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Optimizing Lipid-Based Nanocarriers for the Safe and Efficient Oral Delivery of Tizanidine Hydrochloride

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Abstract:
This study looks into whether nanostructured lipid carriers could be used as an oral preparation to make TZN work better as a medicine. To create TZN-loaded NLCs, the solvent evaporation method called for the employment of solid glyceryl monostearate lipids and liquid oleic acid lipids. Tween 80 and poloxamer 188 were combined to serve as surfactants. Entrapment efficiency and particle size were the primary response criteria utilized in the Box-Behnken design to determine the optimal formulation. The modified blend was evaluated for morphological studies, drug release properties, zeta potential, particle size, polydispersity index, and drug passage/trap efficiency. In in vitro release tests, the NLCs showed significant drug release profiles. This shows that they could be used for better drug delivery with longer release profiles. Based on the results shown above, it looks like the current optimization process can be used to make NLCs that have the right pharmacological qualities. This system might be able to provide a safer and more effective way to deliver TZN in medicines. The study's goal was to improve the therapeutic outcomes and general effectiveness of tizanidine.

Keywords: Tizanidine hydrochloride, solid-lipid, nanocarriers, oral delivery,

Introduction:

Giving medicines by mouth is one of the oldest and most common ways to get them into the body. Oral medicine delivery is better than parenteral drug delivery because it is easier to use, doesn't hurt the patient, is cheaper, and has a high rate of patient cooperation. Oral administration of medicine also lowers the risk of illness transmission and gives more precise control over how often dosages are given (Bharti *et al.,* Keservani *et al.,* 2018; 2012; Khalil *et al.,* 2021; Pramanik and Thakkar, 2020). Recent estimates show that a large amount, between 40% and 70%, of newly created chemicals that are meant to be used as medicines don't dissolve well in water. This makes it harder for them to be absorbed normally in the digestive system at the levels needed for therapy to work. About 40% of new drug options don't dissolve well in water. This makes oral drug delivery difficult because of problems like misaligned dosage, large differences in how well drugs are absorbed between people and within the same person, and lower bioavailability (Ali *et al.,* 2021; Keservani *et al.,* 2010; Keservani *et al.,* 2018).

When you take tizanidine, which is an imidazoline derivative drug, you can also take its hydrochloride salt, which is written as TZN. It works as an adrenergic stimulant that acts on the central nervous system and relaxes muscles. Tizanidine is a muscle relaxant that works on the central nervous system that is often recommended. It mostly affects the spinal cord and is used to treat stiffness that is caused by MS, a spinal cord injury, or illness (Parhi *et al.,* 2023). The drug TZN is in BCS class II and has low solubility and high absorption. When the brain or spinal cord is damaged, muscle strain can happen (Behera *et al.,* 2010; Sharma *et al.,* 2016). This can be painful or uncomfortable and make it hard to speak or move. People often call this condition "spasmia." Spasticity is a situation in which muscles get too tight, making it hard to move or speak, and it may also be painful. This problem happens when nerve cells in the brain or spinal cord can't talk to muscles as well as they should (Aher *et al.,* 2023; Salem *et al.,* 2020). The drug is not very bioavailable when taken by mouth; only about 21% of it gets into the bloodstream because it has strong first-pass metabolic effects. TZN also has an average elimination half-life of about three hours, which is not very long. Because of this, it needs to be given to patients on a regular basis to keep its healing benefits (Ahire *et al.,* 2018; Keservani and Sharma, 2019). NLCs, which are also called third-generation lipid-based nanoparticles, were created by building on the work that solid lipid nanoparticles had already done. Nanostructured lipid carriers are colloidal drug transport systems made up of lipids that are biodegradable and normal in the body. In order to fix the problems with SLN, liquid lipid is added. This creates a solid structure that can hold a lot of drugs and keeps them from leaking out (Coester *et al.,* 2020; Keservani *et al., 2020;* Keservani *et al., 2022*).

Materials and methods:

Materials:

TZN was given to me for free by Sun pharma Limited India. Poloxamer 188, Tween 80, and glyceryl monostearate oleic acid were all provided by BASF (India). Solvents and substances of analytical grade were used in the study.

Lipid and surfactant selection

When making NLCs, the solubility of the medicine was taken into account when picking the solid lipid. The drug was slowly given after 400 mg of different solid fats were weighed out and heated to 5°C above their own melting points. A vortex mixer was used to mix the drug with 1 milliliter of the liquid fat and surfactant that went with it. After the saturation point was reached, the process was continued, which made the mixture solid for 12 hours. The goal is to make sure that no single particle can be seen without any help (Khulbe *et al.,* 2023; Khambete *et al.,* 2016).

Selection of solid-to-liquid

It was possible to make different physical mixtures with solid-to-liquid lipid ratios running from 55:45 to 85:15. The goal was to see how well the solid and liquid lipids worked together. A speed of 250 turns per minute was used to stir the mixes, and they were heated to 100°C ± 1°C for one hour. After that, the mixtures were cooled to room temperature so that they could harden. A small amount of each hardened mixture was put on filter paper to see if the two lipids could mix. After that, a visual study was done to see if there were any oil droplets, which would mean that the lipids did not mix (Goel *et al.,* 2022).

The surfactant ratio selection

The best amount of surfactant to co-surfactant was found by doing the steps below: It was watched that 5 mL of water and 3% Smix were always mixed together, but the surfactant ratio was changed from 0 to 1, 1:2, 2:2, and 3:2. The goal of the experiment with lipid buildup was to find the best ratio (Keservani *et al.,* 2017).

Formulation of TZN-loaded NLC

The liquid evaporation method was used to make the tizanidine NLC. Solid and liquid TZN, soya phosphatidylcholine-90, and lipids were mixed in 100% ethanol, which was the organic phase, in this method. To make the water phase, Tween 80 and poloxamer 188 were dissolved in pure water in a 3:1 ratio. After that, both stages were kept in a water bath at 70 ± 5°C until they were equal. After that, the organic phase was put into a glass tube and slowly added to the water phase while a magnetic stirrer was kept turning at a speed of 1250 ± 25 revolutions per minute. The mixture was made of cold water and was stirred all the time for an hour. It was then lyophilized to get the NLCs, which were then stored in a sealed case for further research (Pathan *et al.,* 2024; Soliman *et al.,* 2016).

Formulation optimization through Box-Behnken design

We used Design-Expert 12 software to find the best NLC recipe by using the "Box-Behnken design." Three separate parts, each with three different levels, were used in this design. In this study, the drug's concentration (B) was set between 2 mg and 10 mg, the lipid's concentration (A) was set between 200 mg and 400 mg, and the surfactant's concentration (C) was set between 1.0% and 5.0%. Things that were measured in this experiment were the particle size (Y1), the

entrapment rate (Y3), and the polydispersity index (PDI) (Y2) (Ahirrao *et al.,* 2023, Ahire *et al.,* 2020).

Process parameters optimization

A number of processing factors that affect the lipid film deposition method used to make NLCs were tweaked. It is important to keep the temperature of a microemulsion at or above the lipid phase transition temperature when making it. You should also think about the temperature at which the organic liquid can evaporate. GMS and oleic acid were mixed in an 8:2 ratio to make TZN-NLCs. The process of making it happened in a water bath that was between 50°C and 70°C. The NLCs were made using a magnetically-driven mixing device that already had the best speed set for spinning (Dindigala and Kodam, 2023, Ahire *et al.,* 2020).

Zeta-potential, PDI, and particle size, Entrapment efficiency

The polydispersity index (PDI), zeta potential, and particle size were all measured by the particle size instrument. The NLC samples were diluted with Milli-Q water before being analyzed, and these tests were done three times to make sure they were correct. We found out how well NLCs loaded with TZN could trap particles by using the sorting method of column chromatography. The mixture was put into a cylinder filled with Sephadex G-50 so that it would be possible to tell the difference between the drugs that were caught and those that were not. After that, the column was centrifuged, and triton X-100 was used to break down the retrieved NLCs in the filtrate. After carefully pipetting and diluting the supernatant samples, they were looked at with a UV spectrophotometer that was set to a range of 320 nm (Keservani *et al.,* 2016, Ahire *et al.,* 2020).

SEM study of NLC

Field emission scanning electron microscopy was used to look at the improved NLCs' structural shape. A mailed sample was attached to a stub and held in place with carbon back tape, and gold vapors were used to cover the optimized NLCs. A 5 kV increased voltage and a 50.7 KX magnification were used to look at the sample (Pathan *et al.,* 2023, Ahire *et al.,* 2020).

DSC Analysis:

A DSC that had been measured by Indium was used to get the test results. Pure medicine samples, ideal drug-loaded nanostructured lipid carriers (NLCs), and a 2.8 mg sample were all looked at. After that, these samples were put in a standard-grade metal pan and sealed. A screening rate of 20°C/min was used to look at the sample over a temperature range of 50–300°C (Vinukonda, 2023; Ahire *et al.,* 2020).

***In-vitro* release study**

A dialysis method with a 12–14 kDa membrane was used to release drug-loaded NLCs and pure drug solutions in a lab setting. A 5 mL sample was put into a dialysis bag. One end was sealed, and dialysis closing clips were used to secure the other end. After that, the dialysis bags were centrifuged at 300 turns per minute at 37°C ± 0.5°C in a 50 mL phosphate buffer saline solution. At each set time period, 5 mL of the sample was taken out and replaced with the same amount of freshly made saline phosphate buffered solution. After that, a UV-visible spectrophotometer was used to look at the sample at a wavelength of 320 nm (Keservani, *et al.,* 2020; Ahire *et al.,* 2020).

RESULTS AND DISCUSSION Lipid and surfactant screening

The TZN solubility in GMS was found to be 3 mg/mL, which means that there were no problems with dissolve. When tests were done with extra solid fats like tristearin, compritol ATO 888, and stearic acid, turbidity was seen at a concentration of 2 mg/mL. TZN could also be dissolved in oleic acid at a concentration of 3 mg/mL and in caprylic acid at a concentration of 2 mg/mL. The results clearly showed that TZN was the most easily dissolved in both oleic acid and GMS. For making NLCs, oleic acid was picked as the liquid lipid and GMS was picked as the solid lipid. When choosing a non-ionic surfactant, things like low toxicity, high stability, compatibility, and not being affected by pH were all taken into account. When TZN was mixed with Tween 80 and poloxamer 188, it didn't dissolve as well as other non-ionic detergents. Table 1 shows the surfactant mix that was chosen for the NLC preparation (Keservani and Sharma, 2018; Rajora *et al.,* 2024).

Table 1: Ratio of surfactant to co-surfactant is selected.

S. No.	Smix ratio	Lipid Qty. (mg)	Surfactant Conc.		Result
			Poloxamer 188	Tween 80	
1.	1:0	5	70	70	Cloudy
2.	1:1	7	50	75	Cloudy
3.	1:2	9	35.0	110.0	Clear
4.	2:2	9	100.0	50.0	Turbid
5.	3:0	9	110.0	35.0	Turbid

Screening of Solid: liquid ratio

At different levels, it was found that GMS and oleic acid were very compatible. When thinking about how much drug is trapped, it is also important to remember that the amount of liquid fat present is important. As a result, it is important to have as much of the chosen fat as possible. This is because more liquid lipid changes the organized crystalline arrangement of the carrier system, making more room for the medicine. Eight parts of GMS and two parts of oleic acid were used, as shown in Table 2.

Table 2: Solid: liquid ratio screening

Sr. No.	Solid-lipid	Solubility	Result
1.	Stearic acid	1.5 mg	Turbid
2.	CompritolATO 888	1.5 mg	Turbid
3.	Glyceryl monostearate	2.5 mg	Clear
4.	Tristearin	1.5 mg	Turbid

Screening of surfactant ratio

The mixture of tween 80 and poloxamer 188 (1:5) was chosen for further study because it had the most noticeable effect on lipid accumulation, leading to the largest amount seen. To make a

stable microemulsion, as shown in Table 3, you need to find the right amount of surfactants.

Table 3: Screening of surfactant

Sr. No.	Surfactant	Solubility	Result
1.	Tween-80	1.5 mg	Turbid
2.	Poloxamer 188	1.5 mg	Turbid

Temperature and RPM optimization of process parameters

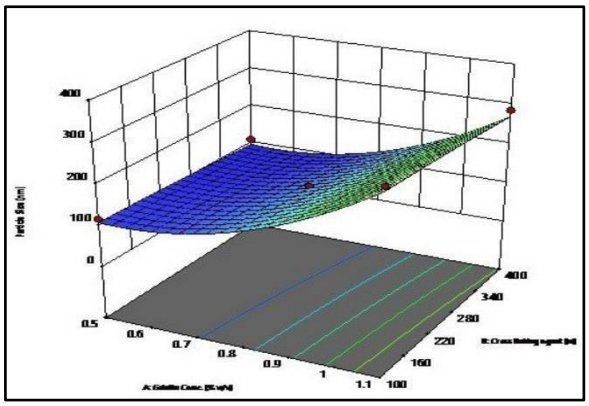
A study was done using an optical lens to look at how temperature and RPM affected the size of NLC. The results show that 70 ± 5°C and 1200 ± 25 RPM are the best temperature and RPM ranges for making NLCs (table 4).

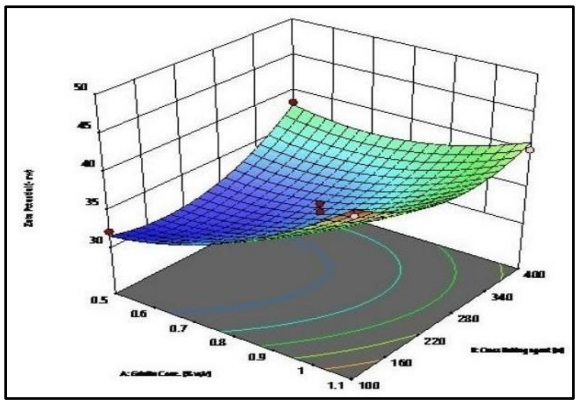
Table 4: Optimum of solid-liquid-lipid

Sr. No.	Lipid ratio (solid: liquid)	Lipid (mg)		Result
		Solid-lipid	Liquid-lipid	
1.	55:45	55	45	Oil stain
2.	60:30	55	40	Oil stain
3.	71:31	65	35	No stain
4.	70:20	70	30	Oil stain
5.	78:18	75	25	No stain
6.	84:14	80	20	Oil stain
7.	85:15	85	15	No stain

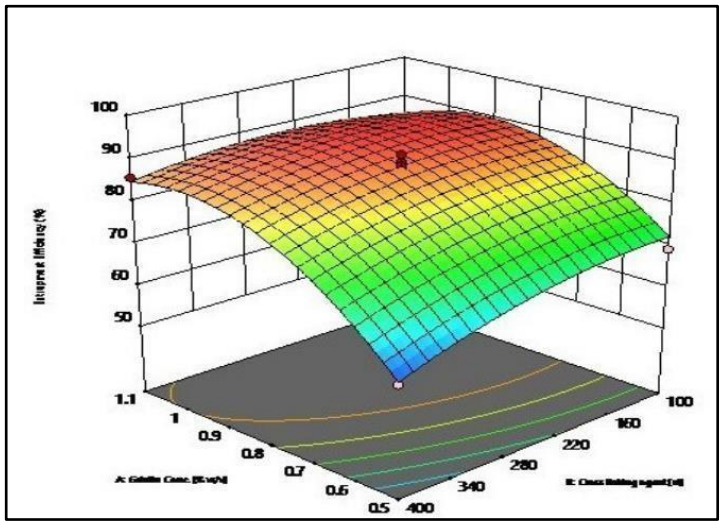
Utilizing Box-Behnken design for optimization

We used a "Box-Behnken experimental design" with three independent factors that each changed at three different levels to find the best formulation. The total lipid concentration (A), the amount of medication (B), and the surfactant concentration (C) were picked as independent variables. The dependent factors that were picked are particle size (Y1), PDI (Y2), and the effectiveness of entrapment (Y3). Figure 1 shows how the correlations between the three answers were shown visually using 3D plots. Table 5 shows the outcomes of the twelve tests that were done (Kattamuri *et al.,* 2012). With the help of non-linear tools, the quadratic model was found to be the best fit for all three dependent variables. The models had a strong link (R^2) that was very close to 1.





(a) (b)



(c)

Figure 1: The effects of independent variables, such as the amount of drug, the concentration of surfactant, and the total lipid concentration, on (a) particle size, (b) polydispersity index, and (c) entrapment efficiency are displayed in a 3D-response surface plot.

Effect of independent variables on (Y1)

The R2 number and adjusted R2 in fit statistics for particle size, which are 0.5716 and 0.5387, show that the model is good enough. It's also very close between the adjusted R^2 and the predicted R^2, with a difference of less than 0.2. It was thought that the coefficient of determination (R^2) would be 0.3851. A number of 17.35 for the F-value shows how important the model is. If the "Probability>F" number is less than 0.0500, it means that the model terms are important. Figure 1a shows a three-dimensional response surface plot that shows how different factors affect the size of particles. This study showed that as the overall lipid content went up, the particles in TZN-loaded NLCs got bigger. On the other hand, as the surfactant content went up, the size of NLC went down (C). The event was caused by the surfactant creating steric hindrance among the microemulsion drops, which stopped the smaller particles from sticking together to make bigger groups during the microemulsion production process.

Effect of independent variables on (Y2)

That the model is important is shown by its F-value of 3.08. When the "Prob>F" numbers are less than 0.0500, the model terms are thought to be important. When the "lack of fit F-value" is 1.68, it means that the lack of fit is not important compared to the pure mistake. This means that the model fits well. The predicted coefficient of determination (R2) was 0.8471, which means that the modified R2 agreed with it well enough. The PDI also had an R2 value of 0.8473. The modified R2 number of 0.5724 means that the fit is very good. The 3-D response plot showed that as the total lipid content went up, so did the PDI of TZN-loaded NLCs. This showed that the PDI was affected by factors that were not related to the lipid content.

Effect of independent variables on (Y3)

The model's model F-value of 14.30 shows how important it is. When the "Prob>F" numbers are less than 0.0500, the "Lack of Fit-value" is 0.41, which means that the lack of fit is not

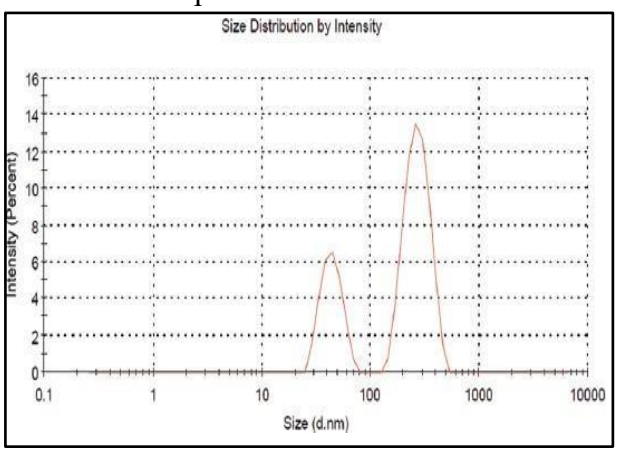
statistically significant. This means that the model fits well. The model has a good fit, as shown by the R2 value of 0.9626, which is very close to the adjusted R2 value of 0.8953. As the total amount of lipids in the cell rises, Figure 1 shows that NLCs become better at capturing and retaining lipids. The observed result is due to the presence of more lipids, which make it easier for drug particles to be completely encapsulated. In addition, the liquid lipid arranges itself in a less ordered way, which makes trapping more effective.

Table 5: Assessment of improved batches of NLCs loaded with tizanidine HCl

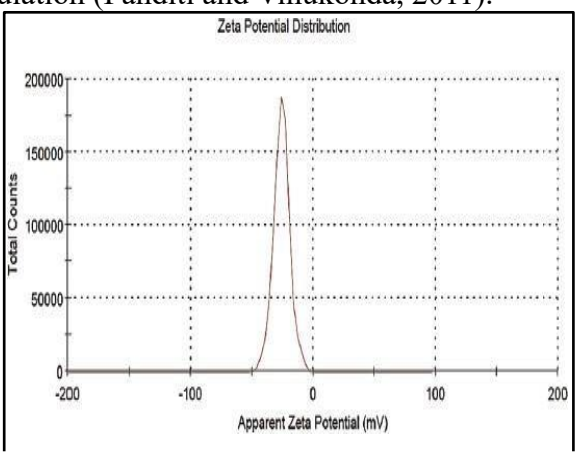
Batches	Lipid (mg)	Drug (mg)	Surfactant conc. (%)	P. size	PDI	% EE
B1	150	1.5	2.5	360.0	0.328	61.10
B2	300	1.5	2.5	495.1	0.355	75.33
B3	150	8	2.5	424.5	0.504	52.21
B4	300	8	2.5	570.2	0.401	79.34
B5	150	5	2.0	545.3	0.420	52.38
B6	400	5	2.0	602.4	0.550	80.45
B7	100	5	5.0	130	0.280	63.56
B8	300	5	5.0	380.2	0.387	79.98
B9	300	1.5	2.0	740.4	0.490	52.79
B10	250	8	2.0	1010.1	0.881	58.35
B11	250	1.5	5.0	360.2	0.316	61.25
B12	250	8	5.0	425.3	0.527	65.13

The optimized formulation's particle size, PDI, zeta-potential, and EE%

Particle size was 318.5 ± 12.35 nm and PDI was 0.365 ± 0.020 in the improved formulation of TZN-loaded NLCs. In Figure 2 (a), we can see the optimized NLC formulation's particle size and size distribution curve. Furthermore, Figure 2 (b) displays the improved formulation's zeta-potential curve, which reveals a measured zeta potential of -26.0 mV for the NLC loaded with TZN. This zeta-potential value is negative, which means the particles in the formulation are stable. Complete electrostatic stabilization is indicated by zeta-potential values falling below -32.1 mV or exceeding 31.0 mV, which should be noted. Also, 79.29 ± 1.88% was found to be the EE of the optimized TZN-loaded NLC formulation (Panditi and Vinukonda, 2011).



(a)



(b)

Figure 3: The optimized tizanidine hydrochloride-loaded nanostructured lipid carriers' particle size distribution (a) and zeta potential (b) are presented.

Morphology of NLC

The picture shown in Figure 3 was taken with field emission scanning electron microscopy to look at the improved makeup of TZN-loaded NLCs. In this picture, you can see both asymmetrical and circle shapes.

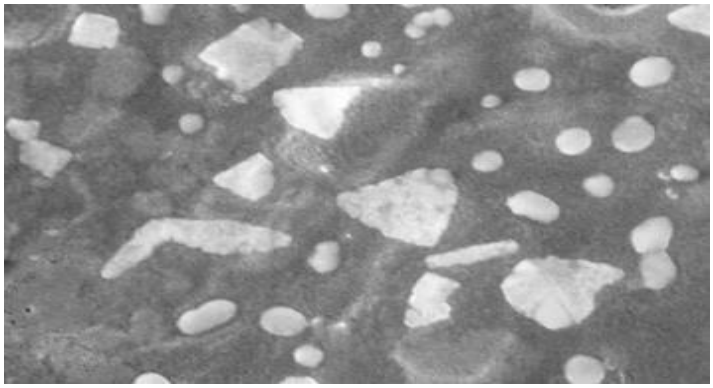


Figure 3: Image of optimized nanostructured lipid carriers obtained using field emission SEM *In -vitro* drug release

Researchers compared the amount of drug released from pure drug and TZN-loaded NLC in a lab setting. They found that the pure drug solution released a lot more drug than the NLC. Figure 4 shows that the difference was most clear in the simulation of intestinal fluid. At the 30-minute mark, the drug solution had an initial percentage total drug release of 32.1 ± 0.8% and the NLCs had 22.8 ± 1.2%. The total amount of drug released rose to 89.2 ± 0.8% for the drug solution and 58.12 ± 1.8% for the NLCs after 24 hours. In particular, the results showed that the drug was released from the NLC over the course of two hours, first quickly and then steadily (Putikam *et al.,* 2012).

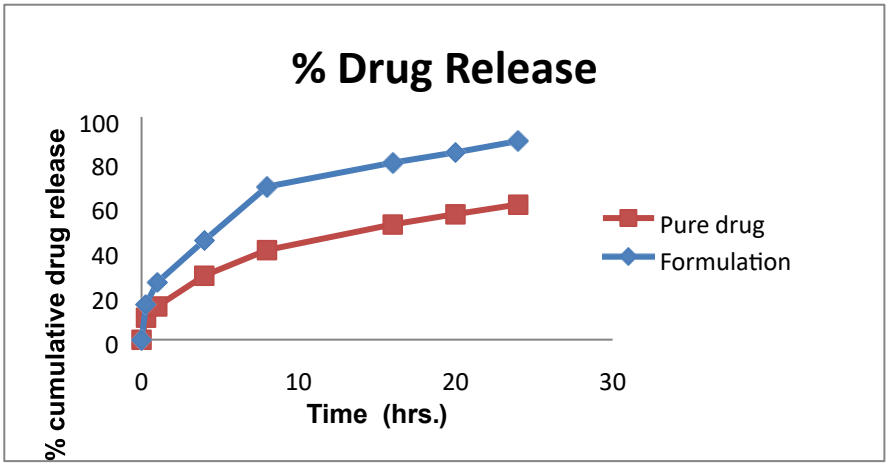


Figure 4: Total percentage of drug release from nanostructured lipid carriers loaded with tizanidine hydrochloride and pure drug

Conclusion:

To successfully make TZN-loaded NLCs, the liquid evaporation method was used, and the formulation was improved using the Box-Behnken design. The NLCs that were loaded with TZN had a polydispersity index (PDI) of 0.365 ± 0.020 and a size of 318.5 ± 12.35 nanometers. The zeta potential of these NLCs was found to be -26.0 mV, and they were able to entrap 79.29% of the particles with an accuracy of ±1.88%. Researchers used the dialysis bag method to study drug release in the lab. The results showed that the drug was released a total of 22.8 ± 1.2% in 30 minutes and 89.2 ± 0.8% after 24 hours. One possible way to give Tizanidine that is both safer and more effective is to put it inside nanostructured lipid carriers (NLCs). Nanostructured lipid carriers (NLCs) could help the drug get around the digestive system without going through it and instead use the lymphatic system. The goal of this approach is to make therapy work better and make tizanidine work better overall. Nanostructured lipid carriers (NLCs) make the medicine more stable by stopping it from breaking down too quickly in the stomach and making it easier for it to get into the lymphatic system. This gives the drug a safe place to be, which lowers the chance of side effects and increases its healing benefits. Based on these results, it looks like the current optimization method can be used to make NLCs that have the right chemical properties to make giving TZN by mouth safer and more effective.

Funding

None

Conflict of Interest

None

References:

1. Aher, P., Surana, K., Ahire, E., Patil, D., Sonawane, D. and Mahajan, S., 2023. Development and Validation of RP-HPLC Method for Quantitative Determination of 4-Amino Benzene Sulphonamide in Sulphonamide Hydrochloride. *Trends in Sciences*, *20*(6), pp.5209-5209.
2. Ahire, E., Thakkar, S., Borade, Y. and Misra, M., 2020. Nanocrystal based orally disintegrating tablets as a tool to improve dissolution rate of Vortioxetine. *Bulletin of Faculty of Pharmacy, Cairo University*, *58*(1&2), pp.11-20.
3. Ahire, E., Thakkar, S., Darshanwad, M. and Misra, M., 2018. Parenteral nanosuspensions: a brief review from solubility enhancement to more novel and specific applications. *Acta pharmaceutica sinica B*, *8*(5), pp.733-755.
4. Ahirrao, S.P., Bhambere, D.S., Ahire, E.D., Dashputre, N.L., Kakad, S.P. and Laddha, U.D., 2023. Formulation and evaluation of Olmesartan Medoxomil nanosuspension. *Materials Today: Proceedings*.
5. Ali, A.A., Hassan, A.H., Eissa, E.M. and Aboud, H.M., 2021. Response surface optimization of ultra-elastic nanovesicles loaded with deflazacort tailored for transdermal delivery: Accentuated bioavailability and anti-inflammatory efficacy. International Journal of Nanomedicine, pp.591-607.
6. Behera, J., Keservani, R. K., Yadav, A., Tripathi, M., & Chadoker, A. (2010). Methoxsalen loaded chitosan coated microemulsion for effective treatment of psoriasis. International Journal of Drug Delivery, 2(2).
7. Bharti. AD., Keservani R. K., Sharma. AK., Kesharwani Rajesh, K. & Mohammed GH. (2012). Formulation and in vitro characterization of metoprolol tartrate loaded chitosan microspheres. Ars Pharmaceutica (53-3), 13-18.

8. Coester, C.J., Langer, K., Von Briesen, H. and Kreuter, J., 2000. Gelatin nanoparticles by two step desolvation a new preparation method, surface modifications and cell uptake. *Journal of microencapsulation*, *17*(2), pp.187-193.
9. Dindigala, A.K. and Kodam, M.R.L., 2023. Application of Lipids in Hot Melt Extrusion Technology. *Journal of Drug Delivery and Therapeutics*, *13*(5), pp.82-86.
10. Goel, H., Siddiqui, L., Mahtab, A. and Talegaonkar, S., 2022. Fabrication design, process technologies, and convolutions in the scale-up of nanotherapeutic delivery systems. In *Nanoparticle Therapeutics* (pp. 47-131). Academic Press.
11. Kattamuri, S.B.K., Potti, L., Vinukonda, A., Bandi, V., Changantipati, S. and Mogili, R.K., 2012. Nanofibers in Pharmaceuticals—A Review. *Am. J. Pharmtech. Res*, *2*(6), pp.188-212.
12. Keservani, R. and Sharma, A.K. eds., 2019. *Nanoparticulate drug delivery systems*. CRC Press.
13. Keservani, R. K., & Sharma, A. K. (2018). Nanoemulsions: Formulation Insights, Applications, and Recent Advances. Nanodispersions for Drug Delivery, 71-96.
14. Keservani, R. K., Sharma, A. K., & Ramteke, S. (2010). Novel vesicular approach for topical delivery of baclofen via niosomes. Lat Am J Pharm, 29, 1364-1370.
15. Keservani, R.K. and Sharma, A.K., 2018. Immunomodulatory and Antimicrobial Effects of Nanoemulsions: Pharmaceutical Development Aspects and Perspectives on Clinical Treatments. In *Nanodispersions for Drug Delivery* (pp. 115-130). Apple Academic Press.
16. Keservani, R.K., Kesharwani, R.K. and Sharma, A.K., 2016. Nanobiomaterials involved in medical imaging technologies. In *Nanobiomaterials in Medical Imaging* (pp. 303-337). William Andrew Publishing.
17. Keservani, R.K., Kesharwani, R.K. and Sharma, A.K., 2017. Introduction to nanotechnology in drug delivery. In *Drug Delivery Approaches and Nanosystems, Volume 1* (pp. 1-19). Apple Academic Press.
18. Keservani, R.K., Sharma, A.K. and Kesharwani, R.K. eds., 2020. *Drug Delivery Approaches and Nanosystems, Two-Volume Set*. CRC Press.
19. Keservani, R.K., Sharma, A.K. and Kesharwani, R.K., 2018. Nanoparticulate nanocarriers in drug delivery. In *Nanobiomaterials* (pp. 3-22). Apple Academic Press.
20. Keservani, Raj K. and Gautam, Surya Prakash. (2020). Formulation and evaluation of baclofen liposome vesicles using lecithin, ARS Pharmaceutica, 61 (3), 175-180.
21. Keservani, Raj K. and Gautam, Surya Prakash. (2022). Skeletal muscle relaxant activity of different formulation of span 60 niosome, ARS Pharmaceutica, 2022. 63 91, 32-44.
22. Khalil, R.M., Abdelbary, A., Arini, S.K.E., Basha, M., El-Hashemy, H.A. and Farouk, F., 2021. Development of tizanidine loaded aspasomes as transdermal delivery system: ex-vivo and in-vivo evaluation. *Journal of liposome research*, *31*(1), pp.19-29.
23. Khambete, H., Keservani, R.K., Kesharwani, R.K., Jain, N.P. and Jain, C.P., 2016. Emerging trends of nanobiomaterials in hard tissue engineering. *Nanobiomaterials in Hard Tissue Engineering*, pp.63-101.
24. Khulbe, P., Singh, D.M., Aman, A., Ahire, E.D. and Keservani, R.K., 2023. The emergence of nanocarriers in the management of diseases and disorders. *Community Acquired Infection*, *10*.
25. Panditi, V.R. and Vinukonda, A., 2011. Development of second order spectroscopic method for the determination of Stavudine in bulk and pharmaceutical Dosage forms. *Journal of Pharmacy Research*, *4*(2), pp.492-493.

26. Parhi, R., Mohanta, B.C., Jena, G.K. and Sahoo, S.K., 2023. Recent advancements in lipid-based nanocarriers for transdermal drug delivery. In *Green Sustainable Process for Chemical and Environmental Engineering and Science* (pp. 347-401). Elsevier.
27. Pathan, A.S., Jain, P.G., Mahajan, A.B., Kumawat, V.S., Ahire, E.D., Surana, K.R., Rajora, A.K. and Rajora, M.A.K., 2023. Beneficial Effects of Water-Soluble Vitamins in Nutrition and Health Promotion. *Vitamins as Nutraceuticals: Recent Advances and Applications*, pp.235-251.
28. Pathan, A.S., Wagh, P.P., Jain, P.G., Sonawane, G.B. and Ahire, E.D., 2024. Functional Foods in Health and Diseases. In *Applications of Functional Foods in Disease Prevention* (pp. 103-117). Apple Academic Press.
29. Pramanik, S. and Thakkar, H., 2020. Development of solid self-microemulsifying system of tizanidine hydrochloride for oral bioavailability enhancement: In vitro and in vivo evaluation. *AAPS PharmSciTech*, *21*, pp.1-11.
30. Putikam, J.K., Rao, Y.N., Anjaneyulu, V. and Undralla, V.K., 2012. Formulation and evaluation of metoprolol succinate extended release tablet. *Research Journal of Pharmacy and Technology*, *5*(1), pp.75-78.
31. Rajora, A.K., Ahire, E.D., Rajora, M., Singh, S., Bhattacharya, J. and Zhang, H., 2024. Emergence and impact of theranostic-nanoformulation of triple therapeutics for combination cancer therapy. *Smart Medicine*, p.e20230035.
32. Salem, H.F., El-Menshawe, S.F., Khallaf, R.A. and Rabea, Y.K., 2020. A novel transdermal nanoethosomal gel of lercanidipine HCl for treatment of hypertension: optimization using Box-Benkhen design, in vitro and in vivo characterization. *Drug delivery and translational research*, *10*, pp.227-240.
33. Sharma, V. K., Koka, A., Yadav, J., Sharma, A. K., & Keservani, R. K. (2016). Self-micro emulsifying drug delivery systems: A strategy to improve oral bioavailability.
34. Soliman, S.M., Abdelmalak, N.S., El-Gazayerly, O.N. and Abdelaziz, N., 2016. Novel non-ionic surfactant proniosomes for transdermal delivery of lacidipine: optimization using 23 factorial design and in vivo evaluation in rabbits. *Drug delivery*, *23*(5), pp.1608-1622.
35. Vinukonda, A., 2023. Determination of Irinotecan enantiomer impurity in Irinotecan Hydrochloride API by using reverse-phase liquid chromatography. *Journal of Drug Delivery and Therapeutics*, *13*(5), pp.41-46.