Indian Journal of Novel Drug Delivery

IJNDD

An Official Publication of Karnataka Education and Scientific Society

Research Article

Development, Optimization and Characterization of Nanostructured Lipid Carriers for Potent Oral Delivery of Furosemide

ANURUGHMA S, NEEMA GEORGE*

Regional Institute of Medical Science and Research Centre for Professional and Advanced studies, Puthuppally, Kottayam, Kerala.

ARTICLE DETAILS

Article history:
Received on 1 November 2018
Modified on 18 December 2018
Accepted on 27 December 2018

Keywords:
Furosemide,
Solvent Diffusion Method,
Nanostructured Lipid Carrier,
Labrafil M 2130,
Capryol PGMC,
2^3 Full Factorial Design,
Entrapment Efficiency,
In Vitro Drug Release.

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). The Furosemide loaded NLC was prepared by solvent diffusion method using labrafil m 2130 as solid lipid, capryol pgmc as liquid lipid and tween 80 as surfactant. The prepared formulations were optimized by 2^3 full factorial design using total lipid: drug ratio, solid lipid: liquid lipid ratio and surfactant concentration (%) as independent variables and %entrapment efficiency and %in-vitro drug release as dependent variables. The optimized Furosemide loaded NLC formulation was evaluated for drug content, entrapment efficiency, drug loading capacity, particle size, PDI , zeta potential, morphology, storage stability, in vitro drug release and mechanism of drug release. Drug content, entrapment efficiency, drug loading capacity, average particle size, PDI and zeta potential of Furosemide NLC were found to be 83.56%, 75.50%, 25.63%, 99.24nm, 0.302 and -31.2mV 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, optimized NLC formulation showed a fast initial release followed by a sustained release, best fitted to Higuchi equation. Pure drug followed Zero order release kinetics. The results obtained showed potential of NLCs for significant improvement in oral bioavailability of poorly soluble Furosemide.

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].

Author for Correspondence:
Email: neemas115@gmail.com

Furosemide is 4-chloro-N-furfuryl-5-sulfamoylanthranilic acid (Fig. 1), it is a white or almost white crystalline powder [2].

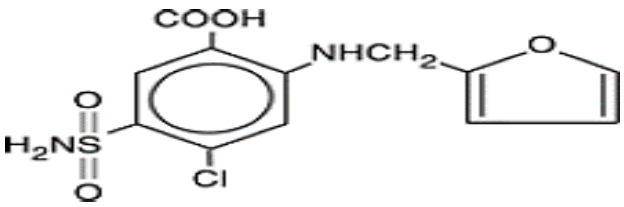


Figure 1: Chemical structural of Furosemide [4-chloro-N-furfuryl-5-sulfamoylanthranilic acid]

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].

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].

Lipid nanoparticles with solid particle matrix are derived from o/w emulsions by simply replacing liquid lipid (oil) by a solid lipid, i.e. being solid at body temperature. They are categorized as first generation nanoparticles: Solid lipid nanoparticles and Second generation nanoparticles: Nanostructured lipid carriers (NLCs) [9, 10].

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, optimized using 2^3 full factorial design and characterized to study feasibility of NLC as a novel carrier system for oral delivery of Furosemide.

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 pH 1.2 acid buffer, pH 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 pH 6.8 phosphate buffer were scanned spectrophotometrically over a range of 200-400 nm to determine the wavelength of maximum absorption (λ_{max}).

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 & capryolpgmc 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 and capryol pgmc were taken.

Design of the Experiment

In this study, a 2^3 full-factorial design was used to optimize NLCs. In order to optimize, total lipid: drug ratio (X1), solid lipid: liquid lipid ratio (X2) and % concentration of surfactant (X3) were selected as independent variables [14]. Each factor was set at a high level and low level (Table 1). The actual values and coded values of different variables are given in Table 2 Eight formulations of Furosemide NLCs (F1–F8) were prepared according to the factorial design. The entrapment efficiency (%) and % *in-vitro* drug release at 7 hours were taken as response parameters. The statistical analysis of responses was made by Design expert® version 11 statistical software trial package.

Table 1: Selection of range of critical parameters for optimization [15]

FACTORS	LEVELS	
	-1	+1
X_1: Total lipid: Drug	1	3
X_2: Solid lipid: Liquid lipid	1.5	4
X_3: Surfactant concentration (%)	0.5	1.5

Preparation of Furosemide Loaded Nanostructured Lipid Carriers (NLC) [8]

NLCs were prepared by the solvent diffusion method. The lipid dispersion was composed specified amount of labrafil m 2130 and capryol pgmc as given in Table 2, where lipids were melted at a temperature 5-10° above its melting point of solid lipid. 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 45-50^{0}C to form the lipid phase. Aqueous phase was prepared by dissolving specified amount of tween 80 as given in the Table 2, in water. This aqueous solution was then stirred and heated to 45-50°C. The lipid phase was slowly added drop wise 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.

Table 2: 2^3 full factorial design of Furosemide loaded NLCs

Runs	Levels	Formulation code	X_1	X_2	X_3	Entrapment efficiency (%)	Drug loading capacity[a] (%)	Drug content[a] (%)	*In-vitro* drug release (%)
1	(+1,+1,+1)	F_1	0.6g:0.2g	0.48g:0.12g	1.5	79.44	20.93	87.01	43.12
2	(+1,+1,-1)	F_2	0.6g:0.2g	0.48g:0.12g	0.5	77.47	20.52	86.02	30.90
3	(+1,-1,+1)	F_3	0.6g:0.2g	0.36g:0.24g	1.5	82.88	21.64	84.55	51.41
4	(+1,-1,-1)	F_4	0.6g:0.2g	0.36g:0.24g	0.5	77.96	20.62	88.98	33.23
5	(-1,+1,+1)	F_5	0.2g:0.2g	0.16g:0.04g	1.5	67.62	40.34	86.96	58.26
6	(-1,+1,-1)	F_6	0.2g:0.2g	0.16g:0.04g	0.5	63.19	38.72	86.02	35.51
7	(-1,-1,+1)	F_7	0.2g:0.2g	0.12g:0.08g	1.5	70.58	41.37	88.98	59.95
8	(-1,-1,-1)	F_8	0.2g:0.2g	0.12g:0.08g	0.5	65.65	39.63	83.07	40.37

[a]Not selected for the optimization, Dose of Furosemide: 2mg/ml

The resultant suspensions were cooled and stored in room temperature. The drug free NLC dispersion was prepared exactly the same manner where drug was excluded.

Characterization of NLC

Entrapment Efficiency and Drug Loading

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 pH 6.8 phosphate buffer.

Entrapment efficiency was calculated using following equation.

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

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

Where,

W_i = weight of drug added initially

W_s = weight of drug in supernatant

W_l = weight of lipid mixture added

Drug Content

1 ml of Furosemide NLC suspension was transferred to 10 ml standard flask. Few drops of DMSO was added, mixed well and made up the volume with pH 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 by UV spectrophotometer at 279nm.

In-Vitro Drug Release Study

In-vitro drug release studies 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 was added to the dialysis bag separately. The cylinder was attached to a stand and suspended in 100 ml of receptor medium (pH 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 was then determined by UV-visible spectrophotometer at 279 nm after suitable dilution with pH 6.8 phosphate buffer.

Statistical Analysis of Responses by Design Expert

Design Expert 11 software was used for the analysis of effect of each variable on the designated response. Quantitative and qualitative contribution of each variable on each of the response was analyzed. The significant polynomial equations generated by Design Expert were used to validate the statistical design. Response surface plots were generated to visualize simultaneous effect of each variable on each response parameter.

Selection of Optimized Formulation

For the selection of optimized formulation, Design Expert 11 software was utilized. It is an advantageous tool, which varies each variable simultaneously and gives all possible optimum selections based on which the optimized formulation is selected.

Characterization of the Optimized Formulation

Entrapment Efficiency (%), drug loading capacity (%), drug content (%) were performed on selected optimized formulation. Additional investigations including particle size, polydispersity index (PDI), scanning electron microscopy (SEM), pharmacokinetics, storage stability study were also performed.

Particle Size and Polydispersity Index (PDI)

Mean particle size (Z-average*)* and polydispersity index (PDI) of the prepared Furosemide loaded optimized NLC sample was measured using Malvern Zeta sizer 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 sample was 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 25°C. 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 Zeta sizer version 7.01. Zeta potential measurements were carried out using zeta dip cell, by applying field strength of 20V/cm at 25 °C after appropriate dilution of optimized sample with double distilled water. All the measurements were done in triplicate**.**

Scanning Electron Microscopy (SEM)

The SEM analysis of the optimized NLC sample was 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. Sample was prepared by placing a small drop of optimized formulation 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, optimized Furosemide loaded NLC 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 (pH 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 sample 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 amounts of Furosemide released from the samples were then determined by UV-visible spectrophotometer at 279 nm after suitable dilution with pH 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 optimized NLC formulations were stored in room temperature in the dark over a period of 60 days. Stability of the optimized formulation 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, odourless powder, the solubility of the received sample of Furosemide was examined in various solvents & buffer solutions. The results observed were shown in Table 3 & 4.

Table 3: 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 4: Solubility of Furosemide in various buffer solutions

BUFFER SOLUTIONS	SOLUBILITY
PH 1.2 acid buffer	Insoluble
PH 5.8 phosphate buffer	Slightly soluble
PH 6.8 phosphate buffer	Freely soluble

The decomposition point of the drug by capillary fusion method and by DSC were found to be 220°C and 221.61°C respectively, equivalent with the monograph value. The DSC thermogram of Furosemide is illustrated in Fig. 2.

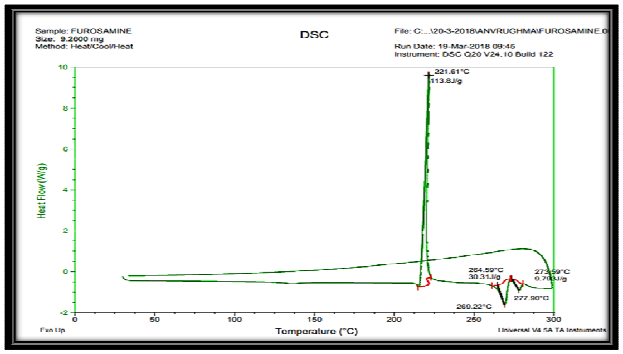
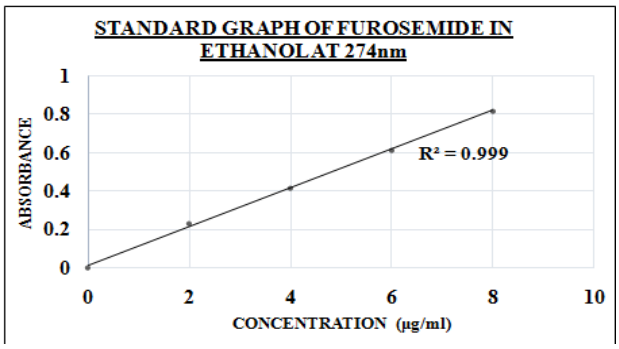


Figure 2: DSC thermogram of Furosemide

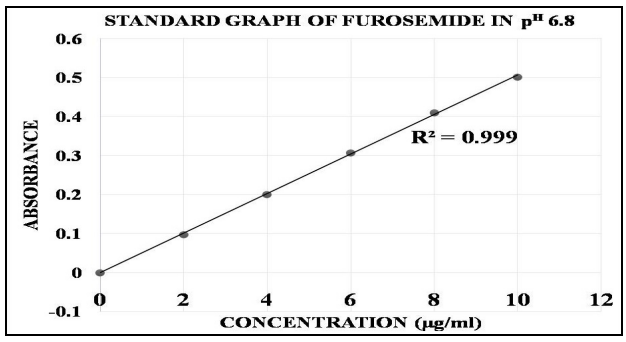
From the DSC thermogram, Furosemide shows a characteristic, sharp exothermic peak at 221.61°C with a heat enthalpy of 113.8J/g, which usually associated with the decomposition of the drug and indicate the crystalline nature of the drug. The degradation product of Furosemide displays an endothermic peak at 269.22°C as is evident from the Fig. 2.

UV Spectrometric Assay of Furosemide

The λ_{max} of the Furosemide ethanolic solution (a) and in DMSO diluted with pH 6.8 phosphate buffer (b) were found to be 274 nm and 279nm respectively. Calibration curves for Furosemide in ethanol (95%) and in DMSO diluted with pH 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 pH 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 5.

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

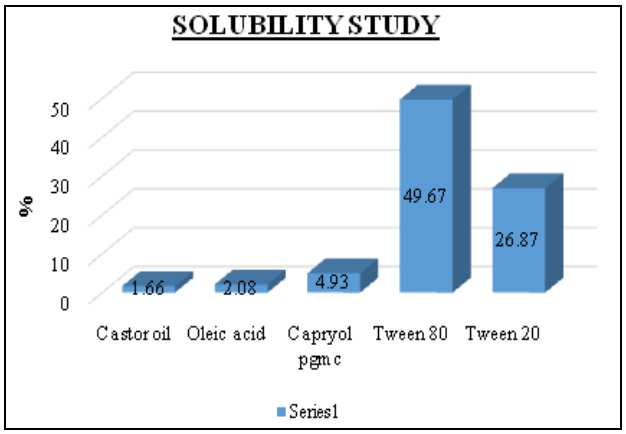
Table 5: Solubility studies of Furosemide in various Solid lipids [16-18]

Solid lipids	Melting point (^{0}c)	Miscibility and clarity
Stearic acid	69-70	Not clear
Cholesterol	148.5	Clear
Labrafil m 2130	35-40	Fairly visible

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 two 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 Fig. 3, 4 and 5, and interpreted in Table 6.

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

Preparation of Furosemide Loaded Nanostructured Lipid Carrier (NLC)

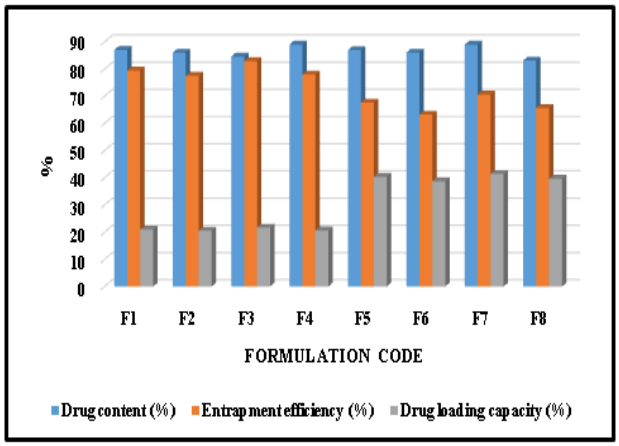
The Nanostructured lipid carrier of Furosemide was prepared by solvent diffusion method using labrafil m 2130 as solid lipid, capryol pgmc as liquid lipid, soy-lecithin as co-surfactant and tween 80 as hydrophilic surfactant.

Analysis of Dependent Variables

Drug Content, Entrapment Efficiency and Drug Loading Capacity

The drug content of Furosemide loaded NLC (as estimated by UV spectrophotometry at 279 nm in pH 6.8 phosphate buffer) was found to be in the range 83.07% to 89.96% (Graph 4). Formulations F5 showed the highest percentage of drug loading that is 89.96%, while the F8 showed the lower drug content of 83.07%.

The entrapment efficiency of Furosemide loaded NLC (as estimated by UV spectrophotometer at 279 nm in pH 6.8 phosphate buffer) was found to be in the range 63.19% to 82.88% (Graph 4). Formulation F3 showed the highest percentage of entrapment efficiency that is 82.88%, while the F6 showed the lower entrapment efficiency of 63.19%.



Graph 4: Entrapment efficiency (%) and drug loading capacity of formulations (F1-F8)

The entrapment is mainly due to the solubility of Furosemide in the solid and liquid lipids and the partition of Furosemide between the oil phase and the aqueous phase. Furosemide is a lipophilic compound, therefore, higher Furosemide loading could be achieved at a high total lipid: drug ratio (X1) and low solid lipid: liquid lipid ratio (X2) which decreases the Furosemide partition in the outer space and leads to higher entrapment efficiency. 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 and results in increasing entrapment efficiency [19].

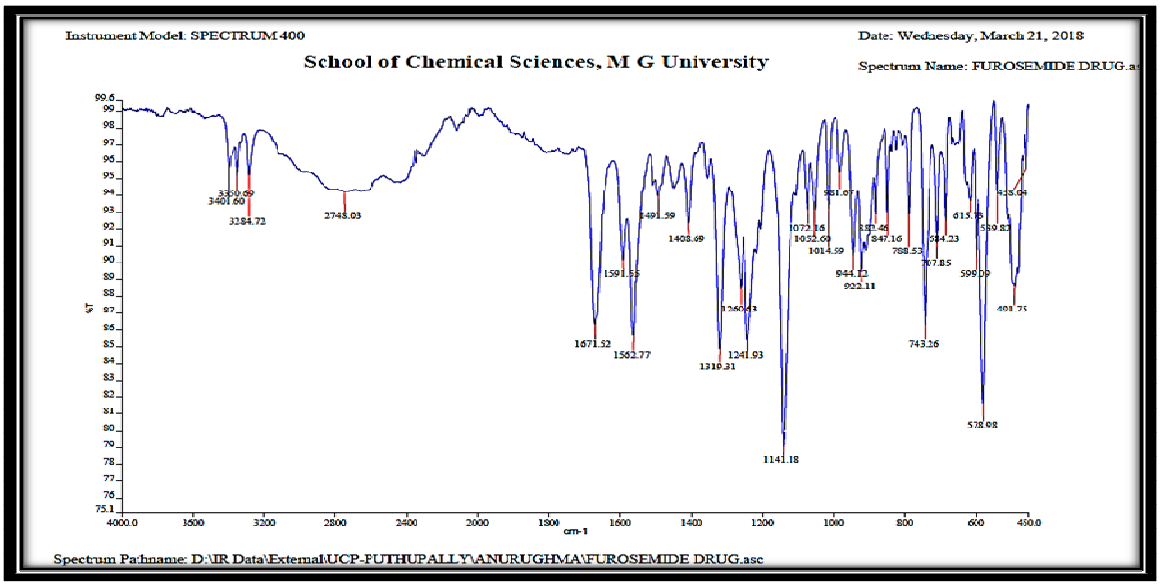


Figure 3: FTIR spectrum of Furosemide

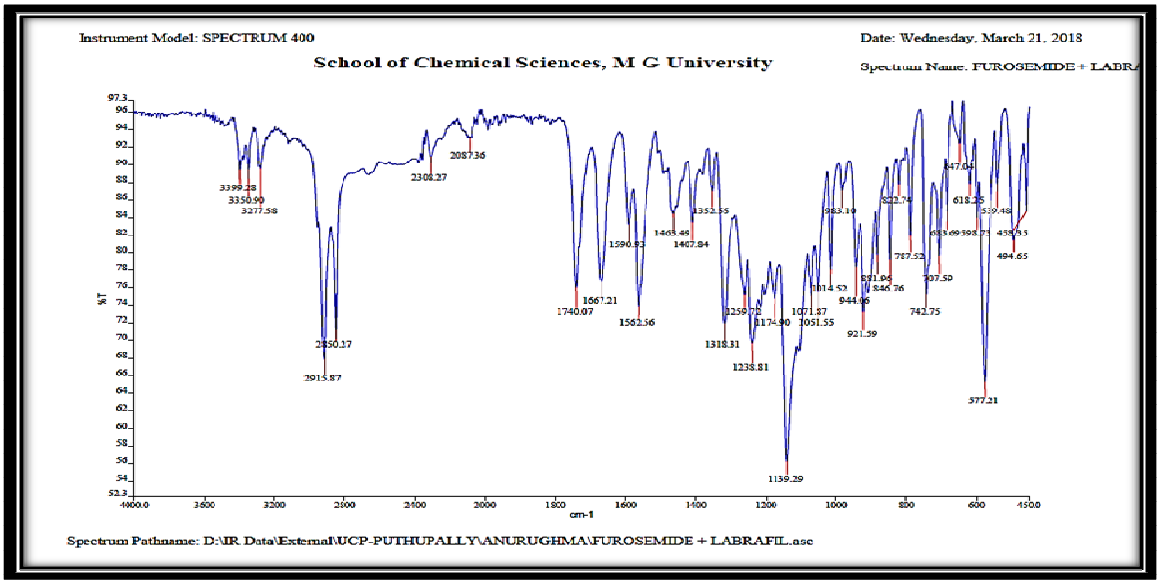


Figure 4: FTIR spectrum of Furosemide with Labrafil m 2130

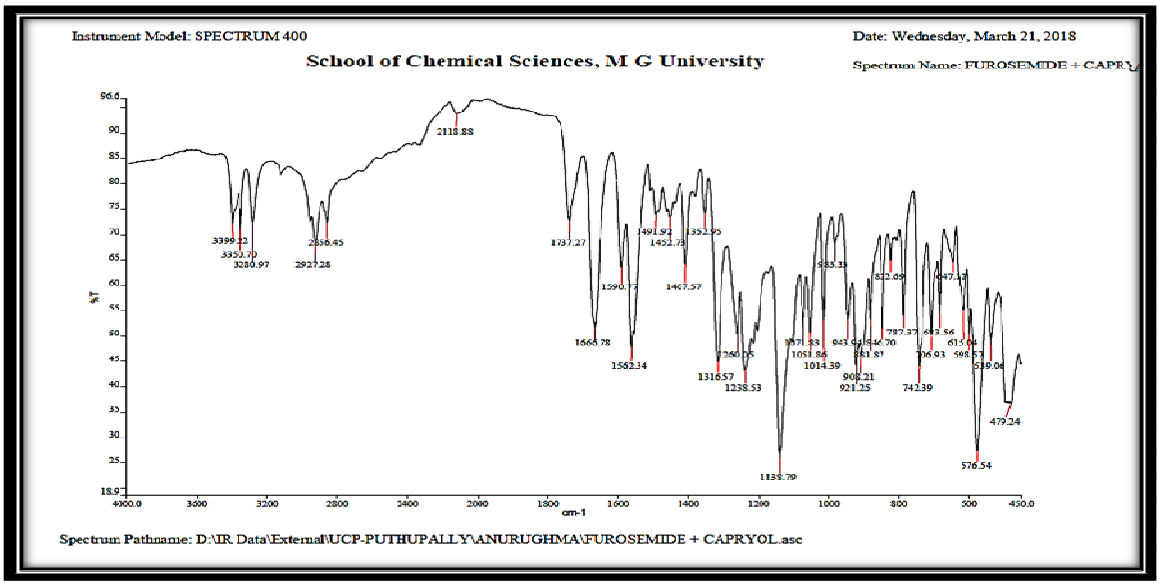


Figure 5: FTIR spectrum of Furosemide with Capryol PGMC

Table 6: Interpretation of FTIR spectrum of Furosemide with lipids

Functional group	Frequency range (cm^{-1})	Observed peaks (cm^{-1})		
		Furosemide	Furosemide + Labrafil m 2130	Furosemide + Capryolpgmc
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

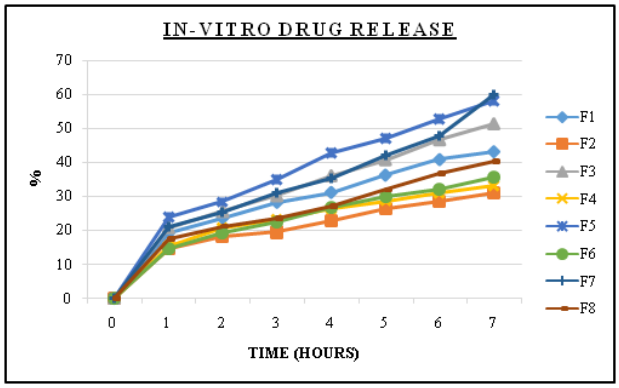
Entrapment efficiency increases when surfactant concentration (X3) increases. The positive relationship of entrapment efficiency with the surfactant concentration can be attributed to the ability of the surfactant system to increase the viscosity of aqueous phase with increasing concentration thereby decreasing the diffusion speed of Furosemide and increasing the entrapment efficiency. The positive effect of surfactant concentration on entrapment efficiency could also be explained by the increased surface area when smaller particles are formed, where Furosemide molecules were adhered or attached. This also could be due to the availability of adequate surfactant which facilitated Furosemide to remain within the lipid particles and/or on the surface of the particles results in high entrapment efficiency [19].

The Drug loading capacity of furosemide loaded NLC (as estimated by UV spectrophotometry at 279 nm in pH 6.8 phosphate buffer) was found to be in the range of 20.52% to 41.37% (Graph4). Formulation F7 showed the highest percent of loading capacity of 41.37% i.e. 1mg NLC contains 0.41mg Furosemide, while the F2 showed lower loading capacity of and 20.52%. A high drug loading is a characteristic for the less stable crystal modification.

There is an inverse proportion between drug loading and total lipid: drug ratio. While an increase in total lipid: drug ratio occurs, a decrease of drug loading as a result of limited capacity of lipid layer takes place. Drug loading increases when solid lipid to liquid lipid ratio decreases. 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 [19].

In-Vitro Drug Release

In- vitro drug release studies were carried out by dialysis method using pH 6.8 phosphate buffer as receptor medium. The cumulative % of drug released plotted against time to obtain drug release profiles as shown in Graph 5. The formulation F5 & F7 showed highest drug release as compared to the other formulations i.e. 59.95% and 58.26% respectively.



Graph 5: Percentage *In-vitro* drug release

NLC exhibit a biphasic drug release pattern that is, initial burst release of drug followed by a sustained release at a constant rate. One possible explanation for special release behaviour of resulted NLC in this study was that liquid lipid was not homogenously distributed in nanoparticles, which formed by employing of solvent diffusion method. In solvent diffusion method, lipids melted at a temperature higher than (5-10°C above) its melting point was applied to prepare NLC and dispersed in aqueous phase. 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 [20].

Validation of Experimental Design

The selected independent variables like the total lipid: drug, solid lipid: liquid lipid and surfactant concentration influenced the entrapment efficiency (%) and % *in-vitro* drug release that is quite evident from the results in Table 2 and has been documented in preceding sections. Consequently, for each dependent variable, the response polynomial coefficients were determined in order to evaluate the effect of each response.

The polynomial equation generated from the experimental design is given below

Entrapment efficiency (%) = + 73.10 + 6.34*A – 1.17*B + 2.03*C – 0.3063*ABC

***In-vitro* drug release (%) =** + 44.09 – 4.43*A – 2.15*B + 9.09*C – 1.14*ABC

Where A is Total lipid: Drug, B is Solid lipid: Liquid lipid and C is Surfactant concentration.

Three dimensional surface plots for the obtained responses were drawn based on the model polynomial functions (Fig. 6 and 7). On carefully observing these plots, the qualitative and interactive effect(s) of independent variables on each response parameter can be visualized.

Selection of Optimized Formulation

Based on the statistical evaluations the software suggested a good number of combinations of which we selected one as optimum batch. The formula selected for the optimum batch is given below (Table 7).

Table 7: Formula for optimum batch based on statistical evaluations

Total lipid: Drug	1.7773
Solid lipid: Lipid liquid	3.2981
Surfactant concentration (%)	1.3444
Entrapment efficiency (%)	72.59
***In-vitro* drug release**	50.47
Desirability	1

Characterization of the Optimized Formulation

Entrapment Efficiency and Drug Loading Capacity

The entrapment efficiency and drug loading capacity of optimized Furosemide loaded NLC (as estimated by UV spectrophotometry at 279 nm in pH 6.8 phosphate buffer) were found to be 75.50% & 25.63%.

Drug Content

The drug content of optimized Furosemide loaded NLC (as estimated by UV spectrophotometry at 279 nm in pH 6.8 phosphate buffer) was found to be 83.56%.

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 (Fig. 8).

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 optimized Furosemide NLC formulation had a zeta potential of -31.2 mV (Fig. 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 value obtained for the NLC is adequate to form a stable nanoparticle suspension.

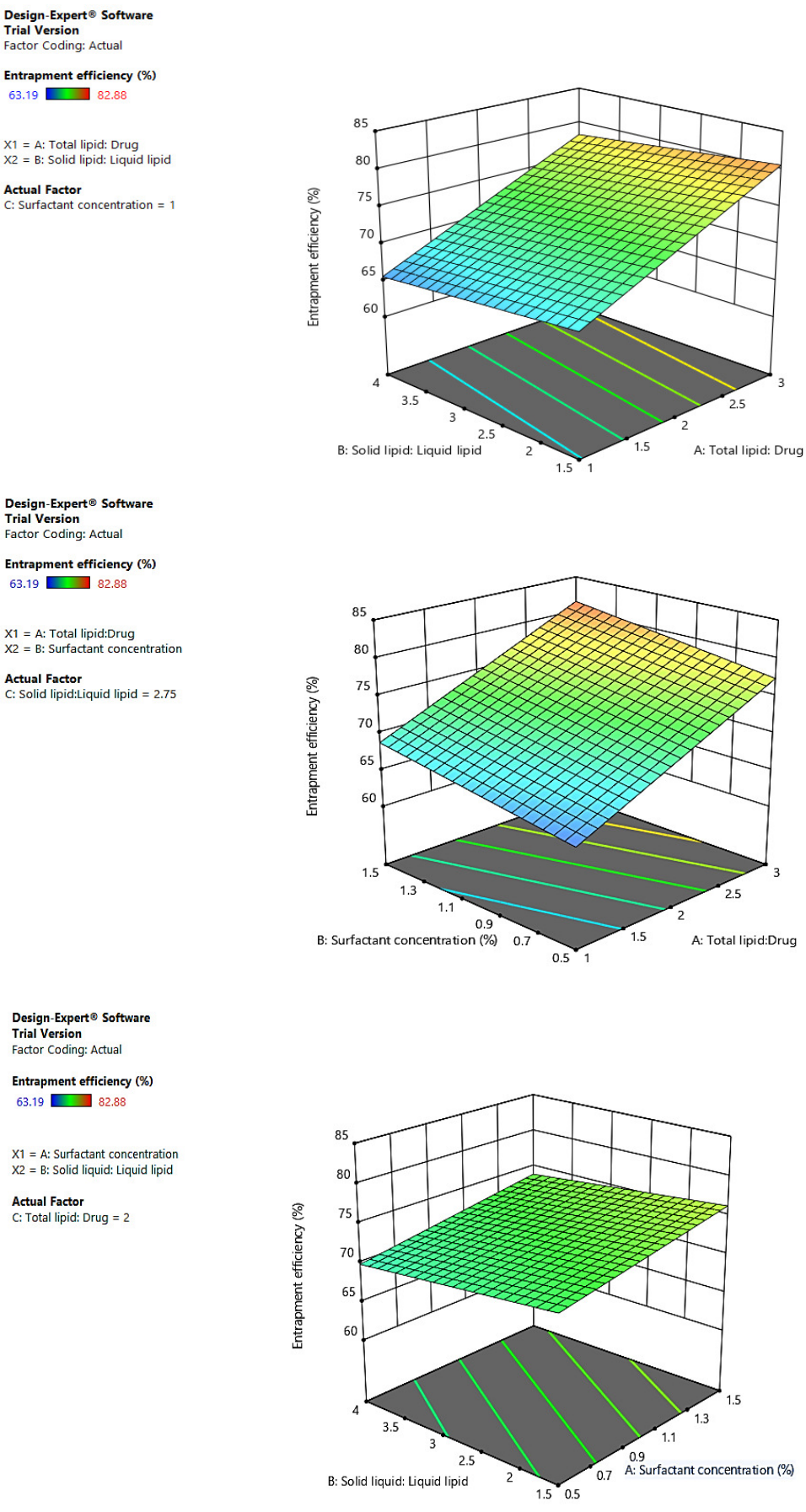


Figure 6: Response surface plot for the effect of total lipid: drug, solid lipid: liquid lipid and surfactant concentration on the Entrapment efficiency

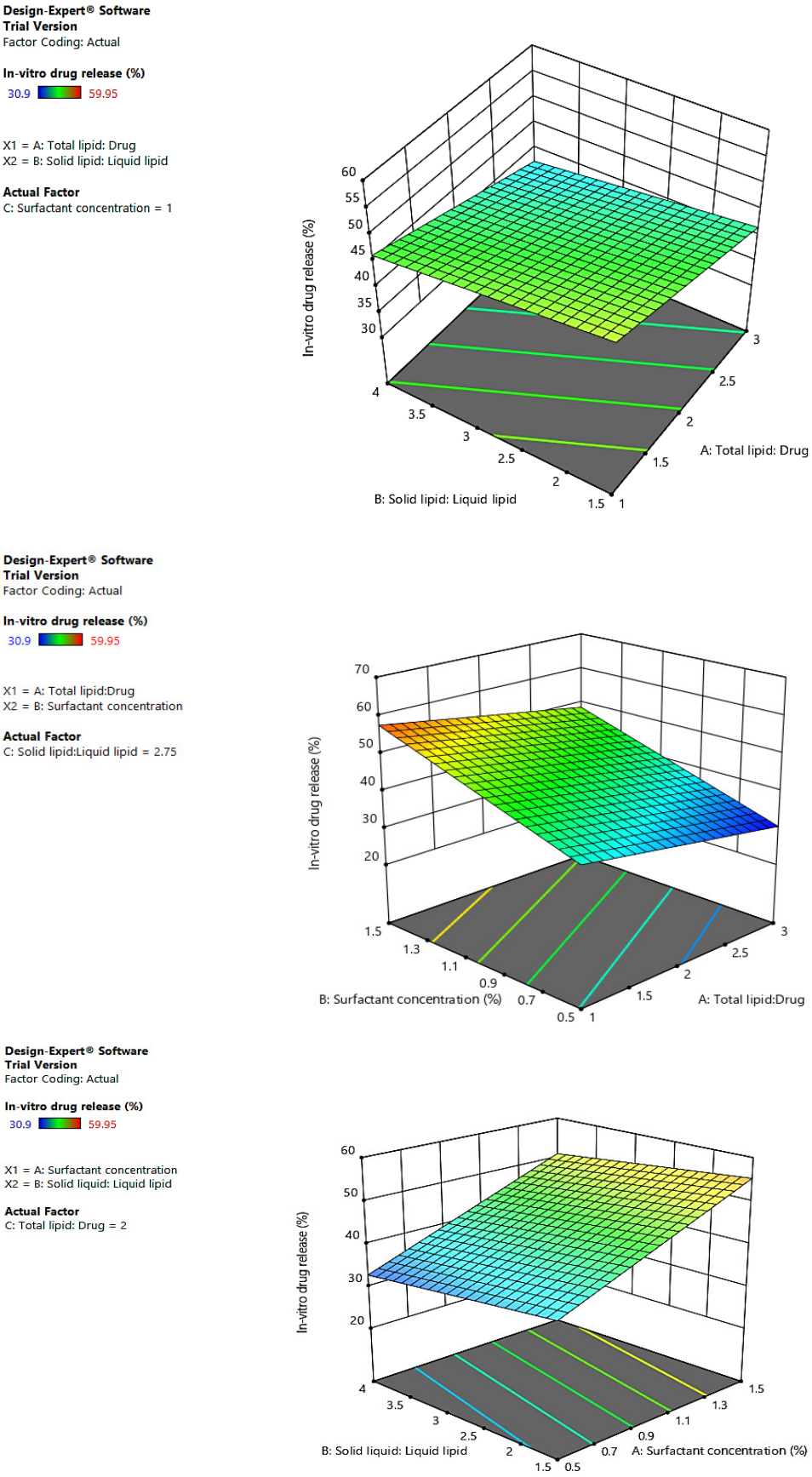


Figure 7: Response surface plot for the effect of total lipid: drug, solid lipid: liquid lipid and surfactant concentration on the In-vitro drug release

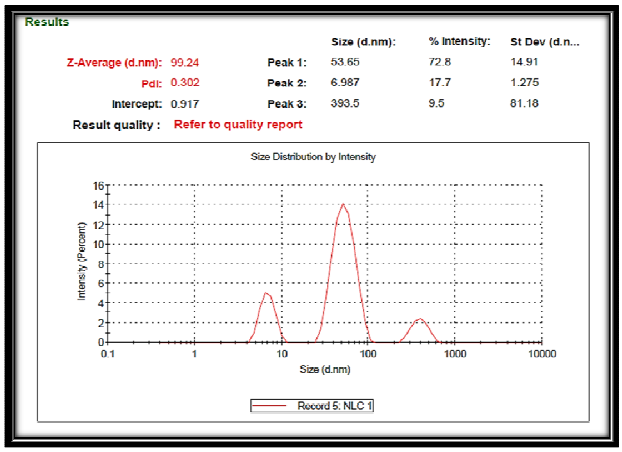


Figure 8: Particle size distribution by intensity of optimized Furosemide NLC

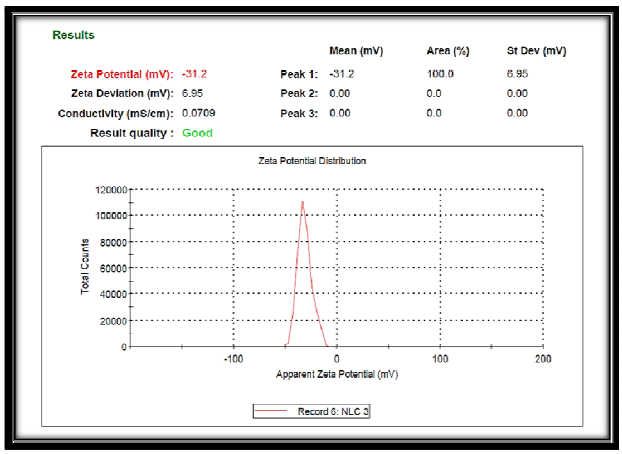


Figure 9: Zeta potential report of optimized Furosemide NLC

Scanning Electron Microscopy (SEM)

The nanoparticulate nature of the NLC dispersion particles was further confirmed by SEM studies. Fig. 10 shows the SEM image of optimized Furosemide NLC.

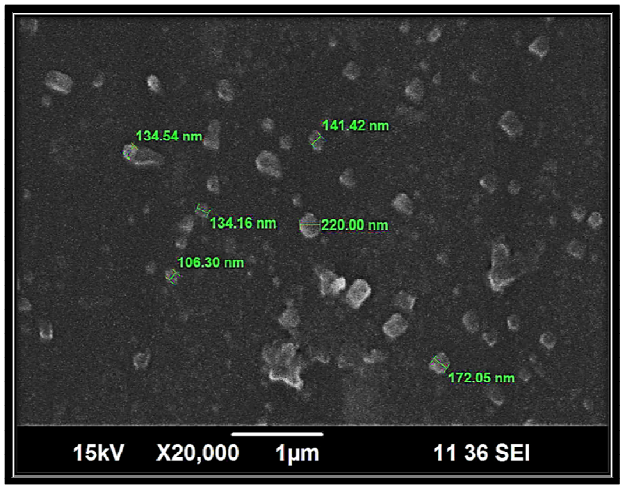


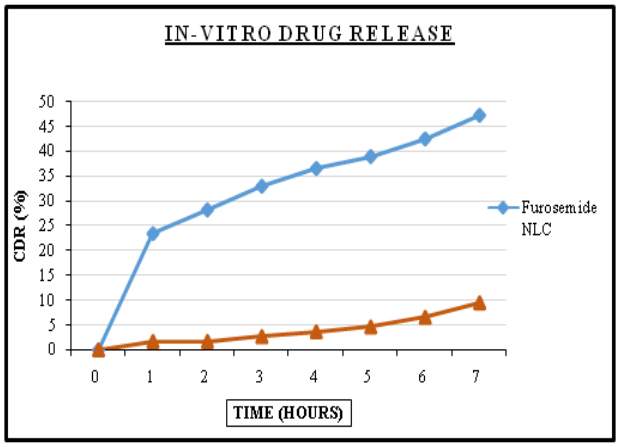
Figure 10: SEM image of optimized Furosemide loaded NLC

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.

Literature survey revealed that if the mean particle size of lipidic nanoparticles 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 NLC in the formulation and attain higher plasma concentration through the lymphatic transport system [4].

In-Vitro Drug Release Study

In- vitro drug release studies of Furosemide drug, optimized Furosemide NLC were carried out by dialysis method using pH 6.8 phosphate buffer as receptor medium. Studies were performed and the results were shown in the Graph 6.



Graph 6: Percentage In-vitro drug release

The *in-vitro* release of optimized NLC showed an interesting bi-phasic release with an initial burst effect. Afterwards, the drug release followed a steady pattern while pure drug showed very slow release.

Kinetics of Drug Release

The *in vitro* drug release data of NLC 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 8.

Table 8: Kinetic release data

FORMULATION		Optimized Furosemide NLC	Furosemide
ZERO ORDER	R^2	0.8633	0.9701
FIRST ORDER	R^2	0.5716	0.8971
HIGUCHI	R^2	0.9572	0.9383
KORSMEYER PEPPA'S	R^2	0.3352	0.6440
	n	0.7716	0.6560

From the Table 8, it is clear that the drug release from NLC shows Higuchi matrix model with R^2 values of 0.9572. Hence the drug release mechanism was assumed to be diffusion controlled for NLC. 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 and pure drug were found to be 0.7716and 0.6560 respectively, indicating the release of drug follows non-fickian diffusion.

Stability of Furosemide Loaded Nanostructured Lipid Carrier

The stability of optimized NLC formulation 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 9.

Table 9: Results of stability studies (60 days)

Parameters	Before stability study	After stability study
Appearance	White colour with characteristic odour	White colour with characteristic odour
Drug content (mg/ml)	1.6713	1.6515
Entrapment efficiency (%)	75.50	74.51
Loading capacity (%)	25.63	25.38
In-vitro drug release (%)	47.26	46.63

CONCLUSION

Furosemide loaded NLC for oral administration was successfully prepared by solvent diffusion method and optimized using 2^3 full factorial design. Drug-excipient interaction study using FT-IR indicated the absence of any drug-excipient incompatibility between Furosemide and excipients. Solvent diffusion method yield nanosized physically stable particles with negative zeta potential value. The PDI values revealed the formation of polydispersed particle. 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 formulations exhibited a biphasic release pattern with initial burst release 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 & pure drug formulations. Optimized NLC formulation was found to be stable for 60 days.

REFERENCES

[1] D. Radhesh Kumar, Mohammed Habeeb, Mangalarapu Shravya, Ramana Reddy, Formulation and evaluation of silica lipid hybrid microparticles containing furosemide, International journal of pharmacy (2015), DOI: 10.7897/2230-8407.06670

[2] IP, Volume 2, 2014, Page no.:1833

[3] http://shodhganga.inflibnet.ac.in/bitstream/10603/176229/12/12_chapter%202.pdf

[4] Veena S. Belgamwar , Jatin D. Dani, Anil U. Tatiya, Mohan G. Kalaskar, Prakash H. Patil, Shailesh S. Chalikwar, 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 8(4), Oct-Dec, 2016, 199-211

[5] https://link.springer.com/chapter/10.1007/978-3-319-41129-3_2

[6] Harsiddhi M. Chaudhary, Kisan R. Jadhav and Vilasrao J. Kadam, Formulation and evaluation of nanostructured lipid carriers containing glipizide, World journal of pharmacy and pharmaceutical sciences, Volume 5, Issue 4, 1424-1437

[7] https://fenix.tecnico.ulisboa.pt/downloadFile/844820067124759/ABSTRACTAnaCartaxo.pdf

[8] Miaojing Wu, Yanghua Fan, Shigang Lv, Bing Xiao, Minhua Ye and Xingen Zhu, Vincristine and temozolomide combined chemotherapy for the treatment of glioma: a comparison of solid lipid nanoparticles and nanostructured lipid carriers for dual drugs delivery, DOI: 10.3109/10717544.2015.1058434

[9] P. Ekambaram, A. Abdul Hasan Sathaliand K. Priyanka, Solid Lipid Nanoparticles: A Review, Sci. Revs. Chem. Commun.: 2(1), 2012, 80-102, ISSN 2277-2669

[10] C. Puglia, Blasi P, Rizza L, Schoubben A, Bonina F, Rossi C, Ricci M, Lipid nanoparticles for prolonged topical delivery: an in vitro and in vivo investigation, Int.J.Pharm.357(2008)295.

[11] M¨uller, R.H., M¨ader, K., Lippacher, A., Jenning, V., 2000b. Festfl¨ussige (halbfeste) Lipid partikel und VerfahrenzurHerstellunghochkonzentrierterLipidpartikel-dispersionen. PCT/EP00/04565.

[12] https://www.ingentaconnect.com/content/govi/pharmaz/2008/00000063/00000012/art00006?crawler=true

[13] Nirmal V. Shah, Avinash K. Seth, R. Balaraman, Chintan J. Aundhia, Rajesh A. Maheshwari, Ghanshyam R. Parmar, Nanostructured lipid carriers for oral bioavailability enhancement of raloxifene: Design and in vivo study, Journal of Advanced Research, DOI: http://dx.doi.org/10.1016/j.jare.2016.03.002

[14] Hetal P. Thakkar, Jagruti L. Desai, Mayur P. Parmar, Application of Box-Behnken design for optimization of formulation parameters for nanostructured lipid carriers of and esartancilexetil, IP: 223.30.225.254

[15] Radheshyam Tiwari, Kamla Pathak, Nanostructured lipid carrier versus solid lipid nanoparticles of simvastatin: Comparative analysis of characteristics, pharmacokinetics and tissue uptake, International Journal of Pharmaceutics 415 (2011) 232– 243

[16] http://www.drugbank.ca/drugs/DB03193

[17] http://www.drugbank.ca/drugs/DB04540

[18] http://patents.google.com/patent/EP0589843A1

[19] Le Quoc Thang, Nguyen DucHanh, Do Quang Duong, Study on cause–effect relations and optimization of exemestane-loaded nanostructured lipid carriers, International Journal of Pharmacy and Pharmaceutical Sciences, DOI: http://dx.doi.org/10.22159/ijpps.2017v9i5.17354

[20] Fu-Qiang Hu, Sai-Ping Jiang, Yong-Zhong Du, Hong Yuan, Yi-Qing Ye , Su Zeng, Preparation and characterization of stearic acid nanostructured lipidcarriers by solvent diffusion method in an aqueous system.