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A Research Study on The Development of Nanostructured Lipid Carriers for Furosemide Delivery: Formulation and Evaluation

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ABSTRACT

Designing and evaluating nanostructured lipid carriers (NLCs) to administer furosemide effectively, a strong loop diuretic with low oral bioavailability because of its substantial first-pass metabolism and low solubility, is the main focus of this study. NLCs were created to increase medication solubility, stability, and simplify controlled release. They are made up of a mixture of liquid and solid lipids stabilized by surfactants. NLCs loaded with furosemide were made via ultrasonography and thermal homogenization. The criteria employed to optimize various formulations included drug entrapment efficiency, zeta potential, particle size, polydispersity index (PDI), and in vitro drug release.

A mean particle size of less than 200 nm was observed in the enhanced NLC formulation, which also had a narrow PDI and a zeta potential that suggested high physical stability. In order to confirm effective drug loading, the entrapment efficiency was noticeably high. The quick release from the pure medication was contrasted with a continuous release profile over a 24-hour period in in vitro release tests. Based on the data, NLCs may be a promising delivery method to increase furosemide's bioavailability and therapeutic effectiveness. It is necessary to conduct additional in vivo research to prove pharmacokinetic benefits

1. INTRODUCTION

Nanostructured lipid carrier Recently,

there has been a lot of interest in lipid nanoparticles, particularly solid lipid nanoparticles (SLNs) and nanostructured lipid carriers (NLC). Solid lipid is used alone to manufacture SLNs, it forms a crystal lattice with minimal space for the therapeutics.Solid Lipid Nanoparticles (SLNs) and Nanostructured Lipid Carriers (NLCs) are advanced lipid-based drug delivery systems designed to improve the bioavailability and stability of lipophilic drugs. While they share similar functions, their structures differ. [1] SLNs consist of solid lipids, which form a stable matrix that encapsulates drugs, offering controlled release and protection from degradation. In contrast, NLCs, as a second-generation lipid nanoparticle, incorporate both solid and liquid lipids. This structural difference enhances drug loading capacity and provides greater stability compared to SLNs. Nanostructured lipid carriers (NLCs) offer notable advantages in drug delivery but encounter several limitations. While NLCs enhance drug solubility, some drugs, like quercetin, face issues with low percutaneous permeability and bioavailability,impacting their effectiveness. Additionally, NLC formulations can become unstable over time, leading to phase separation or precipitation, which affects their clinical reliability. In dermatological applications, although NLCs aim to reduce skin irritation, they may not fully address the issue, potentially causing adverse reactions. Nanotechnology has developed exponentially.[2-3] Nano-technology has practically made its influence in all technical fields, including

pharmaceutics. Nanoparticulate systems such as liposomes were described for the first time in the 1960s by Bangham *et al.*

In 1990s, solid lipid nanoparticles (SLN) were firstly developed by Muller and Gasco by avoiding organic solvents which were involved in the preparations of polymeric nanoparticles. SLNs are sub micronic colloidal nanocarriers containing solids ranging from 1 to 1000 nm.

SLN's use only solid lipids. Recently, SLNs based on a mixture of solid lipid and liquid lipids called nanostructured lipid carriers studied a size range of 1-100nm [1]. NLC's minimized many problems like drug expulsion during storage which are associated with SLN formulation for many drugs due to its high water content.

2. METHODS

Preformulation

As part of preformulation studies, the drug's physical qualities, solubility, melting point, and compatibility with its excipients were looked into. We tested the drug's solubility by seeing how well it dissolved in three different buffer solutions: a pH 1.2 acid buffer, a pH 5.8 phosphate buffer, and a pH 6.8 phosphate buffer. It was also tested to see how well the medicine dissolved in various solvents, including water, acetone, alkali hydroxides, ethanol, methanol, DMSO, chloroform, and ether. The melting point of furosemide was found using both the open capillary tube method and DSC (Nkansah, *et al.,* 2013).

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. [4]

Selection of Excipients

To choose the solid lipid, scientists looked at how well the drug dissolved in melted solid lipid. This could be done with the naked eye in normal lighting. Labrafil, cholesterol, and stearic acid were the fats used in this study. A carefully measured amount of medicine containing different lipids was cooked above the melting point of those lipids in a water bath. Test tubes were used to keep the temperature stable. We checked how well furosemide dissolved in each lipid by looking at them with normal lighting after the lipids had been melted (Saisri, *et al.,* 2021).

Analysing the Dissolvability of Different Liquid Surfactants and Lipids

Castor oil, oleic acid, and capryolpgmc were used as liquid lipids in this work. Tefen 20 and tefen 80 were used as surfactants. To test how well the drug mixed, too much of it was put into small tubes that already had two milliliters of certain oils and surfactants in them. A glass stick was used to mix the drug by hand with the right oil and surfactant. The jars were tightly closed and put in a rotary shaker where they were rotated continuously for 24 hours. For thirty minutes, the liquid lipids were spun at 3000 revolutions per minute. After the right amount of ethanol was added, the liquid that was left over after centrifugation was mixed with it. A UV Spectrophotometer with a 274 nm range was used to test the substance's ability to dissolve (Poovi, *et al.,* 2018). [5-7]

Compatibility Study

How well the drug and excipients work together is the most important factor in determining how stable a mixture is. Because of this, it is very important to find any possible chemical or physical reactions, since they can change how stable and bioavailable the drug is. [8] FTIR was used to find out how Furosemide and the other ingredients in the mixture interacted during the compatibility tests, which were done at room temperature. The drug's FTIR spectrum was recorded in two situations: when it was given by itself and when it was mixed with labrafil m 2130 and capryol pgmc (Ahirrao, *et al.,* 2022).

Design of Experiment

A full-factorial method was used in this study to find the best way to make NLCs. The concentration of surfactant as a percentage, the ratio of solid to liquid lipids, and the ratio of total lipids to drugs were picked as the independent variables for optimization. There was a high level and a low level given to each part. For each variable, Table 1 shows both the real numbers and the encoded values. Using the factorial method, six different forms of furosemide NLCs (B1–B6) were created. The response measures measured the amount of drug released in a controlled lab setting after 7 hours and how well the drug was trapped. [9] The trial version of Design expert statistical software was used to do the statistical study of the answers (Kim, *et al.,* 2022).

Table 1: Complete factorial layout of NLCs loaded with furosemide

Sr. No.	Batch	Entrapment efficiency (%)	DLC (%)	Drug content (%)	Drug release (%)
1	**B1**	78.32	21.34	86.47	42.64
2	**B2**	78.55	21.87	85.69	31.87
3	**B3**	81.58	20.58	86.12	52.54
4	**B4**	78.47	21.67	87.34	32.44
5	**B5**	68.68	41.42	85.77	57.12
6	**B6**	62.32	39.61	85.33	36.34

Preparation of furosemide loaded nanostructured lipid carrier (NLC)

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 5-10^0 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 45-50^0C 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 45-50^0C. 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. The same method was used to make both the NLC dispersion with drugs and the NLC dispersion without drugs (Ahire, *et al.*, 2023). [4,10]

Characterization & comparison of optimized furosemide loaded NLC

a. Drug Content

In a 10 ml standard jar, 1 ml of a suspension of furosemide nanostructured lipid carriers was added. A little DMSO was added, and the mixture was mixed well. Then, pH 6.8 phosphate buffer was added until the right volume was reached. We took one milliliter of this solution and mixed it with fifty milliliters of pH 6.8 phosphate buffer by diluting them.A UV spectrophotometer was used to test the solution's absorbance at 279 nm. Then, the absorption of the similar blank solution was compared to it to find out how much drug was in it (Ahirrao, *et al.*, 2022). [4]

b. Entrapment efficiency (Ee) and Drug loading (Lc)

Entrapment and how well drugs are loaded The NLC suspension was taken out by centrifuging the Furosemide-loaded NLCs at 3000 rpm for 1.5 hours. This made 5ml of the suspension. One milliliter of the liquid that was left over after spinning was taken out and mixed with DMSO after it was diluted with pH 6.8 phosphate buffer. A UV spectrophotometer was then used to measure the drug content at a wavelength of 279 nm (Ali, *et al.*, 2018).

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

c. *In-Vitro* Drug Release

In a lab setting, experiments were done to look into drug delivery using the dialysis method. One end of a dialysis membrane that had been soaked overnight was connected to a custom- made glass cylinder. This made sure that the preparation filled the whole inside circle of the tube. Each sample had 1 milliliter of blood put into the dialysis bag. It was 37 ± 5 ºC, and 100 ml of receptor media was used to mix the cylinder. Attached to the cylinder was a frame that made sure the membrane didn't

touch the media surface very much. An electric mixer set to 100 rpm was used to mix the receptor medium. The cellophane membrane breaks up the NLC and receptor media. When the time was right, 1 ml of the sample was taken out of the receiver section and replaced with fresh medium. The samples were weakened with phosphate buffer with a pH of 6.8. The amount of furosemide released was measured with a UV-visible spectrophotometer at a range of 279 nm. [11-13]

d. 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 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.

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.

A Nanostructured Lipid Carrier Loaded with Furosemide: Stability and Performance

The best NLC mixtures were kept at room temperature and out of the light for 60 days to see how stable they were during storage. The study looked at the materials' physical features, drug content, how well they trapped drugs, how much they could hold, and how the drugs released in vitro over time. The original formulations and the changed formulations were both tested for stability. [13,14,15]

3. 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 2&3.

Table-2: Solubility of Furosemide in various solvents

SOLVENT	SOLUBILITY
Water	Practically insoluble
Acetone	Freely soluble
Methanol	Freely soluble
DMSO	Freely soluble

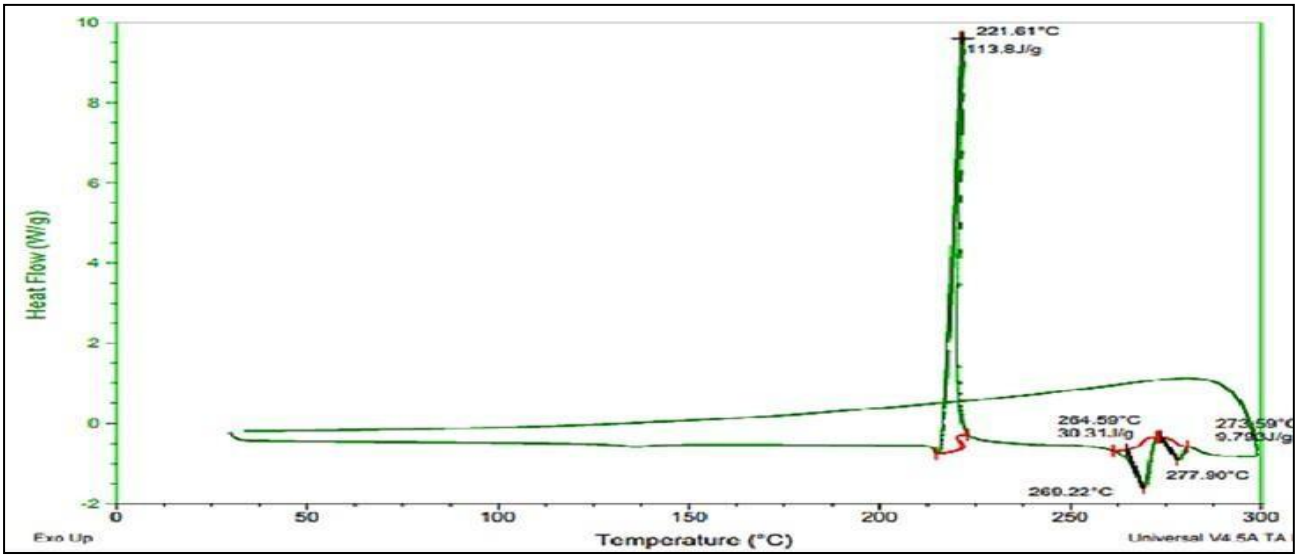
Alkali hydroxides	Freely soluble
Ethanol (95%)	Sparingly soluble
Chloroform	Insoluble
Ether	Insoluble

Table-3: 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

Pre-formulation studies were done to find a good chemical profile and make sure the drug was the right one and that it was pure. The drug is a powder that is clear, white, or almost white. The furosemide sample that was sent was tested in different liquids and buffer solutions to see how well it dissolved. Using the capillary fusion method and DSC, it was found that the drug breaks down at 220°C and 221.61°C, respectively. The value given in the monograph is about the same as these figures. The thermogram of furosemide that was made using differential scanning calorimetry (DSC) is shown in Figure 1. The DSC thermogram shows that furosemide has a rapid and sharp peak in the loss of heat at 221.61°C. With a value of 113.8J/g, this point is marked by heat. This peak typically means that the drug is breaking down, and it shows that the molecule has a crystalline structure. Figure 1 shows that the furosemide breakdown product has a peak at 269.22°C, which means it is absorbing heat.

Figure 1: The DSC graph 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 Figure 2&3

Figure 2: Standard calibration graph of Furosemide in ethanol (95%)

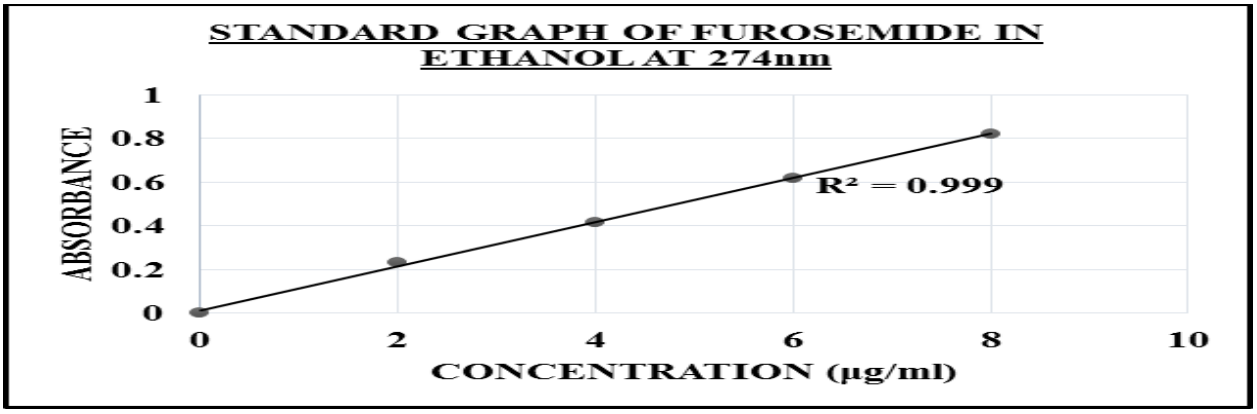
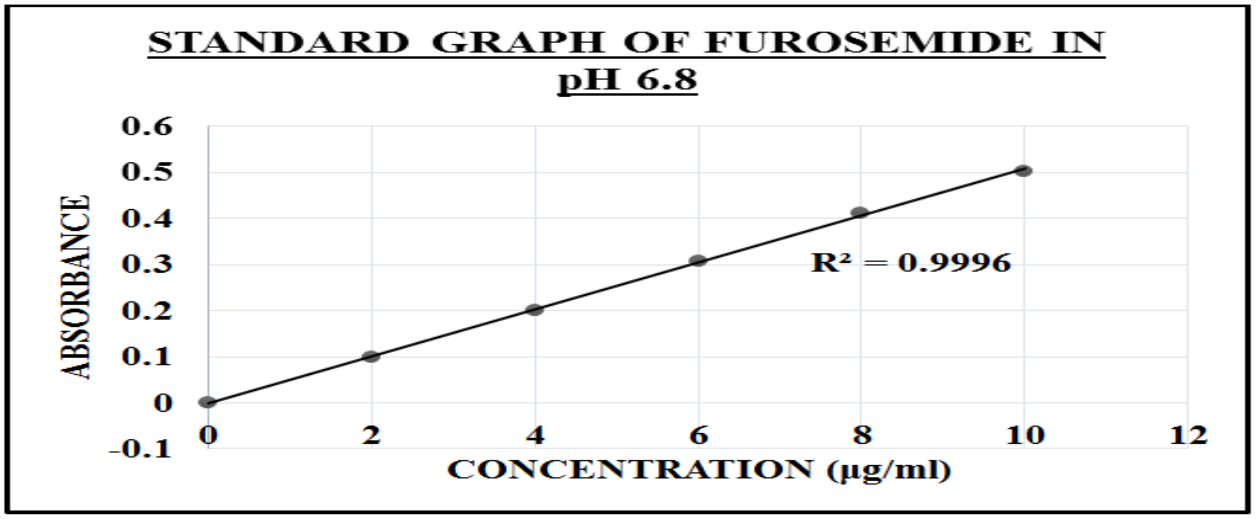


Figure 3: Standard calibration graph of Furosemide in P^H 6.8 phosphate buffer



Selection of Excipients

How well the medicine dissolves is a key factor in choosing the ingredients that are used to make lipid nanoparticles. To find solid lipids, liquid lipids, and surfactants that can dissolve furosemide well, solubility tests were carried out.

Choosing a Solid Lipid

It is very important for the drug to have better solubility in solid lipids so that it can keep its solubilization form. It was found out how well furosemide dissolves in different solid fats. Furosemide was easier for labrafil to dissolve than stearic acid or cholesterol.

Table-4: 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.

Analysing the Dissolvability of Different Liquid Surfactants and Lipids

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 (Figure 4). 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).

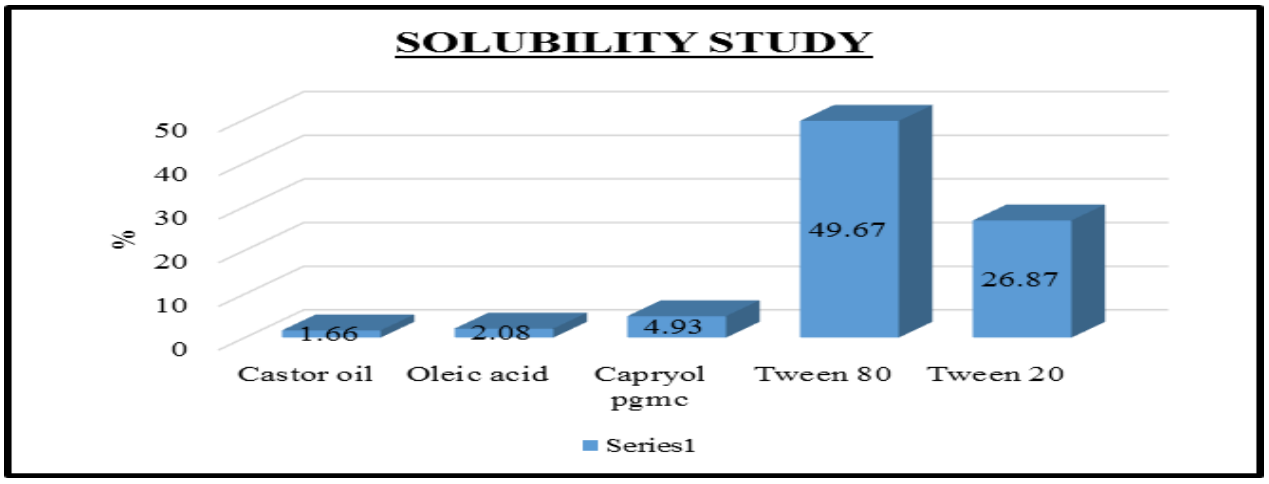


Figure 4: Solubility of Furosemide in liquid lipids and in surfactants

Compatibility Study

Figures 5,6 and 7 show the FTIR spectra of furosemide that is pure and furosemide that has been mixed with different substances. The strong peaks seen in the drug spectrum were also present when the drug and lipids were physically mixed together. This shows that the medicine and lipids are not incompatible.

Figure 5: Analysis of Furosemide's FTIR spectra

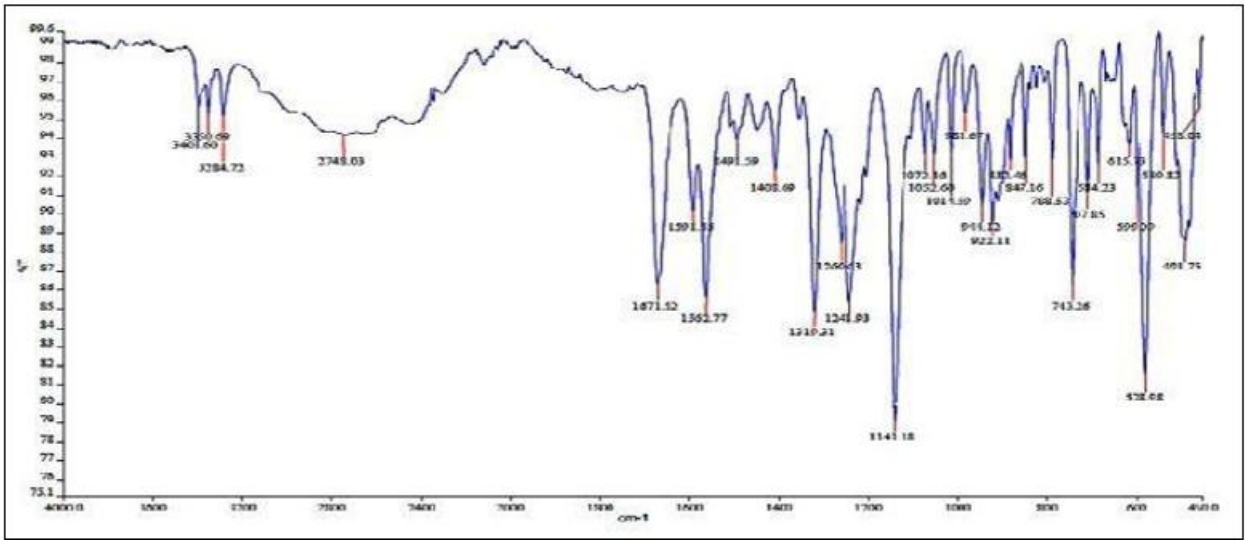


Figure 6: FTIR spectrum of Furosemide with Labrafil m 2130

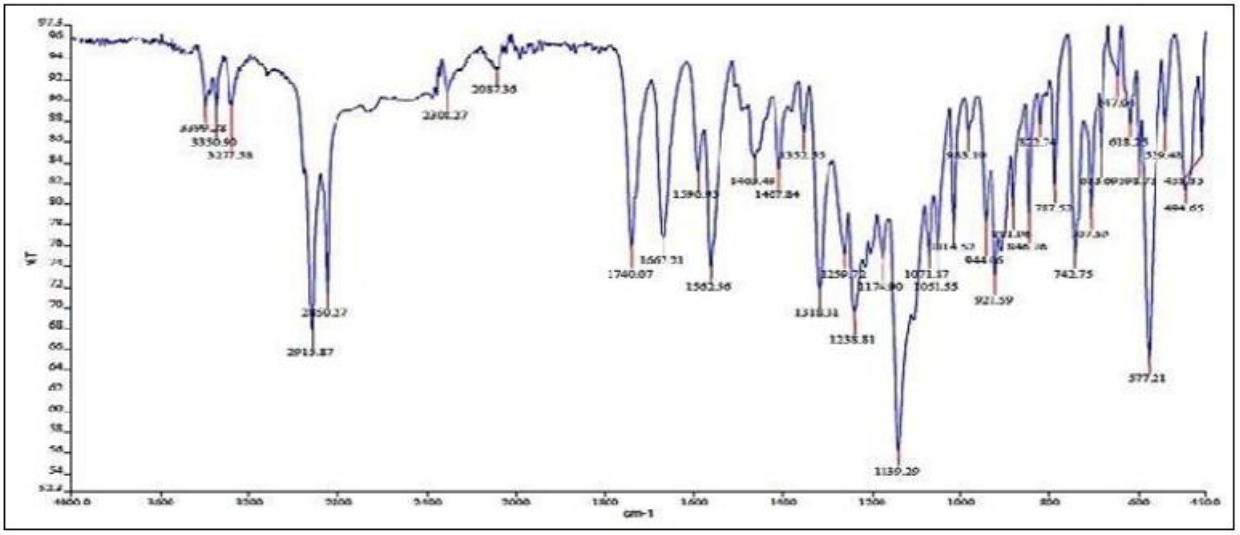


Figure 7: Furosemide and Capryol PGMC FTIR spectra

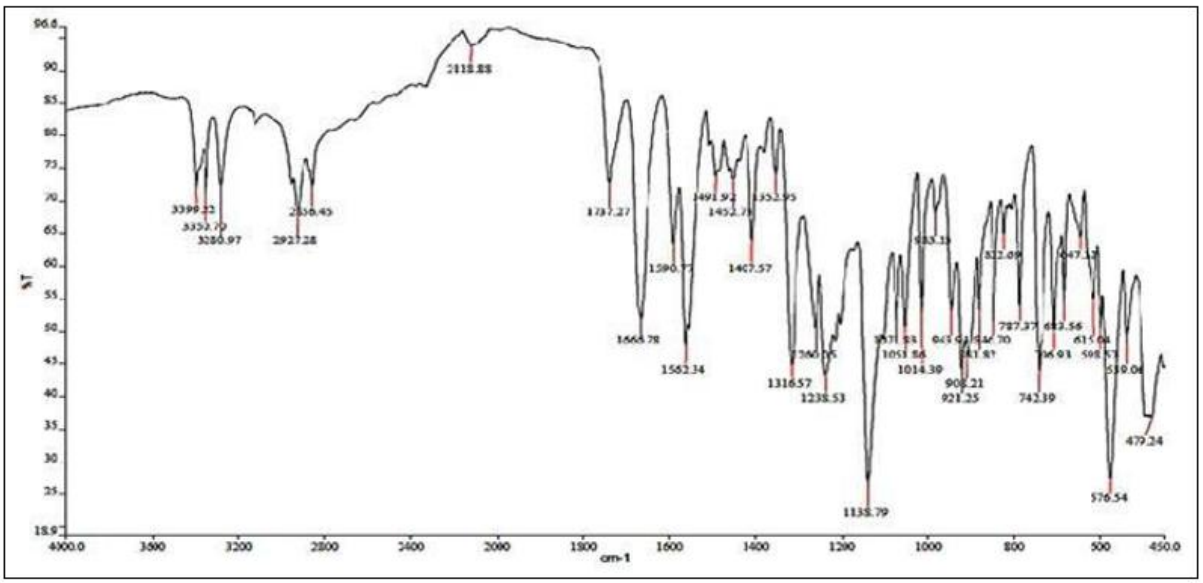


Table-5: Interpretation of FTIR spectrum of Furosemide with lipids

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 ($SO2NH2$)	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)

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

Drug Content,

The amount of furosemide in the NLCs that were loaded with drugs ranged from 84.07% to 89.996%.

Entrapment efficiency (Ee) and drug loading capacity (L_C) Analysis

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% . 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.

The main things that keep furosemide trapped are its ability to dissolve in both liquid and solid lipids and its ability to separate into oily and watery phases. Furosemide works best when the solid-liquid lipid ratio is low and the overall lipid:drug ratio is high. This is because furosemide is lipophilic. This reduces the amount of furosemide that is floating around in space and makes it easier to catch. Adding liquid lipid to solid lipid may cause a decrease in crystallinity and an increase in crystal lattice flaws in order to improve the efficiency of entrapment and make room for the higher amount of furosemide.

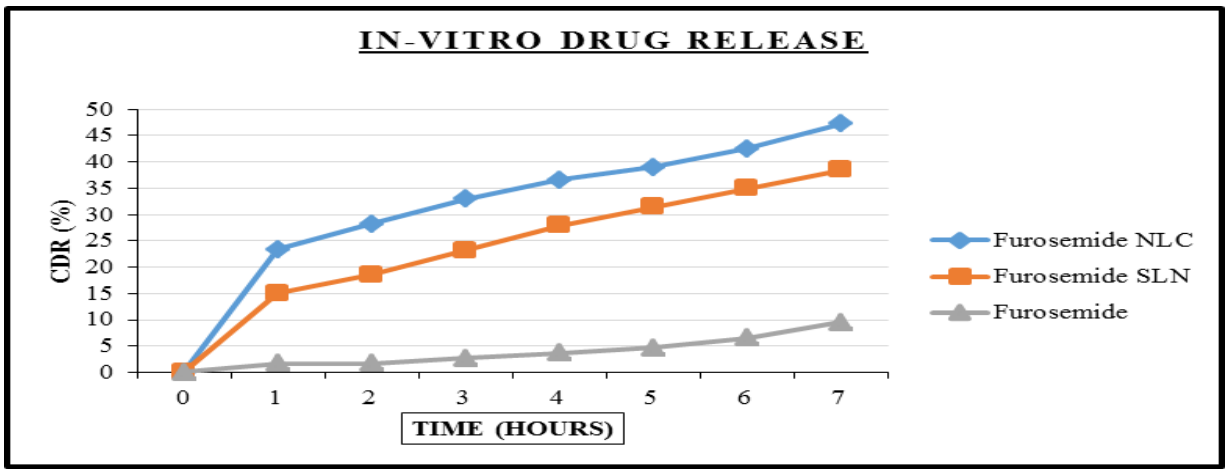
It is known that when the concentration of surfactant goes up, the entrapment effectiveness also goes up. The fact that the surfactant system can make the water phase thicker as the concentration goes up can be used to show that there is a direct link between the concentration and the efficiency of trapping. This slows down the rate at which furosemide diffuses and makes trapping more effective. The fact that surfactant concentration makes trapping more effective may also be due to the fact that Furosemide molecules can connect or link to more surfaces when smaller particles are formed. It's possible that this is because there was enough surfactant, which kept furosemide inside the lipid particles or on their surface, making it very effective at entrapping them. A big drug loading capacity is one of the traits of the less stable crystal modification. It's not a straight line between the amount of total lipid to drug and drug loading. Because the lipid layer can only hold so much, as the overall lipid:drug ratio goes up, drug loading goes down. It is possible for drug loading to go up when the fraction of solid lipids to liquid lipids goes down. At normal temperatures, liquid lipid dissolves furosemide, making it bigger and stopping it from spreading to the outer phase. This makes it easier for the drug to be loaded.

***In-Vitro* Drug Release**

The dialysis method was used for in vitro drug release tests, and a pH 6.8 phosphate buffer was used as the receptor medium. A graph was made of the total amount of medicines released over time in order to make drug release profiles. There are two stages of drug release in NLC: the first stage is a fast release of the drug, and the second stage is a steady release. One possible reason for the different NLC release patterns seen in this study is that the solvent diffusion method used to make the nanoparticles did not equalize the distribution of the liquid lipid. In the solvent diffusion process, lipids that had been heated above their melting point were used to make NLC, which was then spread out in the water phase. In the end, most of the liquid lipid in the nanoparticles' outer layers makes a shell that is full of drugs. This causes the drugs to be released quickly

at first. It is much easier for lipophilic medicines to dissolve in the oilier upper layers. So, the process of drug diffusion or matrix erosion can make it easier for more medicine to be loaded and released

.Figure 8: 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 Figure 8, 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 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-6.

Table-6: Kinetic release data

FORMULATION	ZERO ORDER	FIRST ORDER	HIGUCHI	KORSMEYER PEPPA'S	
	R2	R2	R2	R2	n
Furosemide NLC	0.8633	0.5716	0.9572	0.3352	0.7716
Furosemide	0.9701	0.8971	0.9383	0.6440	0.6560

From the table-6, 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-7.

Table-7: 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.

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-9).

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-10).

Figure 9: Particle size distribution by intensity of Furosemide NLC

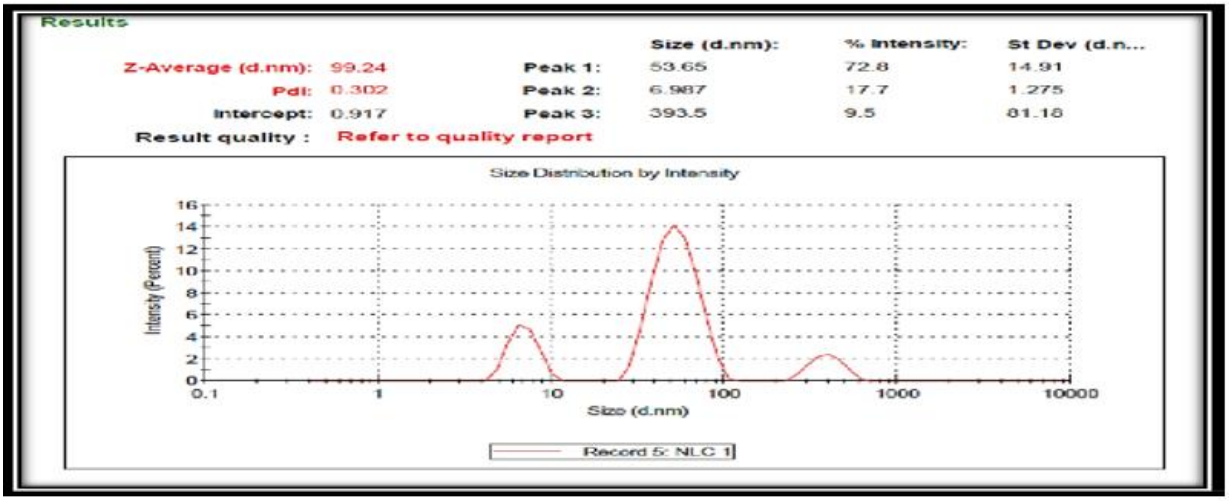
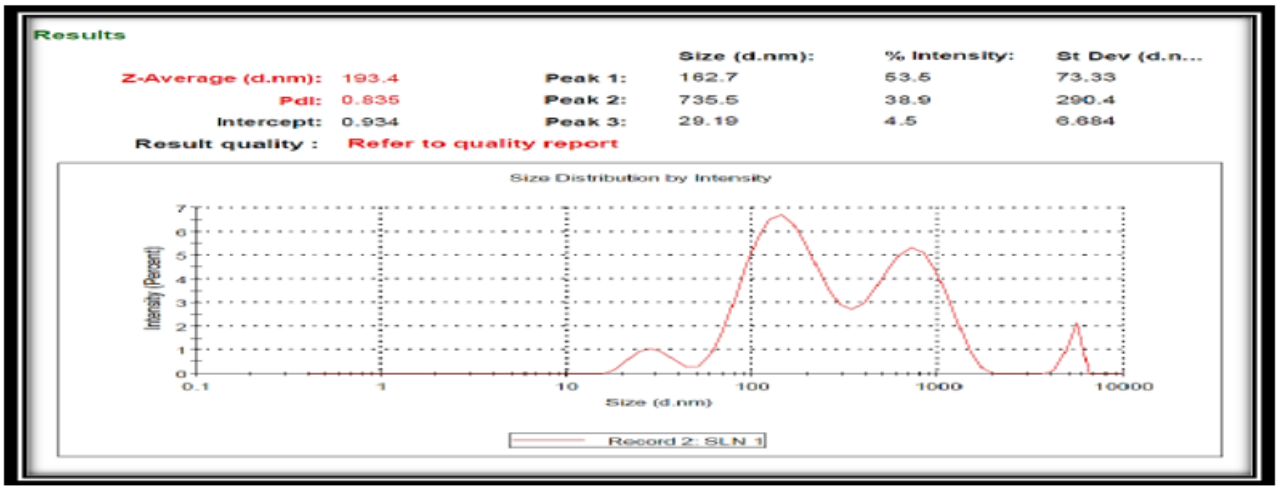


Figure 10: Particle size distribution by intensity of Furosemide SLN



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-11) and that of Furosemide SLN is - 36.1mV (figure-12). 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.

Figure 11: Zeta potential report of Furosemide NLC

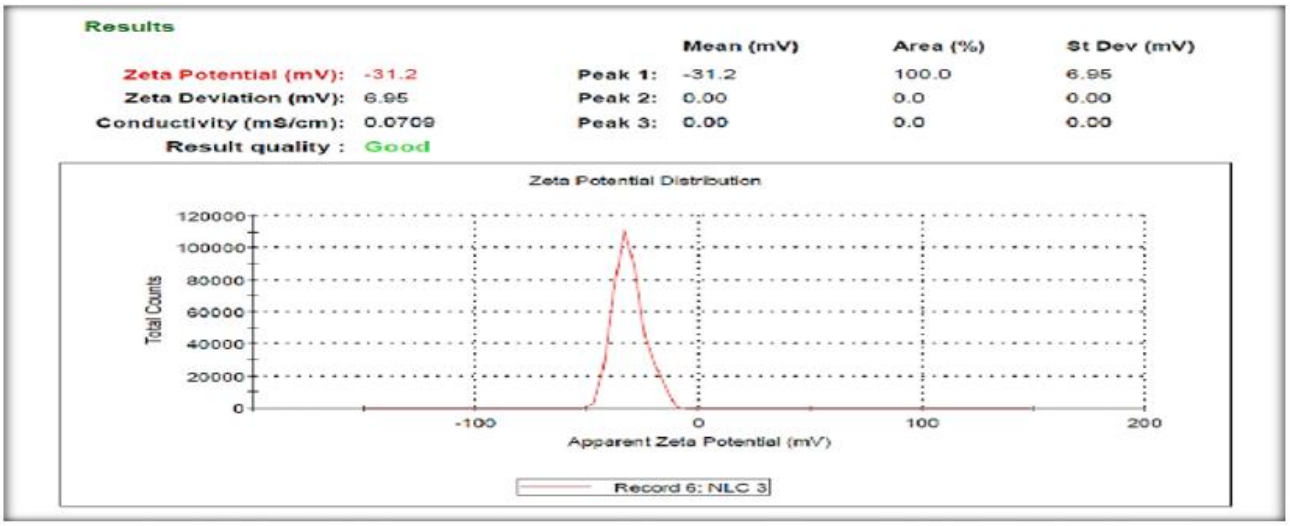
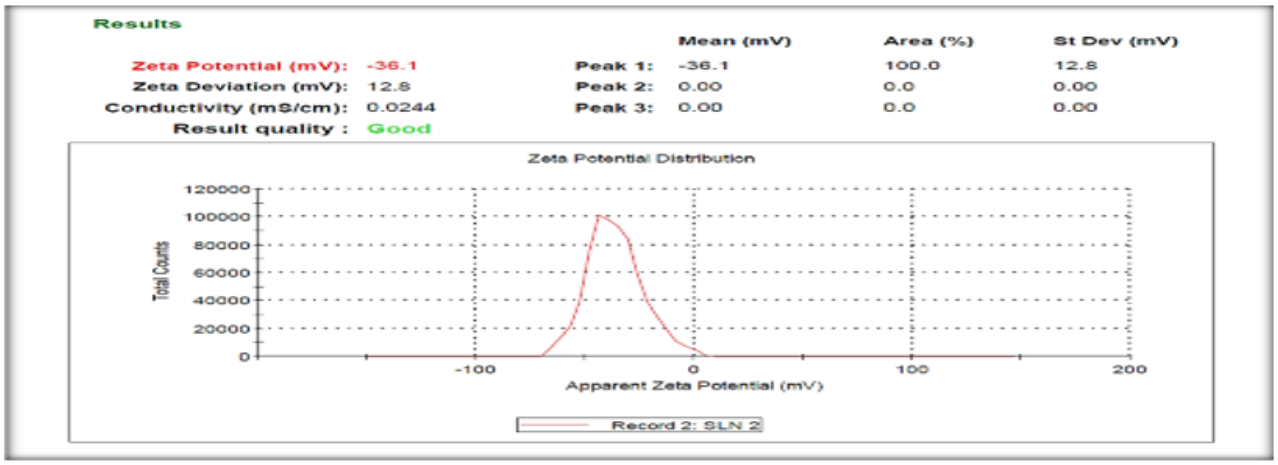


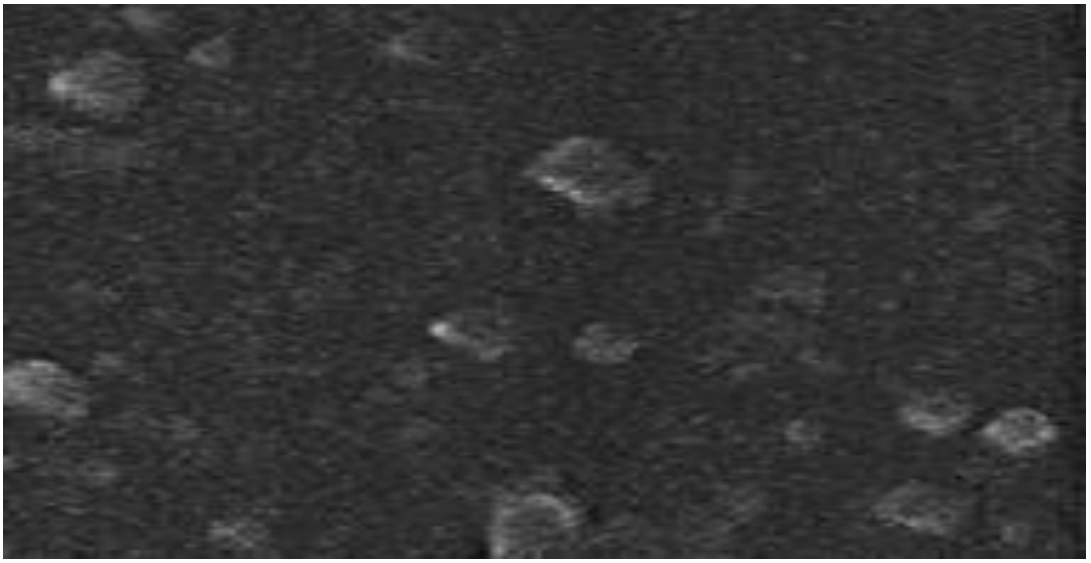
Figure 12: Zeta potential report of Furosemide SLN



Scanning Electron Microscopy (SEM)

For more proof that the NLC dispersion particles are at the nanoparticle scale, SEM studies were conducted. The SEM picture of the better Furosemide NLC is shown in Figure 13. The particles had mostly a spherical shape at the nanoscale, with smooth surfaces and a uniform distribution on a 1µm scale, which matched the size information from the DLS study. The results showed that there were no drug crystals that could be seen in the picture, and they proved that the particles had a spherical shape. The picture shows the link between how the sample was prepared before the SEM test and the fatty makeup of the carriers, which causes the particles to stick together. During the drying step of sample treatment, changes in lipids can cause particles to have shapes other than spheres. There was a study of the literature that showed lipidic nanoparticles with an average size of less than 200 nm would be moved through the lymphatic system instead of the portal vein. They would be able to skip the first pass digestion this way. Additionally, the reticuloendothelial system isn't very good at getting rid of particles in the bloodstream that are smaller than 120 to 200 nm, which stops the spleen and liver from filtering them out. Completely stops the first pass metabolism, which lowers the amount of furosemide NLC in the formulation and raises the concentration in the plasma through the lymphatic transport system.

Figure 13: An enhanced Furosemide-loaded NLC's SEM picture



A Nanostructured Lipid Carrier Loaded with Furosemide: Stability and Performance

The stability of the enhanced NLC formulation was assessed by monitoring the appearance, drug content, entrapment efficiency, drug loading capacity, and in-vitro drug release after being stored in the dark at room temperature for 60 days.

4. CONCLUSION

Using solvent diffusion and a full factorial design, the method for making furosemide-loaded NLC for oral transport was made even better. FT-IR research did not show any signs of drug- excipient incompatibility between furosemide and the excipients. Nanoparticles that are physically solid and have a negative zeta potential are made using the solvent diffusion method. The PDI numbers were used to show how polydispersity particles were made. Using DSC study, it was proven that the pure material was crystalline. The pure drug had zero-order kinetics, but the NLC formulations had a biphasic release pattern with an early burst release and then a constant release that followed the Higuchi equation. Based on the value of n, it looked like the medicine released from both NLC and pure drug formulations would spread using a Fickian process. It was shown that the improved NLC solution stayed stable for 60 days

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