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Optimization and formulation development of nanostructured lipid carriers for furosemide delivery

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Abstract

The goal of this study was to make furosemide more soluble and bioavailable when taken by mouth by adding it to nanostructured lipid carriers. The furosemide-loaded NLC was made using the solvent diffusion method. The solid lipid was labrafil m 2130, the liquid lipid was capryol pgmc, and the lubricant was tween 80. We used a full factorial design to find the best recipes. The concentration of the surfactant, the ratio of solid to liquid lipids, the ratio of total lipids to drugs, and the efficiency of entrapment were all independent factors, and in-vitro drug release was the dependent variable. We tested the new version of Furosemide-loaded NLC to see how much drug it contained, how well it was entrapped, how much drug it could hold, the particle size, PDI, zeta potential, shape, how stable it was in storage, how it released drugs in vitro, and how it did this. Using scanning electron microscopy to look at the particles proved that they were round and had a smooth surface. This was different from the in vitro drug release of pure furosemide. It took zero order dynamics for the medicine to be released. The results showed that NLCs can greatly increase the amount of Furosemide that can be taken through the mouth, even though Furosemide is not easily dissolved.

Keywords: Optimization, nano, lipid carriers, furosemide

Introduction

Nanotechnology in pharmaceuticals is one way to get the therapeutic molecule to the right cell, tissue, or organ at the right time and within the right quantity (Alam, *et al.,* 2018). The best nano drug carrier should have a lot of important qualities, such as the ability to hold enough drugs, be stable in different environments, selectively release drugs at the right time, be safe, work well, and be able to be quickly and cheaply scaled up. Polymeric nanoparticles haven't been used much in clinical medicine because they are expensive and there aren't many approved polymers that are thought to be safe by regulatory officials (Dalal, *et al.,* 2021).

Lipids are often used as the best way to deliver drugs at the nanoscale level, especially for medicines that are lipophilic. This method helps fix the problems that were already mentioned with polymeric nanoparticles (Gul, *et al.,* 2024). The lipid nanoparticles being talked about here are called solid lipid nanoparticles, and pharmaceutical experts are studying them very closely as a smart and new way to give nano drugs (Ahire, *et al.,* 2018). Polymeric nanoparticles were the first nanotechnology idea to improve solubility and, by extension, bioavailability. Their structure is made up of a non-toxic polymer that breaks down naturally and doesn't harm live things. Because the polymers were toxic to cells, there were solvent leftovers from production, there weren't any cheap options, and there weren't many effective ways to make large amounts of nanoparticles, many products couldn't be sold, even though they had good qualities (Fathi, et al., 2023; Thombre, *et al.*, 2022).

The next generation of lipid nanoparticles, called NLC, fixes the problems with SLN, like its small drug capacity, the chance of gel formation, and drug leakage during storage due to lipid polymorphism. NLCs are made up of both liquid and solid lipids (Zewail, et al., 2022). They are created by carefully mixing solid lipids with liquid lipids that don't mix well, which creates a special nanostructure. When solid lipids are mixed with liquid lipids, the structure of the crystals changes in a big way. This is different from how solid lipid nanoparticles are made, which are usually better organized and formed when they are made from solid lipids alone or a mix of solid lipids. The resulting matrix has noticeable flaws in the crystal structure and plenty of room for drug molecules (Natarajan, *et al.,* 2019; Duong, *et al.,* 2020).

This increases the ability to load drugs, stops drugs from leaking out, and improves control over drug release. An important goal of this study was to look into whether NLCs could be used as a new way to deliver furosemide to the stomach. To do this, the liquid diffusion method was used to make furosemide-loaded nanostructured lipid carriers. A complete factorial design was used to improve the synthesis process, and the NLCs that were made were fully described (Poovi, *et al.,* 2018; Gupta, *et al.,* 2023).

Materials and Methods

Furosemide was bought in Mumbai, India, from Yarrow Chem Products. The company Gattefosse in Mumbai, India, gave away free samples of Labrafil and Capryol PGMC. Indian company Central Drug House (P) Ltd provided soy lecithin and stearic acid. The cholesterol came from Specrochem Pvt. Ltd in Mumbai, India. Tween 80 came from the Chemdyes Corporation in Mumbai, India. Otto Chemikabiochemika-reagents in Mumbai, India, is where we got Tween 20 and Tween 2130. DMSO came from Merck Specialties Pvt. Ltd., which is based in Mumbai, India. The rest of the drugs and reagents were all analytical grade.

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).

Excipients Selection

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).

Evaluation of Solubility in 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).

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. 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. 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

Making Nanostructured Lipid Carriers Loaded with Furosemide

The liquid diffusion method was used to make NLCs. The lipids were cooked to a temperature 5–10° higher than their solid lipid melting point. The lipid dispersion was made up of the exact amounts of labrafil and capryol shown in Table 1. A solution of furosemide and liquid soy lecithin was made by mixing them in 5mL of DMSO prior to making the lipid phase. After adding the lipid dispersion to this solution, it was cooked to a temperature between 45 and 50 degrees Celsius. The water phase was made by mixing the right amount of Tween 80 with water, as shown in Table 1. Once the water-based solution had been stirred, it was heated to a temperature between 45 and 50°C. Drop by drop, the fatty phase was added to the water phase in a high-speed homogenizer set to 8000 rpm for five minutes. The mixing took place at room temperature. It was mixed with more liquid until it reached 100 ml, and then it was treated with a probe sonicator for 20 minutes. After the process of cooling, the solutions were kept at 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).

Characterization of NLC

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).

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).

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.

Design Expert's Statistical Analysis of the Responses

The Design Expert software was used to see how each change would affect the intended outcome. There was an analysis done to see how each variable affected each answer in terms of both quantity and quality. The relevant polynomial equations made by Design Expert were used to check the statistical design. To see how each variable affected each reaction parameter at the same time, response surface plots were made.

Choosing the Best Formulation

The best formulation was found with the help of Design Expert program. It's helpful to have this tool because it lets you change all factors at once and gives you a lot of good options from which to choose the best formulation.

Description of the Optimal Composition

The optimized formulation that was picked was tested for how well it captured drugs, how much drug it contained, and how much it could load. In addition to the main study, particle size was analyzed, the polydispersity index was calculated, pharmacokinetics was studied, scanning electron microscopy was used, and the stability of the product was tested.

Particle Size, PDI and Zeta potential

A Malvern Zeta sizer was used to find the average particle size and polydispersity index of the Furosemide-loaded optimized NLC sample that had been made. Photon correlation spectroscopy, which looks at changes in dynamic light scattering caused by the particles' Brownian motion, was used to figure out the average particle size. To get the appropriate scattering intensity, double-distilled water was used to thin out the material. Three sets of measurements were made at a temperature of 25°C and a fixed scattering angle of 90° to the laser beam that was shining on the sample. A polystyrene cuvette that was only used once was used to put the material into the machine. Before adding the new sample, the sample that was meant for that experiment was used to clean the cuvette.

Because it measures the amount of electric charge on the surface of particles, the zeta potential

is a very useful tool for checking how stable colloidal systems are physically. To find out, a method called electrophoretic light scattering was used. The Malvern Zeta Sizer version 7.01 was used to measure the zeta potential of the mixtures. A zeta dip cell was used to measure the zeta potential at 25 °C. A field strength of 20 V/cm was used for the readings, and the best sample was diluted with double-distilled water. Three times were used for each test.

SEM Study

SEM was used to look at the improved NLC sample and see how regular and round the particles were on the surface of the formulations. A scanning electron microscope with a 15kV accelerating voltage was used to look at the materials' shape and structure. The sample was made by using double-sided tape to stick a small amount of the best mixture to a metal specimen stub. The sample was allowed to dry out and be coated with gold sputter before it was imaged.

***In-Vitro* Drug Release**

In-vitro drug release tests using the dialysis method were done on both optimized Furosemide-loaded NLC and pure Furosemide medicine. 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 was put into the dialysis bag in a volume of 1 milliliter. 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. A magnetic mixer set to 100 rpm was used to mix the receptor medium. Between the sample and the receptor medium, the cellophane membrane works as a wall. When the time was right, 1 ml of the sample was taken out of the receiver section and replaced with fresh medium. A pH 6.8 phosphate buffer was used to thin the samples, and a UV-visible spectrophotometer set to 279 nm was used to measure the amount of furosemide that was released.

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.

Results and Discussion

Preformulation Studies

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.

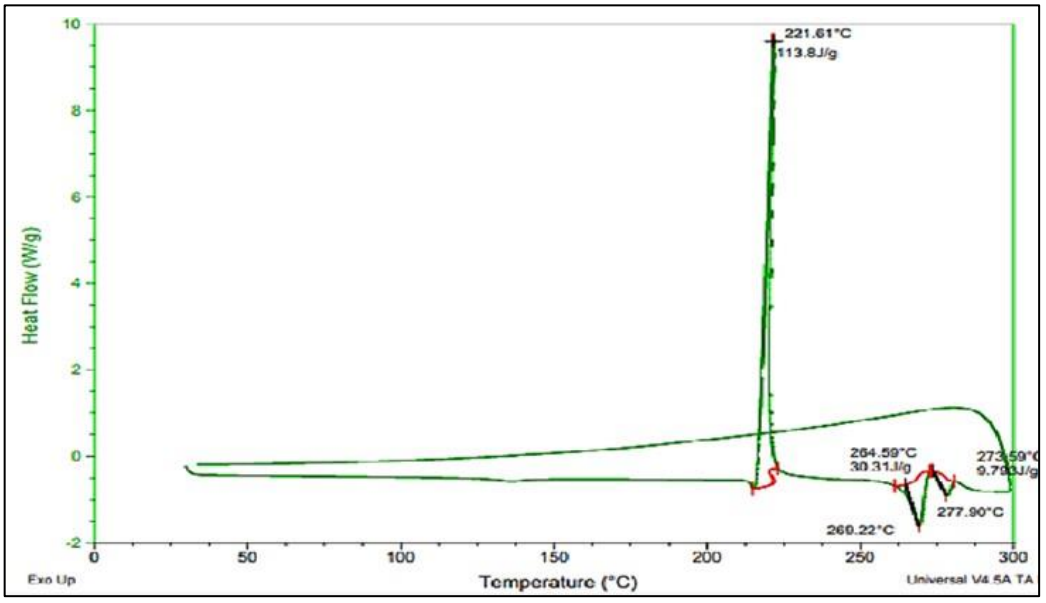

Figure 1: The DSC graph of furosemide

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.

Analysing the Dissolvability of Different Liquid Surfactants and Lipids

The solubility tests showed that of all the liquid lipids that were tried, Capryol PGMC had the best solubility, measuring 4.93 mg/ml. It was found that oleic acid and castor oil had solubilities of 1.66 and 2.08 mg/ml, respectively, which means they were less soluble. It was important to carefully pick a surfactant that lowers the interfacial tension between the lipid phase and the water phase so that the NLCs would be the right size and keep their shape over time. Furosemide dissolves better in Tween 80 than in Tween 20, the two detergents that were tested.

Compatibility Study

Figures 2, 3, and 4 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.

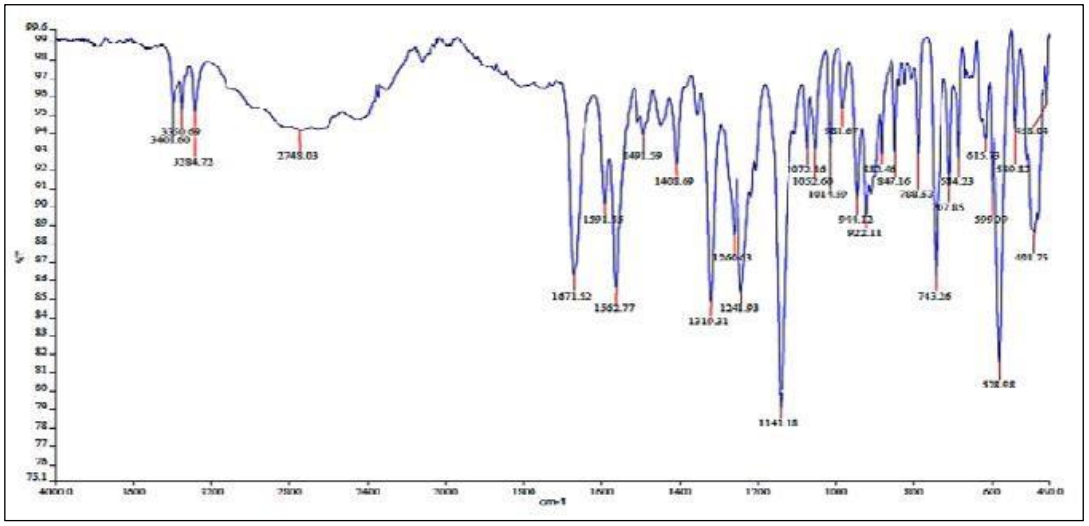

Figure 2: Analysis of Furosemide's FTIR spectra

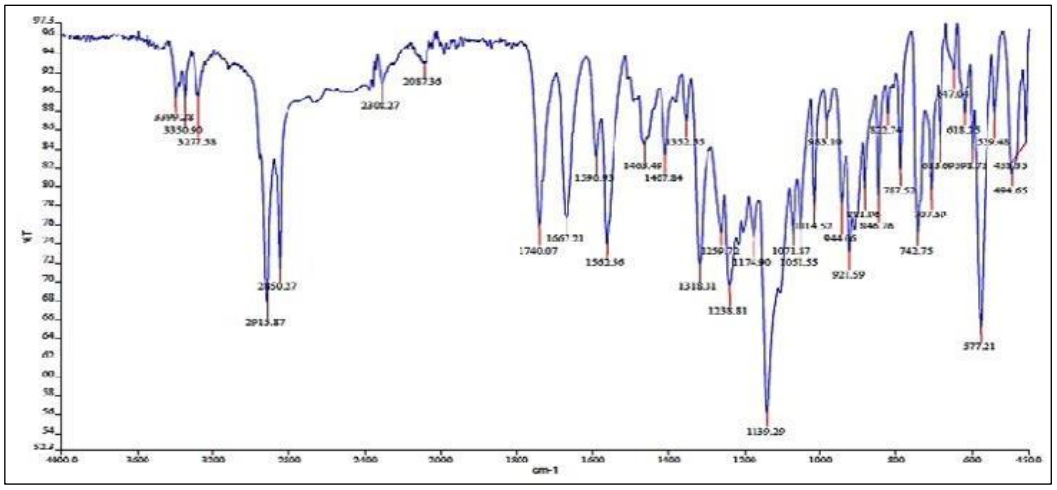

Figure 3: Labrafil-Furosemide FTIR spectra

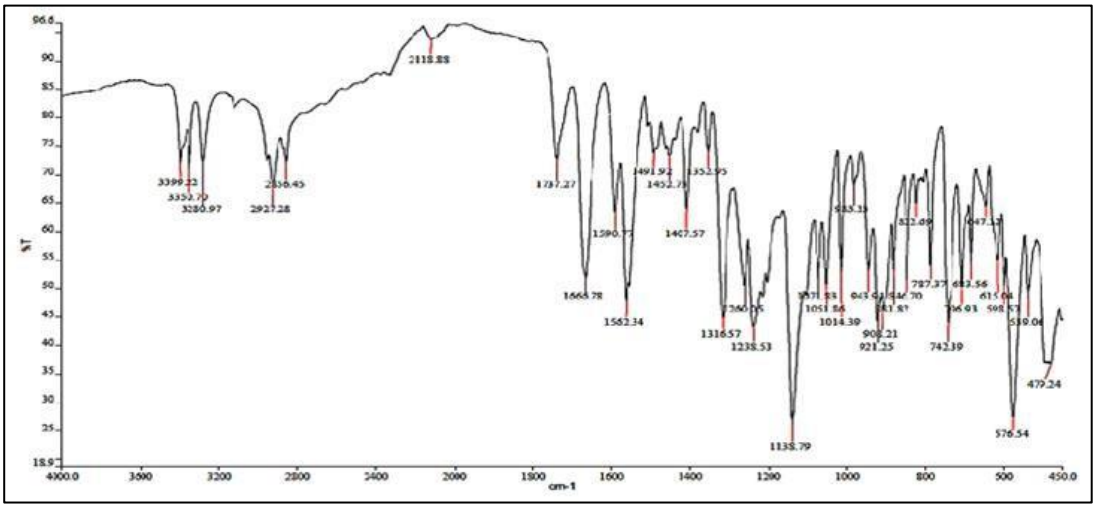

Figure 4: Furosemide and Capryol PGMC FTIR spectra

Encapsulation of Furosemide in Non-Liquid Carrier

Furosemide's nanostructured lipid carrier was made using the solvent diffusion method. Tween 80 was used as a water-loving surfactant, labrafil was used as a solid lipid, and capryol pgmc was used as a liquid lipid.

Drug Content, Entrapment Efficiency, and Loading Capacity Analysis

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

Verification of the Experimental Plan

The chosen independent factors, such as the concentration of surfactant, the solid lipid:liquid lipid ratio, and the total lipid:drug ratio, affected the percentage of in-vitro drug release and how well the drug was trapped. These effects were easily seen and talked about in earlier sections. So, the polynomial coefficients for each dependent variable were found so that the effect of each answer could be judged. We used the model polynomial functions to make three-dimensional surface plots that show the answers we got. The charts show in great depth how the separate factors impact on each response factor, both in a qualitative way and in a way that involves interaction.

Choosing the Best Formulation

The software gave us a lot of choices, and we chose the best one based on scientific studies. This is the recipe that was chosen for the best batch.

Description of the Optimal Composition

Drug Content

How well drugs are trapped and how much they can be loaded Researchers improved the Furosemide-loaded NLC and found that it could entrap 75.50% of the drug and hold 25.63% of it. The research showed that the Nanostructured Lipid Carrier (NLC) that was designed for Furosemide had an 83.56% drug content.

Particle Size, PDI and Zeta Potential

The way particle sizes are spread out is a key factor that affects how stable colloidal systems are. The particles in the NLC that contained furosemide were 99.24 nm on average. The PDI shows how evenly the particle sizes are spread out in the system. Polydispersity is a way to measure how uniform the particles are. Its numbers range from 0 to 1. A low PDI value means that the sizes in the system are not spread out very widely. On the other hand, a high value means that the sizes in the system are spread out widely. The mixture had a PDI of 0.302, which means that the particles were evenly spread out and would stay stable over time (figure 5).

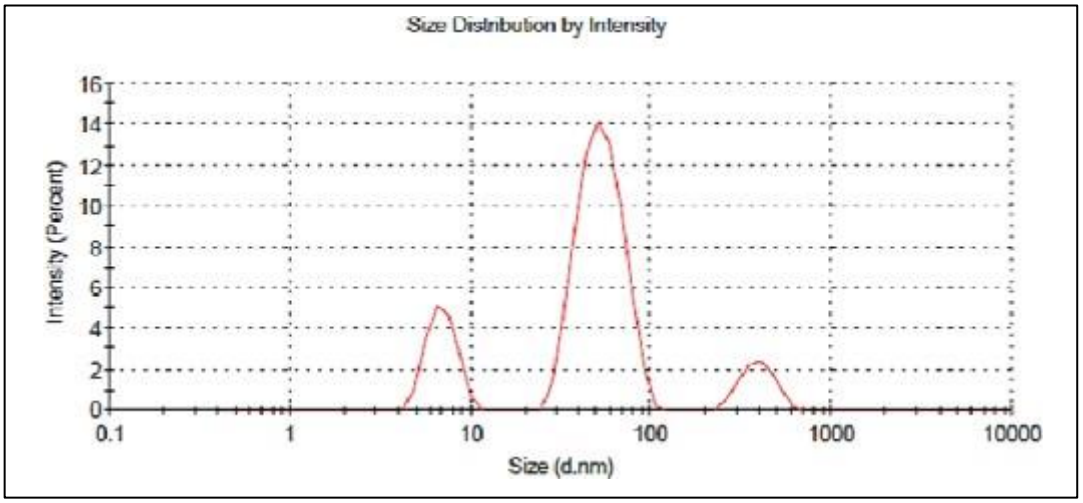

Figure 5: Distribution of particle sizes according to optimum Furosemide NLC intensity

The zeta potential is the difference in potential between the medium for dispersion and the layer of particles that stay still. It tells you how charged the surface of a particle is. Along with the zeta potential, the particle surface charge goes up. Due to the electric force between particles, zeta potential is very important for keeping them stable in suspension. It is good to have a zeta potential of at least 30 mV. We found that the improved Furosemide NLC formulation had a zeta potential of -31.2 mV, which you can see in Figure 6. Particles with the same charge will push away from each other because of electrostatic forces when the negative zeta potential level is high. This pushing away keeps the particles from sticking together and helps keep particle mixtures stable. The NLC value that was found is good enough to make a stable mixture of nanoparticles.

SEM study

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 6. 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.

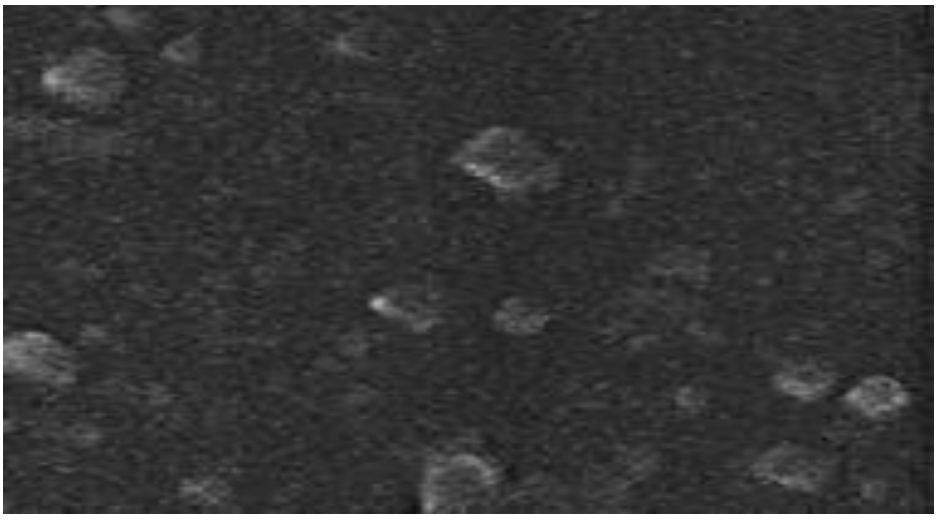

Figure 7: An enhanced Furosemide-loaded NLC's SEM picture

***In-Vitro* Drug Release**

The tests to see how much furosemide and modified furosemide NLC were released were done in a dish with a pH 6.8 phosphate buffer as the receptor medium. For these tests, the dialysis method was used. Figure 7 shows the results of the research that was done. In vitro release of the improved NLC showed an interesting two-phase release with a fast burst effect at the start. Once that was done, the drug was released regularly, but the pure drug was released slowly.

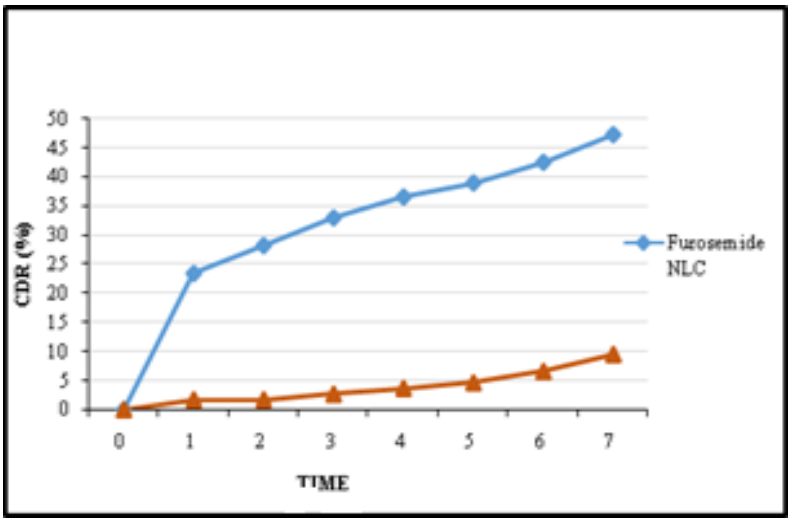

Figure 7: % drug release

Stability study

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.

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.

Reference:

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