent Oral Delivery of Furosemide

Anurughma S*, Mrs. Neema George
Department of Pharmaceutical Sciences, Regional Institute of Medical Science and Research, Centre for Professional and Advanced studies, Puthuppally, Kottayam, Kerala, India

Original Research Article

***Corresponding author**
Anurughma S

Article History
Received: 01.11.2018
Accepted: 07.11.2018
Published: 30.11.2018

DOI:
10.36348/sjmps.2018.v04i11.002

Abstract: The aim of the present study was to increase the solubility and thereby improve the oral bioavailability of Furosemide by incorporating the drug in nanostructured lipid carriers (NLC) and in solid lipid nanoparticle (SLN) and also to compare the efficiency of NLC over SLN. Both the NLC and SLN were prepared by solvent diffusion method using labrafil m 2130 as solid lipid, capryol pgmc as liquid lipid, and tween 80 as surfactant. Properties of Furosemide loaded NLCs & SLNs such as drug content, entrapment efficiency, loading capacity, particle size, PDI , zeta potential, morphology, storage stability, in vitro drug release and mechanism of drug release were investigated and compared. Drug content, entrapment efficiency, loading capacity, average particle size, PDI and zeta potential of Furosemide NLC were found to 83.56%, 75.50%, 25.63%, 99.24nm, 0.302 and -31.2mV and that of Furosemide SLN were found to 84.55%, 71.07%, 24.62%, 193.4nm, 0.835 and -36.1mV respectively. Morphology study by scanning electron microscopy (SEM) analysis showed spherical particles with smooth surfaces. As compared to in-vitro drug release of Furosemide pure drug, both the NLC and SLN showed fast initial release followed by a sustained release, best fitted to Higuchi equation. Pure drug followed Zero order release kinetics. Furosemide NLC showed higher entrapment efficiency, drug loading capacity, in-vitro drug release, reduced the drug expulsion in storage when compared to SLN. This investigation demonstrated the efficiency of NLC over SLN for improved oral bioavailability of Furosemide and it was deduced that the liquid lipid (capryol pgmc)was the principal formulation factor responsible for the improvement in characteristics and pharmacokinetics of NLCs.

Keywords: Furosemide, Solvent diffusion method, Nanostructured lipid carrier, Solid lipid nanoparticles, Labrafil M 2130, Capryol PGMC, In-vitro drug release.

INTRODUCTION

Of all the drug delivery systems oral route is the most convenient and non-invasive method of drug administration which receives the highest degree of patient compliance. For a drug substance that to be well absorbed following oral administration, it has to: (i) be sufficiently soluble in the gastrointestinal fluids and (ii) easily permeate across the GI membrane without undergoing significant elimination mediated by GI enzymes and enterocyte transporters. According to recent estimates, nearly 30% of the oral immediate release drug products and 40-70% of the newly discovered chemical entities are poorly soluble in water. Drugs with poor aqueous solubility and dissolution properties are not suitable for oral delivery using conventional tablet formulations as it produces low and variable bioavailability, which leads to erratic biological effects [1].

Furosemide is 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid (figure1), it is a white or almost white crystalline powder [2]. Furosemide is a very efficient loop diuretic used in draining all kinds of oedemas (of cardiac, hepatic or renal origin), in mild or moderate hypertension (itself or combined with other antihypertensive drugs), or used in greater doses in acute and chronic renal failure, in oliguria. Erratic oral absorption (11–90%) is the main problem associated with the formulation and effectiveness of the Furosemide. According to Biopharmaceutical Classification System (BCS), Furosemide is classified as a class IV drug having low solubility and low permeability [3].

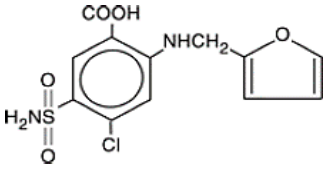


Fig-1: Chemical structural of Furosemide

Nanotechnology is an emerging interdisciplinary technology and widely used as a drug carrier system, which is designed in such way that it can achieve adequate stability, improved absorption, controlled release, quantitative transfer and, therefore, the expected pharmacodynamic activity [4]. Nanotechnology offers drugs in the nanometer size range which enhances the performance in a variety of dosage forms [5].

Recently, several approaches have been investigated to develop nanosized drug delivery system. These systems can generally be divided into two groups: polymeric and lipidic systems [6].

Polymeric nanoparticle was the first emerging nanotechnology for the enhancement of solubility and thereby bioavailability [1]. They consist of a biodegradable polymer which is biocompatible and nontoxic. Despite their interesting properties, not many products made it to market because of the presence of solvent residues left over from production, the cytotoxicity of the polymers, the lack of low-cost, and unavailability of some good techniques for the production of nanoparticles at large scale [7].

In order to overcome these problems, lipids have been put forward as an alternative carrier [6]. The emerging field of lipid-based oral drug delivery systems is expected as promising carriers because of their potential to increase the solubility and improve oral bioavailability of poorly water soluble, lipophilic drugs and has attracted considerable academic attention [4].

Lipid-based nanoparticles have attracted a large attention as possible alternatives to polymeric ones due to their highly biocompatible and biodegradable natural components. Due to the physicochemical properties of lipids, lipid-based nanocarriers can be easily obtained by direct emulsification of the molten lipids and subsequent recrystallization, avoiding the use of potentially toxic solvents that are commonly required for the preparation of other kinds of nanocarriers [8].

Solid lipid nanoparticles (SLNs) are considered to be the most effective lipid based colloidal carriers, introduced in early nineties. This is the one of the most popular approaches to improve the oral bioavailability of the poorly water soluble drugs [9]. SLN are defined as lipidic nanocarriers generally spherical in shape with an average diameter between 10–1000 nm containing biocompatible solid lipid core matrix (mono-di and tri glycerides, fatty acids, steroids and waxes) stabilized by various classes of emulsifiers [10]. SLNs are composed of the lipid matrix which is solid at body and room temperature [8].

NLC, the new generation of lipid nanoparticles, overcome the limitations associated with the SLN, namely, limited drug loading, risk of gelation and drug leakage during storage caused by lipid polymorphism [11]. NLCs are constituted of blends of lipids in solid and liquid states, produced by controlled mixing of solid lipids with spatially incompatible liquid lipids, leading to a specific nanostructure [8]. In contrast to the more or less highly ordered SLN being yielded from solid lipids or blends of solid lipids, the incorporation of liquid lipids to solid lipids leads to massive crystal order disturbance. The resulting matrix shows great imperfections in the crystal lattice and leaves enough space to accommodate drug molecules, leading to improved drug loading capacity, preventing its leakage and giving more flexibility for modulation of drug release [12].

In present study Furosemide loaded NLCs were prepared by solvent diffusion method. The physicochemical properties of obtained NLC, such as drug loading capacity, stability in storage, in-vitro release behaviour were investigated and compared with those of Furosemide loaded SLN.

MATERIALS

Furosemide was purchased from Yarrow chem products, Mumbai, India; Labrafil M 2130 and Capryol PGMC were obtained as gift samples from Gattefosse, Mumbai, India; Soy lecithin was purchased from Tokyo chemical industry co .Ltd, Tokyo, Japan; Stearic acid was purchased from Central drug house (P) Ltd, New Delhi, India; Cholesterol was purchased from Specrochem Pvt Ltd, Mumbai, India; Tween 80 was purchased from Chemdyes corporation, Rajkot, India; Tween 20 was purchased from Otto Chemika- biochemika- reagents, Mumbai, India. DMSO

was purchased from Merck specialities Pvt Ltd, Mumbai, India. All other reagents and chemicals obtained were of analytical grade.

METHODS

Preformulation Studies

Preformulation studies were carried out to assess the physical appearance of drug, solubility, melting point and the compatibility with its excipients. Solvents like water, acetone, alkali hydroxides, ethanol (95%), methanol, dimethyl sulfoxide (DMSO), chloroform, ether and buffer solutions like P^H 1.2 acid buffer, P^H 5.8 phosphate buffer, and PH 6.8 phosphate buffer were used to determine the solubility of pure drug. The melting point of Furosemide was determined by open capillary tube method and by Differential scanning calorimetry (DSC).

UV spectrometric assay of Furosemide

Two Furosemide standard solutions (10µg/ml) namely: a) Furosemide ethanolic solution, b)Furosemide in DMSO diluted with P^H 6.8 phosphate buffer were scanned UV spectrophotometrically over a range of 200-400 nm to determine the wavelength of maximum absorption (λmax).

The calibration curves were constructed over a concentration range of 2-10µg/ml, for standard solutions (a&b). The absorbance was recorded at their respective wavelengths and graph was plotted with concentration against absorbance.

Selection of excipients

Selection of solid lipid

Solid lipid was selected by checking the solubility of the drug in melted solid lipid by means of visible observation with the naked eyes under normal light. Lipids used for this study were stearic acid, cholesterol and labrafil m 2130. Weighed quantity of drug (50mg) separately with various lipids (5g each) was heated above the melting point of lipid in a water bath by regulating temperature in test tubes. After melting of lipid, the solubility of Furosemide in each lipid was observed visually under normal light [13].

Determination of solubility in various liquid lipids and surfactants

Liquid lipids used for this study were castor oil, oleic acid & capryol pgmc and surfactants used were tween 20 & tween 80. The solubility of drug was determined by adding excess amount of the drug in small vials containing 2ml of selected oils, and surfactants separately. The drug was mixed in respective oil and surfactant manually with glass rod. The vials were tightly stopper and were continuously stirred for 24 hours in rotary shaker. Liquid lipids were centrifuged at 3000 rpm for 30 min. The supernatant was separated and dissolved in ethanol and solubility was quantified by UV-Spectrophotometer at 274 nm after appropriate dilution with ethanol [3, 13].

Compatibility study

The stability of a formulation primarily depends on the compatibility of the drug and excipients. Hence it is important to detect any possible chemical or physical interaction, since they can affect the bioavailabity and stability of the drug. The compatibilty studies were carried out at room temperature by FTIR to determine the interaction of Furosemide with the excipients used in the formulation. The FTIR spectra of drug alone and the combination of drug with labrafil m 2130 cs and capryol pgmc were taken.

Preparation of furosemide loaded nanostructured lipid carrier (NLC) and solid lipid nanoparticle (SLN)

NLCs were prepared by the solvent diffusion method. The lipid dispersion was composed of 355.4mg labrafil m 2130 cs and 82.7mg capryol pgmc, where lipids were melted at a temperature $5\text{-}10^0$ above its melting point. Furosemide (200g) and liquid soya lecithin (0.5g) were dissolved in 5mL of DMSO and added to the lipid dispersion with heating at the temperature of $45\text{-}50^0$C to form the lipid phase. Aqueous phase was prepared by dissolving tween 80 in 100mL of water. This aqueous solution was then stirred and heated to $45\text{-}50^0$C. The lipid phase was slowly added dropwise into the aqueous phase at room temperature and mixed using high speed homogenizer at 8000 rpm for 5 minutes. The volume was made to 100ml and further treated using a probe sonicator for 20 minutes. The resultant suspensions were cooled and stored in room temperature.

SLNs were prepared by the same method, only labrafil m 2130 was used as lipid (liquid lipid was omitted). The drug free SLN or NLC dispersion was prepared exactly the same manner where drug was excluded.

Characterization and comparison of NLC and SLN

Drug content

1 ml of Furosemide NLC suspension and Furosemide SLN suspension were transferred to 10 ml standard flasks separately. Few drops of DMSO was added, mixed well and made up the volume with p^H 6.8 phosphate buffer. From this solution, 1ml was taken and diluted to 50 ml with p^H 6.8 phosphate buffer. The absorbance of the solution was measured against the corresponding blank solution and drug content was determined UV spectrophotometer at 279nm.

Entrapment efficiency (E_e) and Drug loading (L_c)

5ml of prepared Furosemide-loaded NLCs were separated from the NLCs suspension by centrifugation at 3000 rpm for 1.5 h. Then 1ml supernatant was taken and dissolved in DMSO, drug content was analysed at 279 nm using a UV-spectrophotometer after suitable dilution with p^H 6.8 phosphate buffer.

Entrapment efficiency was calculated using following equation.

$$E_e = [\frac{W_i - W_s}{W_i}] \times 100$$

$$L_c = [\frac{W_i - W_s}{(W_i - W_s) + W_l}] \times 100$$

Where,

W_i = weight of drug added initially
W_s = weight of drug in supernatant
W_l = weight of lipid mixture added

Particle size and Polydispersity index (PDI)

Mean particle size (Z-average) and polydispersity index (PDI) of the prepared Furosemide loaded NLC sample and SLN sample were measured using Malvern Zetasizer version 7.01. The mean particle size was measured based on photon correlation spectroscopy technique that analyses the fluctuations in dynamic light scattering due to Brownian motion of the particles. The samples were diluted suitably with double distilled water to produce a suitable scattering intensity. All the measurements were done in triplicate, at a fixed scattering angle of 90˚ to the incident laser beam and at a temperature of 25ºC. Disposable polystyrene cuvette was used for placing the sample inside the instrument. Before putting the fresh sample, cuvette was rinsed using the sample to be measured for each experiment.

Zeta potential

Zeta potential, reflecting the electric charge on the particle surface, is a very useful way of evaluating the physical stability of any colloidal system. It was determined based on an electrophoretic light scattering technique. Zeta potential of the formulations were measured by using Malvern Zetasizer version 7.01. Zeta potential measurements were carried out using zeta dip cell, by applying a field strength of 20V/cm at 25 °C after appropriate dilution of samples with double distilled water. All the measurements were done in triplicate.

Scanning Electron Microscopy (SEM)

The SEM analysis of the samples were performed to investigate the surface morphology and homogeneity of the particles in the formulations. The samples were examined morphologically by scanning electron microscope (JSM-6490LV, JEOL) with 15kV accelerating voltage. Samples were prepared by placing a small drop of dispersion onto an aluminium specimen stub using double-sided adhesive tape , dried and sputter coated with gold prior to imaging.

In-vitro drug release study

In-vitro drug release studies of pure Furosemide drug, Furosemide loaded NLC, Furosemide loaded SLN and pure Furosemide were performed using dialysis method.

Dialysis membrane (cellophane membrane), previously soaked overnight, was tied to one end of a specially designed glass cylinder (open at both ends) such that the preparation occupies inner circumference of the tube. 1ml of samples were added to the dialysis bag separately. The cylinder was attached to a stand and suspended in 100 ml of receptor medium (p^H 6.8 phosphate buffer + 0.02% tween 80) maintained at 37 ± 5ºC so that the membrane just touched the receptor medium surface. The receptor medium was stirred at 100rpm using magnetic stirrer. The cellophane membrane acts as a barrier between the NLC and receptor medium (sink condition). An aliquot of 1ml of the sample was withdrawn from the receiver compartment at predetermined time intervals and replenished with fresh medium. The amount of Furosemide released from the samples were then determined by UV-visible spectrophotometer at 279 nm after suitable dilution with p^H 6.8 phosphate buffer.

Kinetics of drug release

In order to understand the mechanism of drug release, in vitro drug release data were treated to kinetic models such as Zero order, First order, Higuchi model and Korsmeyer- Peppa's model. Criteria for selecting the most appropriate model was based on best goodness of fit.

Stability of Furosemide loaded nanostructured lipid carrier

To investigate storage stability, the NLC, SLN formulations were stored in room temperature in the dark over a period of 60 days. Stability of the formulations was periodically monitored & evaluated the appearance, drug content, entrapment efficiency, drug loading capacity, in-vitro drug release during storage and compared with the initial formulations depicted.

RESULTS AND DISCUSSION

Preformulation studies

Preformulation studies were done for confirming the identity, purity and to establish a suitable drug profile. The drug is white or almost white in colour and odourless powder. The solubility of the received sample of Furosemide was examined in various solvents & buffer solutions. The results observed were shown in table 1& 2

Table-1: Solubility of Furosemide in various solvents

SOLVENT	SOLUBILITY
Water	Practically insoluble
Acetone	Freely soluble
Methanol	Freely soluble
DMSO	Freely soluble
Alkali hydroxides	Freely soluble
Ethanol (95%)	Sparingly soluble
Chloroform	Insoluble
Ether	Insoluble

Table-2: Solubility of Furosemide in various buffer solutions

BUFFER SOLUTIONS	SOLUBILITY
P^H 1.2 acid buffer	Insoluble
P^H 5.8 phosphate buffer	Slightly soluble
P^H 6.8 phosphate buffer	Freely soluble

The decomposition point of the drug by capillary fusion method and by DSC were found to be 220^0C and 221.61°C respectively, equivalent with the monograph value. The DSC thermogram of Furosemide is illustrated in figure 2. From the DSC thermogram, Furosemide shows a characteristic, sharp exothermic peak at 221.61^0C with a heat enthalpy of 113.8J/g, which usually associated with the decomposition of the drug and indicate the crystalline nature of the drug. The degradation product of Furosemide displays an endothermic peak at 269.22^0C as is evident from the figure.

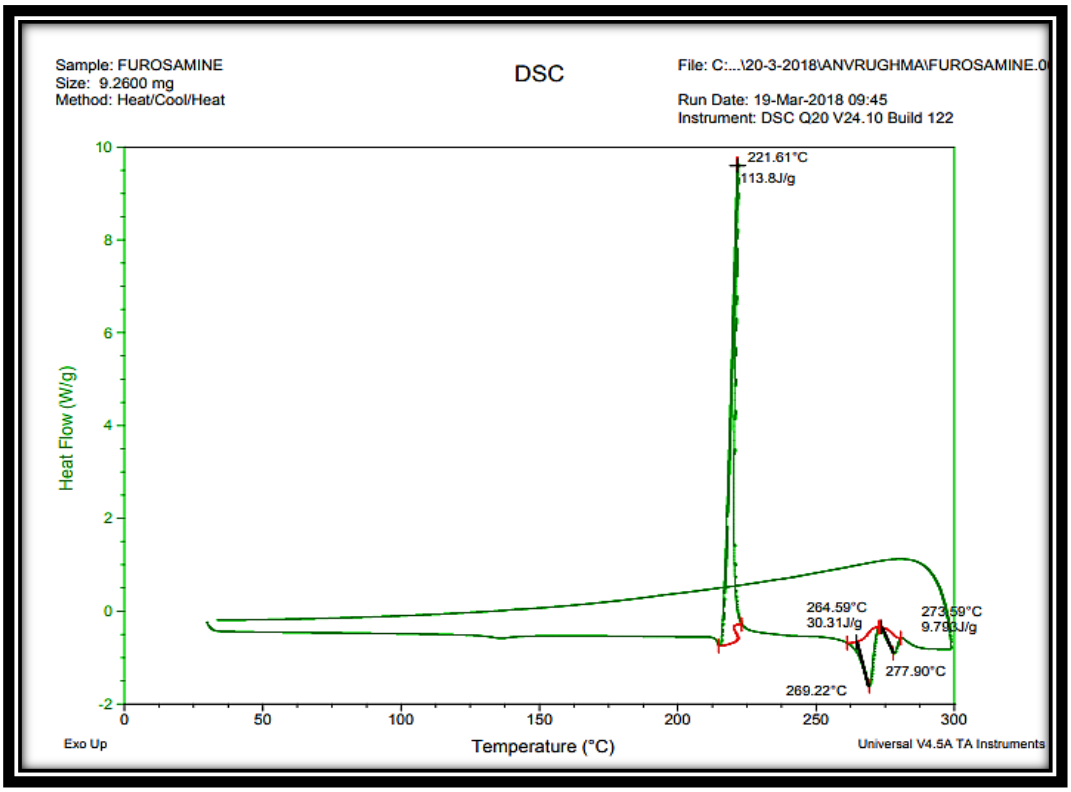
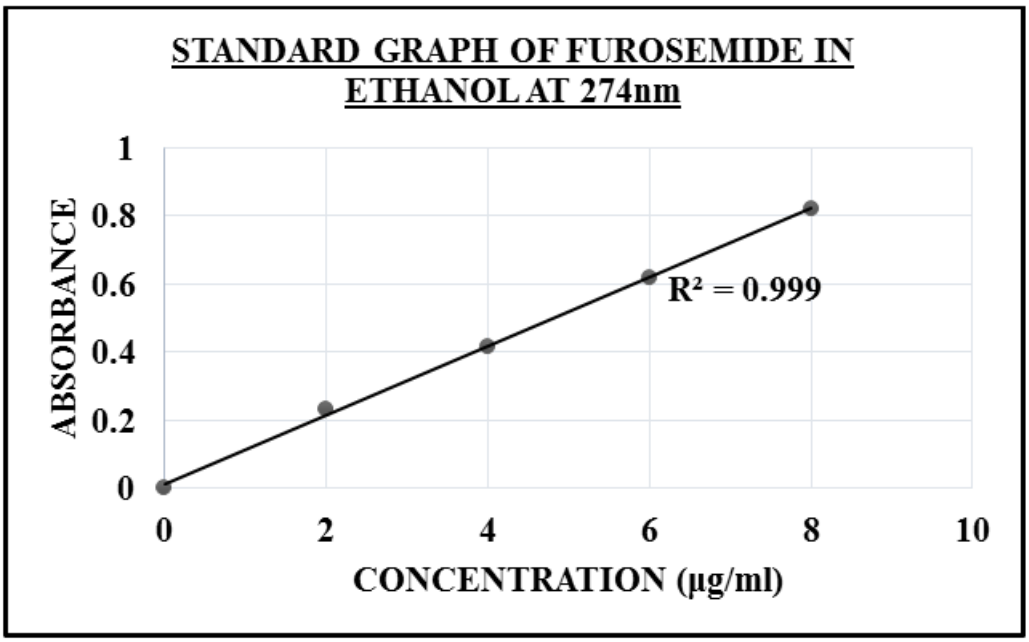


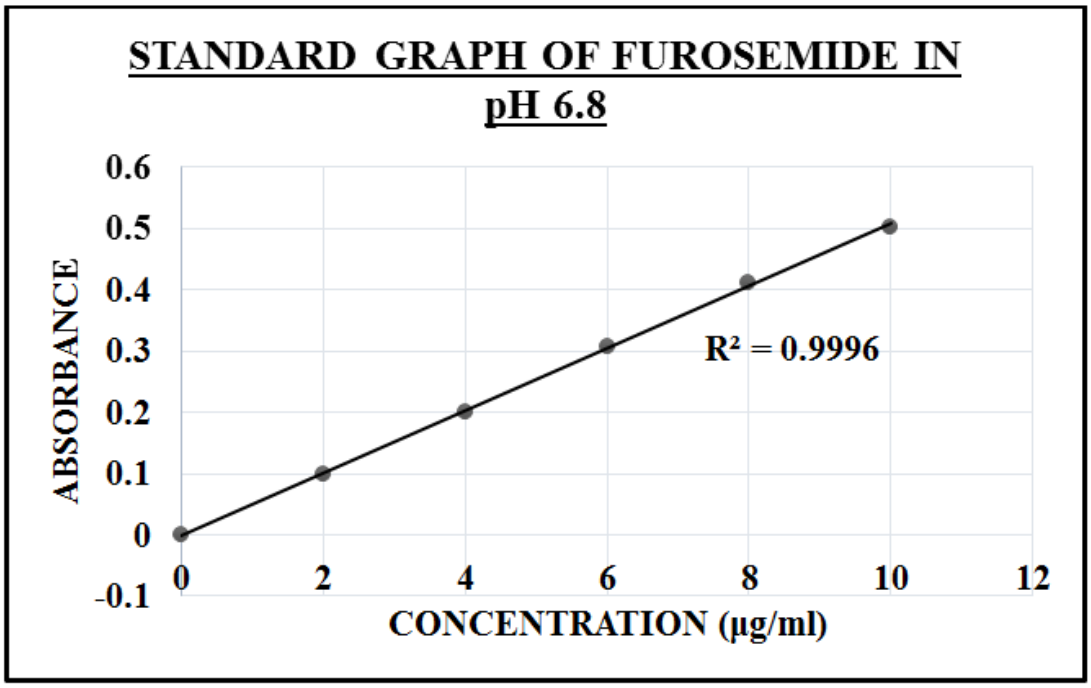
Fig-2: DSC thermogram of Furosemide

UV spectrometric assay of Furosemide

The λ_{max} of the Furosemide ethanolic solution (a) and in DMSO diluted with P^H 6.8 phosphate buffer (b) were found to be 274 nm and 279nm respectively. The calibration curves for Furosemide in ethanol (95%) and in DMSO diluted with P^H 6.8 phosphate buffer were shown in graph 1 & 2



Graph-1: Standard calibration graph of Furosemide in ethanol (95%)



Graph-2: Standard calibration graph of Furosemide in P^H 6.8 phosphate buffer

Selection of excipients

Solubility of drug substance is a key criterion for selection of components for developing lipid nanoparticles. Solubility studies were performed to identify suitable solid lipids, liquid lipids& surfactants that possess good solubilizing capacity for Furosemide.

Selection of solid lipid

To keep the drug in solubilization form, it is of prime importance that drug has higher solubility in solid lipid. The solubility of Furosemide was determined in various solid lipids and results were shown in table-3.

Table-3: Solubility studies of Furosemide in various Solid lipids

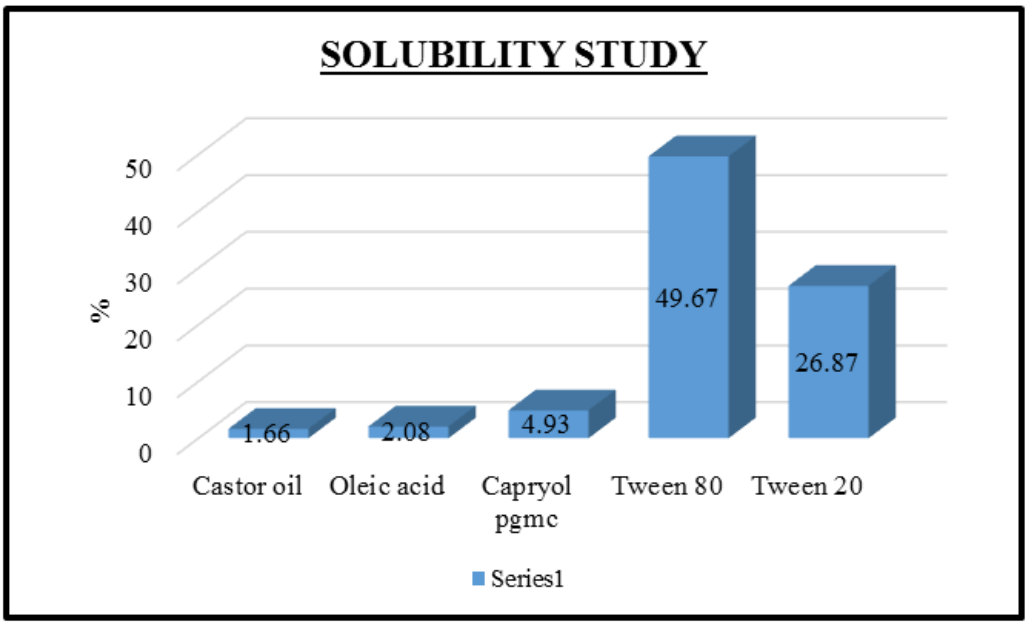
SOLID LIPIDS	MELTING POINT (^{0}C)	MISCIBILITY AND CLARITY
Stearic acid	69-70	Not clear
Cholesterol	147-150	Clear
Labrafil m 2130	35-40	Fairly visible

As compared with stearic acid and cholesterol, Furosemide was more soluble in Labrafil m 2130.

Determination of solubility in various liquid lipids and surfactants

According to the results of solubility studies in liquid lipids, Capryol pgmc exhibited the highest solubility of 4.93 mg/ml. Castor oil and Oleic acid showed the lower solubilities of 2.08 mg/ml and 1.66 mg/ml respectively (graph 3).

Surfactant reduces the interfacial tension between the lipid phase and the aqueous phase, therefore it was important to choose appropriate surfactant to obtain the desired size and the long-term physical stability of NLCs. Among 2 surfactants, the solubility of Furosemide in Tween 80 (49.67 mg/ml) was found to be higher than Tween20 (26.87 mg/ml).



Graph-3: Solubility of Furosemide in liquid lipids and in surfactants

Compatibility study

The FTIR spectrum of pure Furosemide and drug with different excipients used in formulation are shown in figure 3, 4 & 5 and interpreted in table-4.

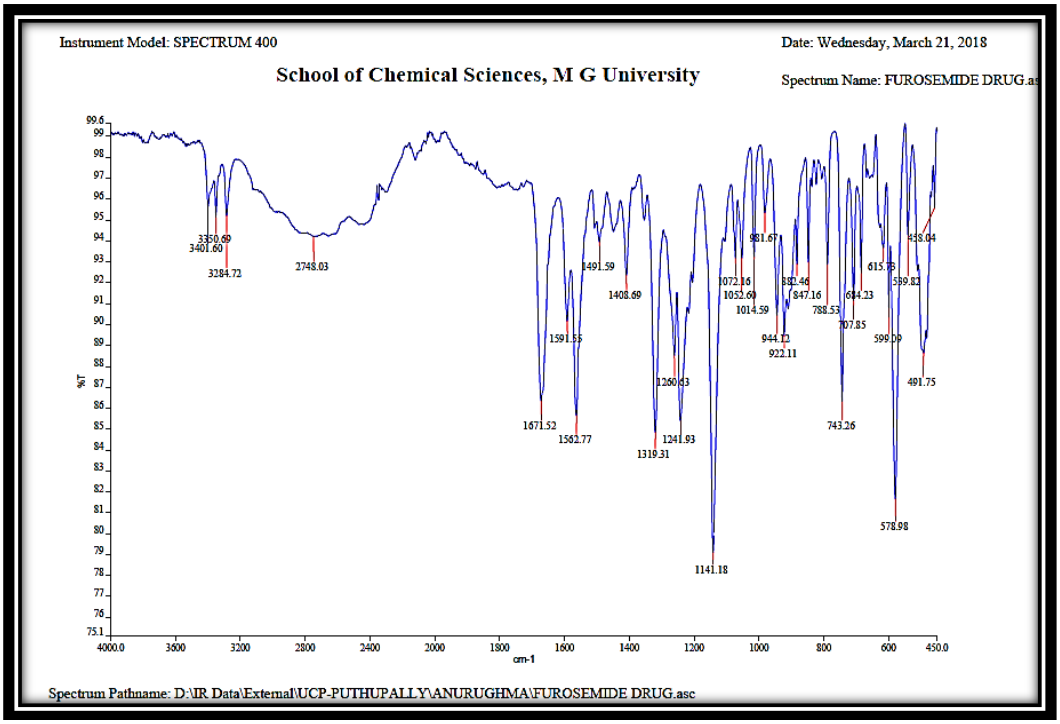


Fig-3: FTIR spectrum of Furosemide

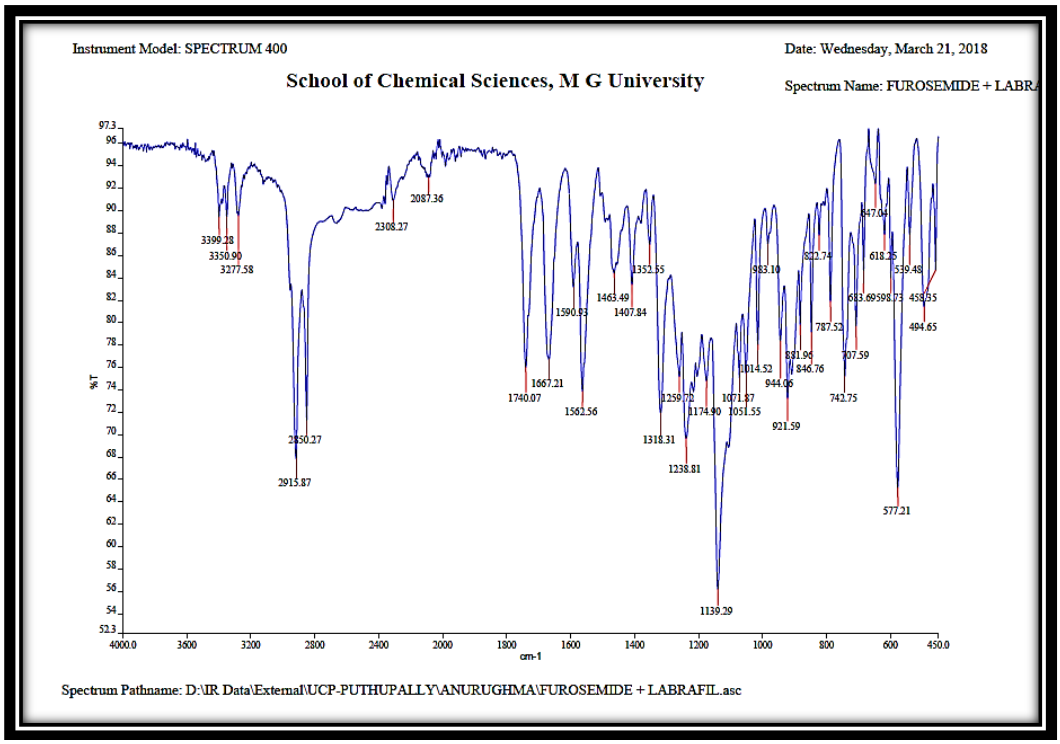


Fig-4: FTIR spectrum of Furosemide with Labrafil m 2130

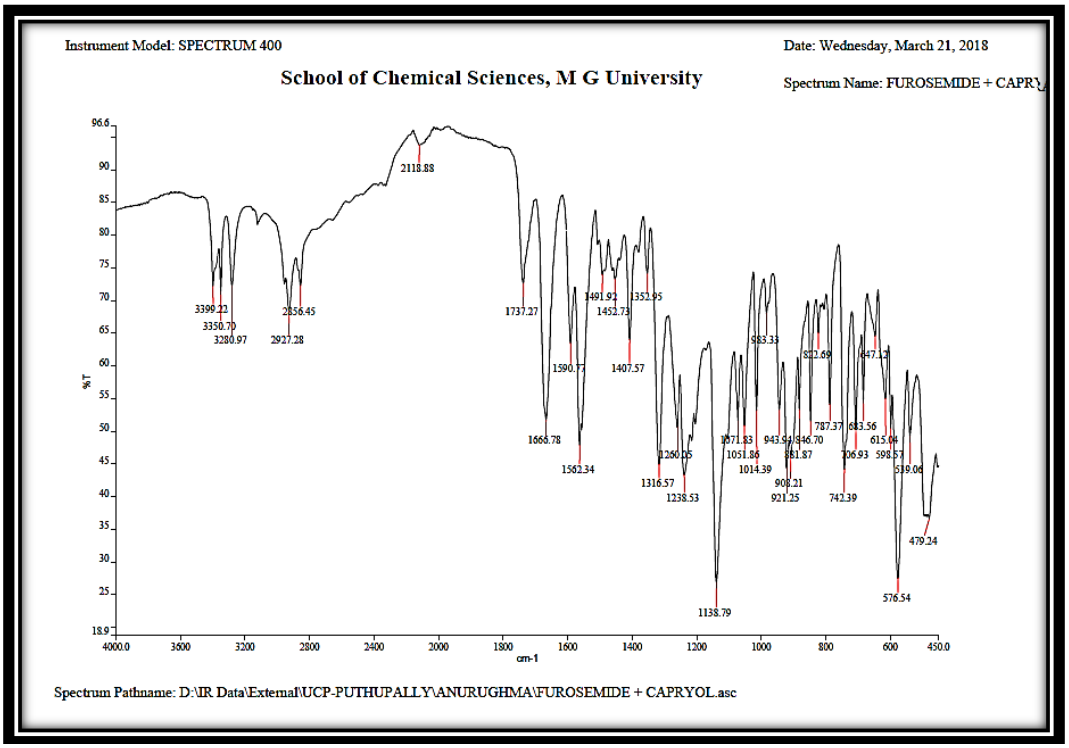


Fig-5: FTIR spectrum of Furosemide with Capryol PGMC

Table-4: Interpretation of FTIR spectrum of Furosemide with lipids

Functional group	Frequency range (cm^{-1})	OBSERVED PEAKS (cm^{-1})		
		Furosemide	Furosemide + Labrafil m 2130	Furosemide + Capryol pgmc
N-H Bending vibration	Near 1515	1562.77	1562.34	1562.56
O=S=O Stretching vibration	1390-1290	1319.31	1316.57	1316.31
N-H Stretching vibration (SO_2NH_2)	3390-3330	3350.69	3350.70	3350.70
C=O Stretching vibration	1600-1800	1671.52	1666.78	1666.78
C-O Stretching vibration	1320-1210	1241.93	1238.53	1238.81
O-H Bending vibration	1440-1395	1408.69	1407.57	1407.84
C-Cl Stretching vibration	850-550	578.98	577.21	576.54

The major peaks observed in drug spectrum were also observed in spectrum of physical mixture of drug and lipids, it indicate there was no incompatibility between drug and lipids.

Preparation of furosemide loaded nanostructured lipid carrier (NLC) and solid lipid nanoparticle (SLN)

The Nanostructured lipid carrier of Furosemide was prepared by solvent diffusion method using labrafil m 2130 as solid lipid, capryol pgmc as liquid lipid, soy-lecithin as co-surfactant and tween 80 as hydrophilic surfactant.

Characterization & comparison of optimized furosemide loaded NLC & SLN

Drug content

The drug content of Furosemide loaded NLC and Furosemide loaded SLN (as estimated by UV spectrophotometry at 279 nm in P^H 6.8 phosphate buffer) were found to be 83.56% & 84.55% respectively.

Entrapment efficiency (E_e) and drug loading capacity (L_C)

The entrapment efficiency and drug loading capacity of Furosemide loaded NLC (as estimated by UV spectrophotometry at 279 nm in P^H 6.8 phosphate buffer) was found to be 75.50% & 25.63% and that of Furosemide loaded SLN was found to be 71.07% & 24.62% respectively. From the results Furosemide loaded NLC formulation showed highest percentages of entrapment efficiency and drug loading capacity.

The entrapment is mainly due to the solubility of Furosemide in the lipids and the partition of Furosemide between the oil phase and the aqueous phase. The incorporation of liquid lipid into solid lipid could lead to a reduction of crystallinity and increase the imperfections in the crystal lattice which helps to accommodate the higher amount of Furosemide in NLC and results in increasing entrapment efficiency. Liquid lipid acts as a solubilizing agent for Furosemide at room temperature and provides the additional spaces for Furosemide to accommodate and prevents Furosemide from diffusing to the external phase, results in increasing drug loading.

Particle size and Polydispersity index (PDI)

Particle size distribution is one of the most important characteristics for the evaluation of the stability of colloidal systems. The average particle size of the Furosemide loaded NLC was estimated to be 99.24nm. The PDI gives information about the homogeneity of particle size distribution in the system. Polydispersity is measure of particle homogeneity and it varies from 0 to 1. A small value of PDI is indication of narrow size distribution in the system whereas large value indicates wide size distribution in the system. The PDI of formulation was found to be 0.302 which indicates that there is narrow particle size distribution and hence stable for longer duration of time (figure-6).

The average particle size of the Furosemide loaded SLN was estimated to be 193.4nm with a PDI of 0.835, indicating wide particle size distribution (figure-7).

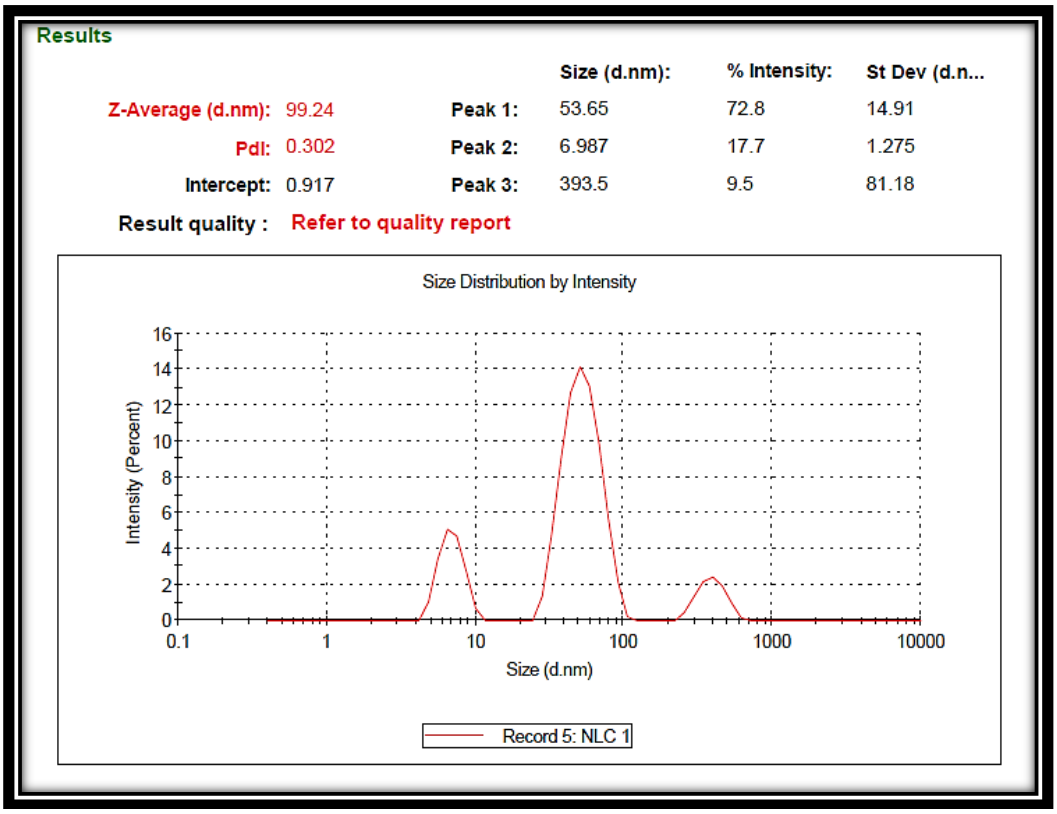


Fig-6: Particle size distribution by intensity of Furosemide NLC

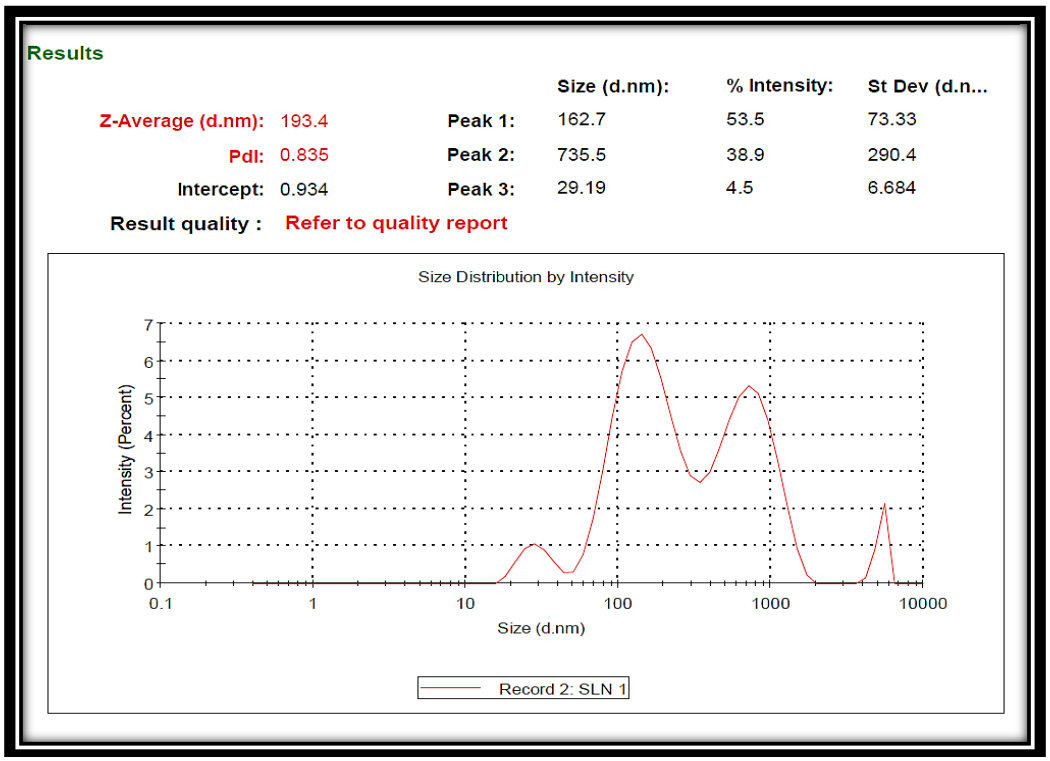


Fig-7: Particle size distribution by intensity of Furosemide SLN

The average particle size of Furosemide loaded NLC formulation is smaller than that of Furosemide SLN. The addition of liquid lipid was found to cause a decrease in particle size. As compared to the PDI values, it is found that SLN is more polydisperse than NLC.

Zeta potential

Zeta potential is the potential difference between the stationary layer of the dispersed particle and dispersion medium. It measures the surface charge of particles. As the zeta potential increases, the particle surface charge also increases. Zeta potential greatly influences particle stability in suspension through the electrostatic repulsion between particles. A zeta potential value of equal to or more than 30 mV is desirable.

The Furosemide NLC suspension had a zeta potential of -31.2 mV (figure-8) and that of Furosemide SLN is -36.1mV (figure-9). High negative charges of zeta potential indicate that the electrostatic repulsion between particles with the same electrical charge will prevent the aggregation of the particles and could stabilize particle suspensions. Thus, the values obtained for the NLC and SLN are adequate to form a stable nanoparticle suspension.

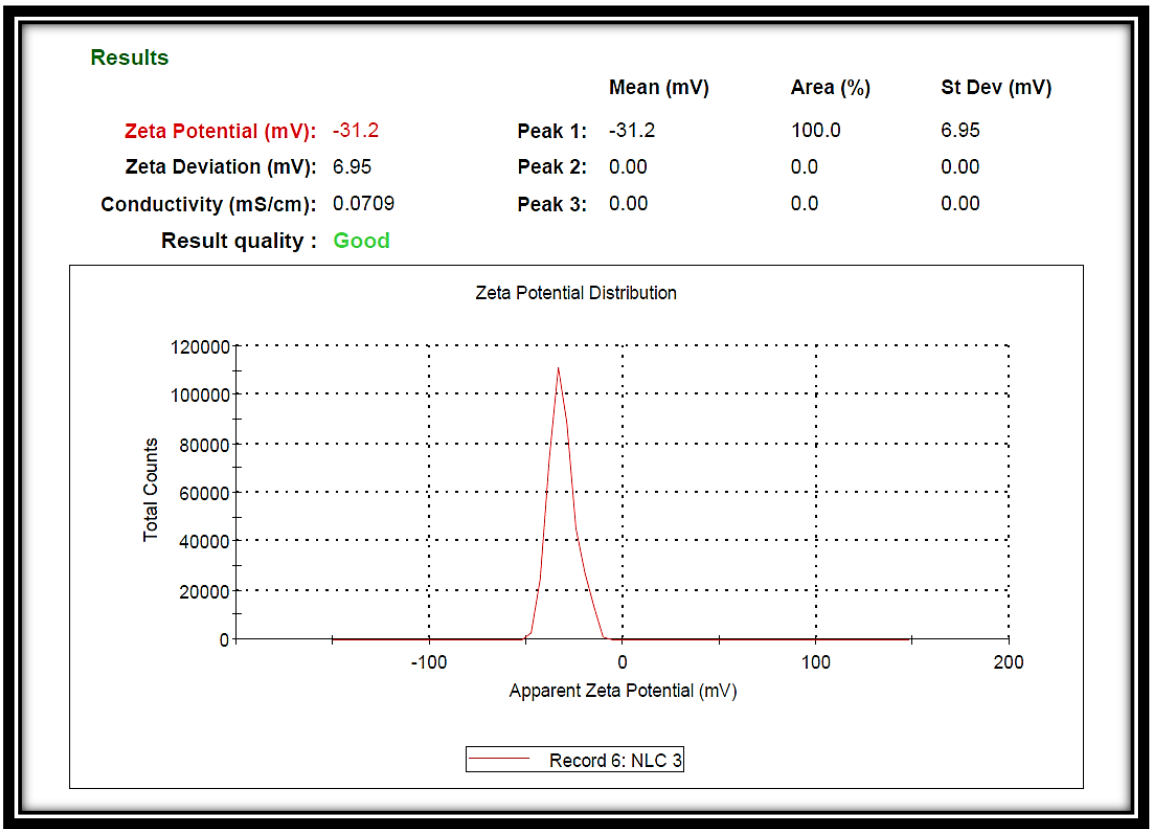


Fig-8: Zeta potential report of Furosemide NLC

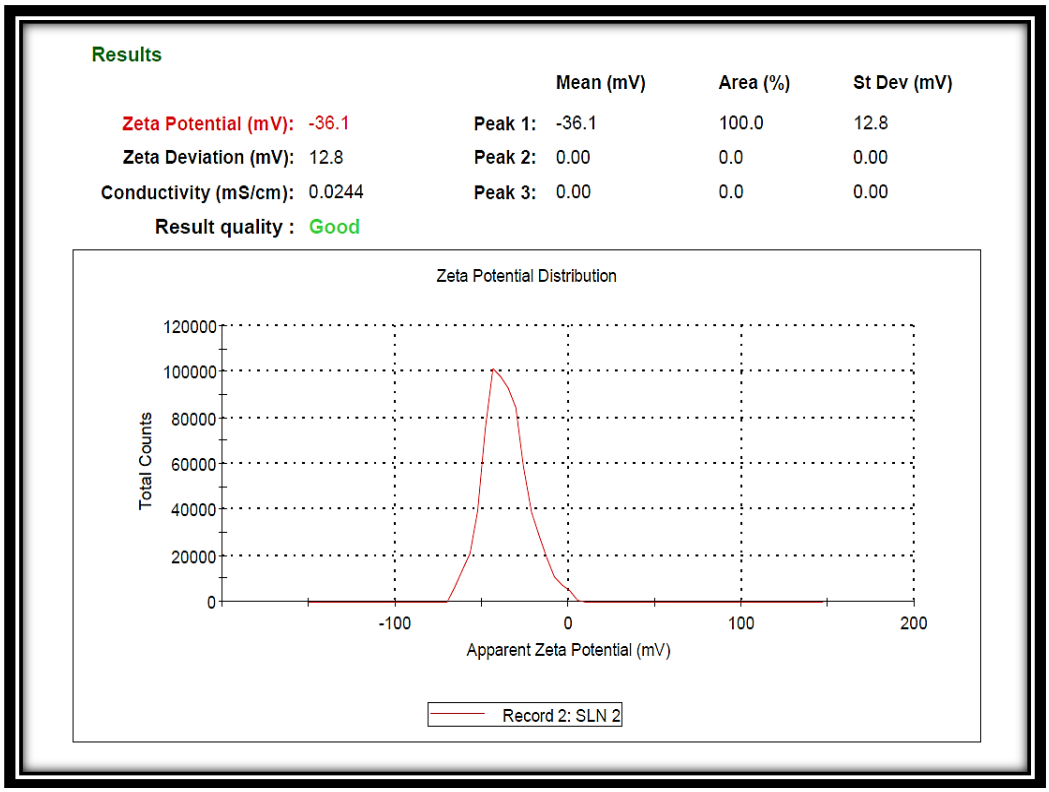


Fig-9: Zeta potential report of Furosemide SLN

Scanning Electron Microscopy (SEM)

The nanoparticulate nature of the NLC dispersion particles was further confirmed by SEM studies. Figure 10& 11shows the SEM images of Furosemide NLC & SLN. The particles are almost spherical in shape in the nanometer range with smooth surfaces and uniform distribution on a scale of 1µm which was in agreement with the size data determined by DLS. The results indicated that the particles were spherical and no drug crystal of particles visible in the figure. The picture shows agglomeration of particles due to the lipid nature of the carriers and sample preparation prior to SEM analysis. Some particle shapes deviating from sphericity might be due to the lipid modification during the drying process of sample treatment.

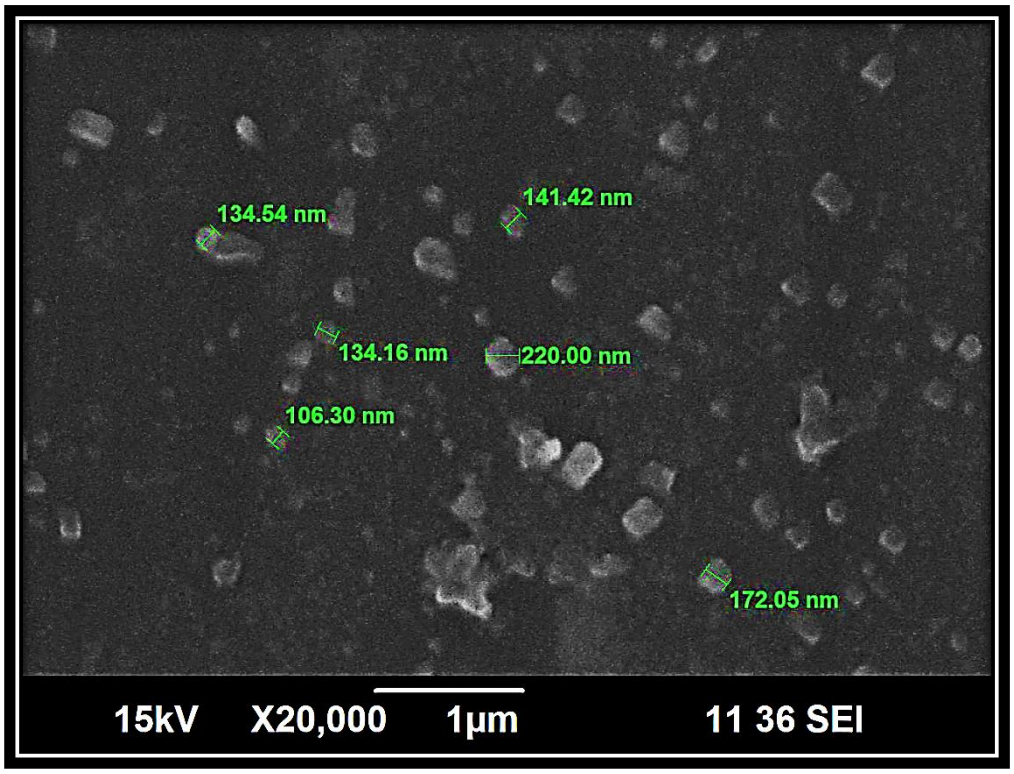


Fig-10: SEM image of Furosemide loaded of optimized NLC

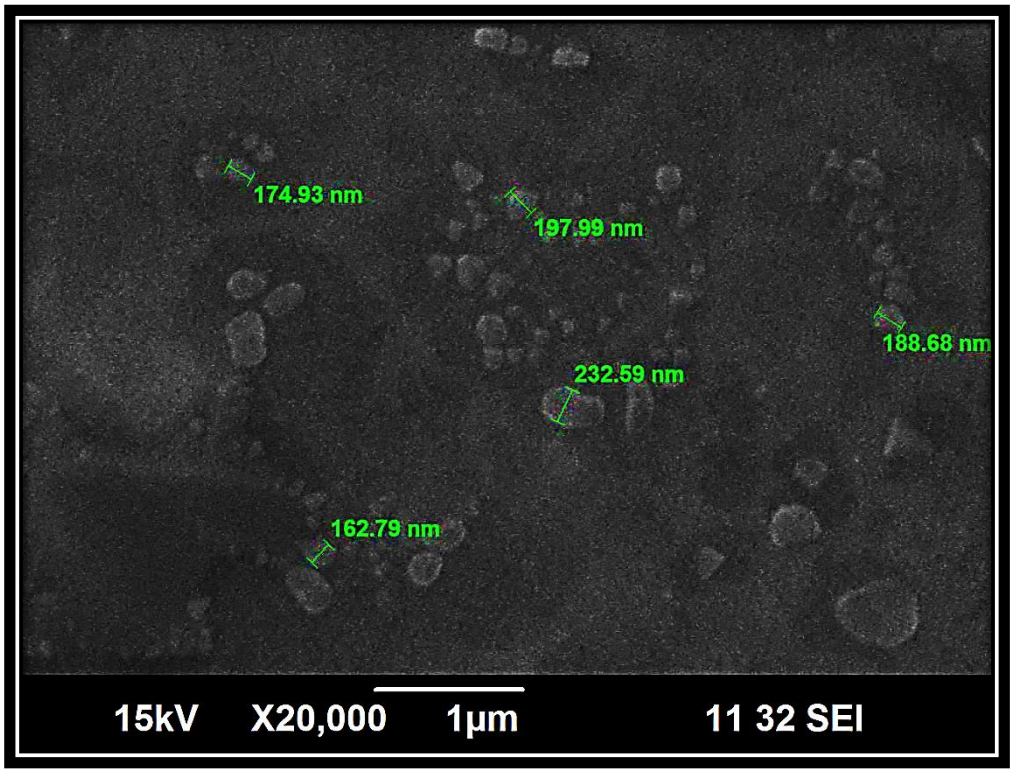


Fig-11: SEM image of Furosemide SLN

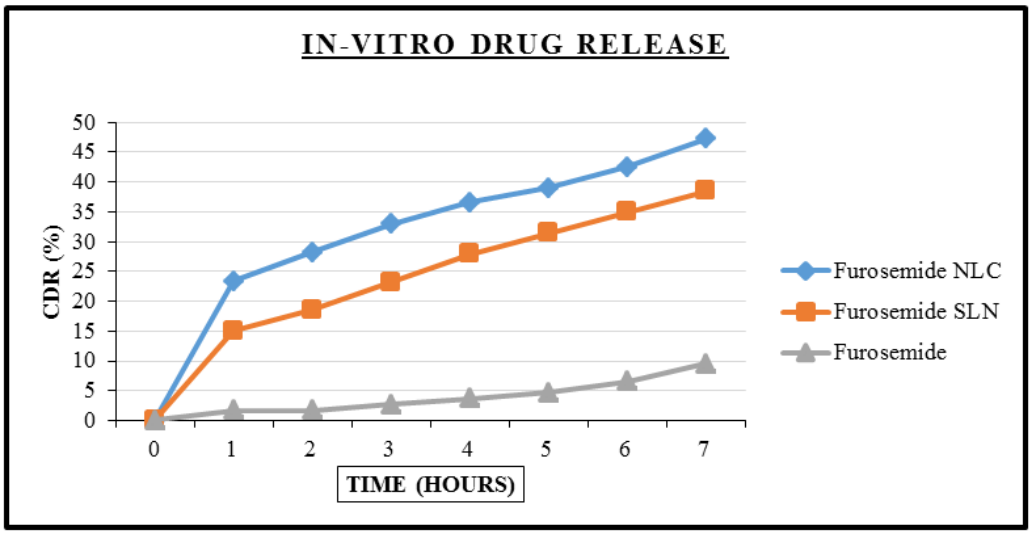
Literature survey revealed that if the mean particle size of lipidic nanoparticles (of both SLNs/NLCs) were below 200 nm, it would be transported via lymphatic transport system instead of portal vein thus avoiding the first pass metabolism. Moreover, small particles ranging between 120 – 200 nm rarely undergo blood clearance by the reticuloendothelial system i.e. liver and spleen filtrations are avoided. Thus, altogether, avoids first pass metabolism that

will in turn decrease the dose of Furosemide lipid particles in the formulation and attain higher plasma concentration through the lymphatic transport system.

In-vitro drug release study

In- vitro drug release studies of Furosemide drug, Furosemide loaded NLC, and furosemide loaded SLN were carried out by dialysis method using P^H 6.8 phosphate buffer as receptor medium. Studies were performed and the results were shown in the Graph-3.

The in-vitro release of both the NLC and SLN showed an interesting bi-phasic release with an initial burst effect. Afterwards, the drug release followed a steady pattern. In SLN, The initial burst release may be due to the desorption of drug associated with the surface of nanoparticles and the later stage was attributed to the fact that solubilized drug can only be released slowly from the lipid matrices due to dissolution and diffusion.



Graph-3: Percentage In-vitro drug release

When solvent diffusion method at a temperature higher than (5-10^0) the melting point of lipids was applied to produce NLC, liquid lipid was not homogenously distributed in nanoparticles matrix. During cooling down process from the melted lipid droplet in dispersed medium to the formation of a nanostructured lipid carrier at room temperature, because of the different melting point between solid lipid and liquid lipid, the solid lipid (labrafil m 2130) which owns higher melting point could crystallize first, forming a liquid lipid free or little lipid core. Finally, most of the liquid lipid (capryol pgmc) located in the outer layers of the nanoparticles forms drug-enriched casing which leads to burst release of the drug at the initial stage. The oil-enriched outer layers possess substantially higher solubility for lipophilic drug. Therefore, a higher amount of drug could be easily loaded, as well as released by the drug diffusion or the matrix erosion.

From the graph 3, NLC showed an increased drug release rate as compared to both SLN and pure drug.

Kinetics of drug release

The in vitro drug release data of NLC, SLN and pure drug were subjected to the drug release kinetics and release mechanism. The formulations were studied by fitting the drug release time profile with the various equations such as Zero order, First order, Higuchi and Korsmeyer pappas. Results are shown in the table-5.

Table-5: Kinetic release data

FORMULATION	ZERO ORDER	FIRST ORDER	HIGUCHI	KORSMEYER PEPPA'S	
	R^2	R^2	R^2	R^2	n
Furosemide NLC	0.8633	0.5716	0.9572	0.3352	0.7716
Furosemide SLN	0.9169	0.6355	0.9742	0.4029	0.7760
Furosemide	0.9701	0.8971	0.9383	0.6440	0.6560

From the table-5, it is clear that the drug release from NLC and SLN shows Higuchi matrix model with R^2 values of 0.9572 and 0.9742 respectively. Hence the drug release mechanism was assumed to be diffusion controlled for both the NLC and SLN. In the case of pure drug, the drug release follows Zero order kinetics (R^2=0.9701). When

analyzed according to Kosmeyer Peppas model, the release exponent for NLC, SLN and pure drug were found to be 0.7716, 0.7760 and 0.6560 respectively, indicating the release of drug follows non-fickian diffusion.

Stability of Furosemide loaded nanostructured lipid carrier

The stability of NLC and SLN formulations was ascertained by monitoring appearance, drug content, entrapment efficiency, drug loading capacity and in-vitro drug release after stored in room temperature in the dark over a period of 60 days. Results are shown in the table-6.

Table-6: Results of stability studies (60 days)

PARAMETERS	BEFORE STABILITY STUDY		AFTER STABILITY STUDY	
	SLN	NLC	SLN	NLC
Appearance	White colour with characteristic odour	White colour with characteristic odour	White colour with characteristic odour	White colour with characteristic odour
Drug content (mg/ml)	1.6910	1.6713	1.6615	1.6515
Entrapment efficiency (%)	71.07	75.50	69.59	74.51
Loading capacity (%)	24.49	25.63	24.11	25.38
In-vitro drug release (%)	38.49	47.26	36.78	46.63

From the above result it can be concluded that Furosemide NLC formulation is more stable than SLN Formulation.

CONCLUSION

Furosemide loaded NLC and SLN for oral administration were successfully prepared by solvent diffusion method using labrafil m 2130 as solid lipid, capryol pgmc as liquid lipid & tween 80 as surfactant. Drug-excipient interaction studies using FT-IR indicated the absence of any drug-excipient incompatibility between Furosemide and excipients. The particles formed in both the formulations were physically stable and in nanosize range. The PDI values revealed both the formulations were polydisperse, among them, NLCs were less polydisperse than SLNs. DSC study showed the crystalline nature of pure drug. The SEM study confirmed the conversion of crystalline drug to amorphous form, appeared as spherical particles with smooth surfaces. The NLC & SLN exhibited a biphasic release pattern with burst release at the initial stage and followed by sustained release fitted to Higuchi equation while the pure drug followed Zero order kinetics. The n value suggested fickian diffusion mechanism of drug released from NLC, SLN & pure drug formulations. As compared to Furosemide SLN, NLC had higher entrapment efficiency, drug loading capacity, in-vitro drug release, reduced the drug expulsion in storage as well as lower particle size and PDI. From these results, we can concluded NLC obtained in this study increases the bioavailability of Furosemide.

REFERENCES

1. Kumar, D. R., Mohammed, H., Mangalarapu, S., & Ramana, R. (2015). Formulation and evaluation of silica lipid hybrid microparticles containing furosemide, *International journal of pharmacy*.
2. IP, Volume 2, 2014, Page no.:1833
3. http://shodhganga.inflibnet.ac.in/bitstream/10603/176229/12/12_chapter%202.pdf
4. Belgamwar, V. S., D Dani, J. A. T. I. N., Tatiya, A. U., Kalaskar, M. G., & Patil, P. H. (2016). A novel anionic atorvastatin loaded nanostructured lipid carriers for oral delivery: formulation development, in vitro and in vivo pharmacodynamic study, Indian Journal of Novel Drug Delivery. *Indian Journal of Novel Drug Delivery*, *8*(4), 199-211.
5. https://link.springer.com/chapter/10.1007/978-3-319-41129-3_2
6. Chaudhary, H. M., Jadhav, K. R., & Kadam, V. J. (2016). Formulation and evaluation of nanostructured lipid carriers containing glipizide, *World journal of pharmacy and pharmaceutical sciences*, 5(4), 1424-1437.
7. https://fenix.tecnico.ulisboa.pt/downloadFile/844820067124759/ABSTRACTAnaCartaxo.pdf
8. Wu, M., Fan, Y., Lv, S., Xiao, B., Ye, M., & Zhu, X. (2016). Vincristine and temozolomide combined chemotherapy for the treatment of glioma: a comparison of solid lipid nanoparticles and nanostructured lipid carriers for dual drugs delivery. *Drug delivery*, *23*(8), 2720-2725.
9. Ekambaram, P., Sathali, A. A. H., & Priyanka, K. (2012). Solid lipid nanoparticles: a review. *Sci Rev Chem Commun*, *2*(1), 80-102.

10. Shah, B., Khunt, D., Bhatt, H., Misra, M., & Padh, H. (2015). Application of quality by design approach for intranasal delivery of rivastigmine loaded solid lipid nanoparticles: effect on formulation and characterization parameters. *European Journal of Pharmaceutical Sciences*, *78*, 54-66.
11. Joshi, M., & Patravale, V. (2008). Nanostructured lipid carrier (NLC) based gel of celecoxib. *International journal of pharmaceutics*, *346*(1-2), 124-132.
12. https://www.ingentaconnect.com/content/govi/pharmaz/2008/00000063/00000012/art00006?crawler=true
13. Shah, N. V., Seth, A. K., Balaraman, R., Aundhia, C. J., Maheshwari, R. A., & Parmar, G. R. (2016). Nanostructured lipid carriers for oral bioavailability enhancement of raloxifene: design and in vivo study. *Journal of advanced research*, *7*(3), 423-434.