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Formulation and Characterization of Solid Lipid Nanoparticles for Nasal Delivery of Donepezil to the Brain

Raju Rajapandi[1], Pathan Dilnawaz[2], Sharabu Ravichandra[3], Ramakrishna Kavati[4], Minhas Paramjeet Singh[5], Firake Bhushan Murlidhar[6] and Sethi Pranshul[7]*

[1]Department of Pharmaceutical Analysis, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil 626126, Viruthunagar Dist, Tamil Nadu, India
[2]Department of Pharmaceutics, KJEI' Trinity College of Pharmacy, Pune 411048, India
[3]Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur 522019, Andhra Pradesh, India
[4]Pulipati Prasad College of Pharmaceutical Sciences, Telangana 507305, Near Ammapalem(v), Konijerla(m), Thanikella, khammam (d), Telangana 507305, India
[5]Priyadarshini College of Pharmacy, Koratagere Tumkur 572129, Karnataka, India
[6]KJEI Trinity College of Pharmacy, Pune 411048, India
[7]Department of Pharmacology, College of Pharmacy, Shri Venkateswara University, Gajraula 244235, Uttar Pradesh, India

**Corresponding Author*

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Abstract: The aim of this study was to make solid lipid nanoparticles that are filled with donepezil so that they can be delivered to the brain through the nose. Solid lipid nanoparticles were made using a surfactant mix of tween 80 and poloxamer 188 and a lipid called glyceryl behenate. This was done through a liquid emulsification diffusion method. The solid lipid nanoparticles were tested for their stability, drug release *in vitro*, zeta potential, particle size, and their ability to work in living things. We found that the improved version released 96.72 ± 5.89% of the drug *in vitro* in 24 h. The Higuchi model fit the data the best, with an r^2 value of 0.9504. The best mixture of solid lipid nanoparticles containing donepezil was kept at 4±2 °C (refrigerator) and 25±2 °C/60 ±5% RH for up to six months. Stability studies showed that there were no major changes in the mixture's zeta potential, drug loading, or ability to entrap other molecules. When the improved mixture was kept at 40±2 °C and 75±5% RH, however, the particles got a lot bigger. It seems that the optimized formulation is better at hitting the brain than other formulations because it has higher drug targeting efficiency and direct transfer %.

Keywords: Nose to Brain Delivery, Donepezil, Pharmacokinetic, Solid Lipid Nanoparticles, Glyceryl Behenate

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Introduction

When certain barriers are present, like the blood-brain barrier, the blood-cerebrospinal fluid barrier, and efflux transporters, taking medicine by mouth might not always be able to get the right amount of medicine to the brain (Yasir *et al*., 2018). These walls control the movement of cerebrospinal fluid between the vascular system and the blood flow to the rest of the body. Other factors, like the physical properties of the drug, make it harder to get to the central nervous system (Keservani *et al*., 2016). So, drugs are sent to the brain using different methods, such as breaking the blood-brain barrier (BBB), changing the drugs, and using different ways to send them, like intracerebroventricular, intrathecal, and olfactory pathways (intranasal route) (Topal *et al*., 2020). The intranasal method is a new way to get around the blood-brain barrier that is being developed because it is useful, easy, and does not hurt the brain. It also lowers overall exposure, which means fewer bad effects on the body as a whole (Ahire *et al*., 2020). Because the nasal mucosa and brain are connected neurologically, drugs that are given through the nose can reach the olfactory epithelium part of the nasal mucosa. This section opens up for chemicals to enter the central nervous system (Patil *et al*., 2023).

A number of composition strategies have been made to get around the BBB through the nose, but each has its own problems. Because of this, the idea of lipid-based nanoparticles was developed, like solid lipid nanoparticles (Yasir *et al*., 2022).

Because of these factors, creating a donepezil delivery method that does not involve the mouth will be helpful because it will limit the bad effects of oral treatment, stop systemic exposure, and stop drug distribution to areas that are not supposed to get it (Surana *et al*., 2022). Because donepezil is meant to work in the brain, the method that was made should also send the right amount of medicine to where it needs to be (Arora *et al*., 2022). The aim of this study was to make solid lipid nanoparticles that are filled with donepezil so that they can be delivered to the brain through the nose.

Materials and Methods

Lipid Selection:

One important factor that affects how well the drug encapsulates in the lipid is how well it dissolves in melting lipid. But it is not possible to study equilibrium solubility at this time. So, we used a different method to find the solid fat that had the best chance of dissolving the medicine (Nguyen and Maeng, 2022).

It was taken in a screw-capped tube that contained 20 mg of donepezil. Warming was used to make the solid lipids melt above their melting point. Over time, these lipid melts were added to the donepezil-containing vial in parts while it was being mixed with a vortex mixer and kept at the same temperature (Singh *et al*., 2017). The solubility ended when a clear, light yellow lipid solution was made. Visual observation showed how much melted fat was needed to dissolve the donepezil. Three people, or "triplicates," took part in the experiment (Kopparam *et al*., 2020).

Development of Solid Lipid Nanoparticles Loaded with donepezil:

A basic lab study kept a few things the same and looked at how they changed particle size and how well they were trapped. The drug to lipid ratio was 1:5, or 40 mg to 200 mg. Other factors included the surfactant content (2% w/v), the chloroform: ethanol ratio (5% v/v) as the solution for the drug and lipid, the homogenization time (20–30 min), the stirring time (3 h) and speed (3000 rpm), and the sonication time (10 min) (Bhandari *et al*., 2022). In this study, factors like the drug-to-lipid ratio, the amount of surfactant, and the speed of the stirring were improved even more. We used averages to figure out what happened, and we did three copies of each trial. Table 1 shows the different batches made (Aher *et al*., 2023).

Table 1: Donepezil loaded solid lipid nanoparticles composition

Formulation ID	Parameter				
	Drug (mg)	Lipid (mg)	Surfactant % (w/v)	Stirring time (h)	Stirring Speed (rpm)
F1	40	200	2.0	3.0	3000
F2	40	200	2.0	3.0	3000
F3	40	200	2.0	3.0	3000
F4	40	200	2.0	3.0	3000
F5	40	200	2.50	3.0	3500
F6	40	250	2.50	3.5	3500

Evaluation of Optimized Formulation:

The following characteristics of the optimized formulation of solid lipid nanoparticles loaded with donepezil (F5) were assessed:

Characterization of the optimized formulation:

The measurements of zeta potential, PDI, and average particle size were made using photon correlation spectroscopy. At 250°C, measurements were made at a 90° angle (Espinoza *et al*., 2019). Using a transmission electron microscope, the surface morphology of the optimized solid lipid nanoparticles formulation was examined. Solid Lipid Nanoparticles dispersion was put onto a 300-mesh copper grid covered with carbon film, and the grid was let to air dry for ten minutes in order to conduct the TEM observation (Tekade *et al*., 2023). Following full drying, the sample was repeatedly dyed with a 2% w/v phosphotungstic acid solution and allowed to air dry. Software for soft imaging viewers and digital micrographs were utilized for the picture acquisition and analysis (Sonawane *et al*., 2023).

Drug release kinetic studies:

In vitro release tests with solid lipid nanoparticles loaded with donepezil were used to see how the medicine released from the optimized product and to compare it to the pure drug. A dialysis membrane and the dialysis bag diffusion method were used to do it. A specific number of solid lipid nanoparticles containing donepezil and donepezil fluid were put into a dialysis bag and both ends were shut (Butani, 2018). Each nanoparticle contained 5 mg of the drug. The sealed bag was then put in a beaker with 100 ml of phosphate buffer (pH 7.4, the same as CSF fluid pH) and stirred constantly at 50 rpm at 37±0.5°C. To keep the sink state, 5 ml portions were taken out of the receiver section (beaker) at set times for up to 24 h and replaced with the same amount of fresh medium. Spectrophotometric research was done on the materials at λ max of 270.5 nm (Pardeshi *et al*., 2024).

Stability evaluation:

The goal of the stability studies was to find out how the formulation ingredients changed the stability of the medicine and how the physical stability of the formulation changed with storage temperature and relative humidity (Keservani *et al*., 2017).

Brain targeting Investigation:

Numerous metrics might be used to assess the degree of transport from the nose to the brain, which circumvents the blood-brain barrier and is made possible by a direct link between the brain and the olfactory part of the nasal cavity (Keservani *et al*., 2023). In order to verify the validity of the created formulation for brain targeting, all five factors were identified-- the brain/blood ratio after intravenous and intranasal injection, after 0.5 h; the proportion of relative bioavailability (RB) in the blood and brain after intranasal delivery; the index for drug targeting (DTI); the proportion of drug targeting efficiency (DTE) and the proportion of direct transfer from nose to brain (DTP) (Behera *et al*., 2010).

Results and Discussion

Lipid Selection:

Table 2: Solubility of donepezil in different lipids

Lipid	Melting point of lipid (°C)	Amount(mg) of lipid required to solubilize 20 mg Donepezil
Glyceryl behenate	72	53.79± 8.11
Precirol ATO 5	58	62.41± 5.31
Palmitic acid	64	132.1± 4.20
Stearic acid	70	149.11± 8.10

Table 3: Release kinetic models for the optimal formulation of donepezil-solid lipid nanoparticles

Optimized Donepezil solid Lipid Nanoparticles	Zero order		First order		Higuchi Model		Korsmeyer-Peppas	
	R^2	K_0 (h^{-1})	R^2	K_0 (h^{-1})	R^2	K_0 (h^{-1})	R^2	n value
F5	0.7619	3.4620	0.9210	0.0601	0.9601	20.110	0.9217	0.3911

The ingredients used to make the mixture must not irritate or sensitize the body and must be cleared by pharmaceuticals. They should be thought of as safe in general (GRAS). How solid lipid nanoparticles are made depends on which detergent and lipid are used. How well lipid nanoparticle packaging works depends on how well the drug dissolves in the lipid. It is thought that high lipid solubility could lead to high encapsulation efficiency.

The study on solubility showed that glyceryl behenate was the best at breaking down donepezil, compared to glyceryl palmitostearate, stearic acid, and palmitic acid (Table 2).

Solid Lipid Nanoparticles Preparation:

Table 1 shows how several batches of solid lipid nanoparticles were made using a modified solvent emulsification diffusion process, and Table 3 lists the different observed reactions.

Evaluation of Optimized Formulation:

The following outcomes of an evaluation of the optimized donepezil-solid lipid nanoparticles formulation (F5) for various parameters were observed:

Characterization of the optimized formulation:

Zeta potential curve and particle size distribution of the mixture that was found to work best (F5). Table 3 shows that the average particle size of different groups of solid lipid nanoparticles ranges from 99.42±7.26 nm to 217.19±23.49 nm. The average particle size of the improved version was found to be 99.42±7.26 nm, which means it can be used to target the brain through the nose. There were particles in all batches that were in the nano range, as shown by the PDI readings. The PDI value of 0.173 for the improved formulation was found, which suggests that the solid lipid nanoparticles that were made had pieces of the same size. The zeta potential shows how charged the particles are which mixed in with the solid lipid nanoparticles. A zeta potential between +30 mV and -30 mV makes the particles stable because they don't stick together. The zeta potential of -24.5 mV for the improved Solid Lipid Nano-particles mixture showed that it was very stable. The TEM picture showed a thick, nearly circular structure of solid lipid nanoparticles that had been optimized. It was found that the particle size result and the TEM analysis result were pretty close to each other.

Drug release kinetic studies:

An *in vitro* release study was conducted on the optimized formulation (F5). Solid lipid nano-particles loaded with donepezil showed a burst

Table 4: Results of Brain/Blood Ratio at 30 min, DTI, DTE (%) and DTP (%)

Formulation and route of administration	Brain/blood ratio at 30 min	DTI	DTE (%)	DTP (%)
Donepezil loaded solid lipid nanoparticles i.n.	1.60	3.11	289.71	66.44
Donepezil loaded solid lipid nanoparticles i.n.	0.164	1.61	157.20	37.13
Donepezil loaded solid lipid nanoparticles i.v.	0.03	-	-	-

release at first, followed by a gradual release, according to their dissolving profile. While the delayed release may be due to the drug being contained inside the lipid matrix, the first burst release may be related to the presence of free drug in the external phase and adsorbed drug onto the surface of particles. The optimized solid lipid nanoparticles loaded with donepezil demonstrated an initial burst release of 31.46±9.11% after one hour. Subsequently, they demonstrated sustained drug release, reaching a maximum total drug release percentage of 95.98±6.11% in a 24-h period. The Higuchi model was determined to be the best fitted model for the optimized formulation (F5), with the highest correlation coefficient (R2= 0.9601). Finding the value of the release exponent "n" to be 0.3911 seems to point to a diffusion-controlled release mechanism, or "Fickian diffusion".

Stability evaluation:

The donepezil-loaded solid lipid nanoparticles did not change much in size when stored at 4.0± 2.0 °C (refrigerator) and 25± 2.0 °C /60 ± 5.0% RH for six months. However, the particle size went up a lot ($P < 0.001$) when it was stored at 40± 2.0 °C /75 ± 5.0% RH because the particles clumped together. The average particle size was found to be 2210.50 nm after six months at 40±2.0 °C and 75±5% RH. The PDI number was 0.810. Zeta potential is an important part of body health. Solid Lipid Nanoparticles were kept in a refrigerator at 4.0±2.0 °C and at 25±2.0 °C/60 ±5.0% RH for six months. The zeta potential did not change significantly. However, it did change significantly at 40±2.0 °C/75 ±5.0% RH. This change was related to time and temperature. This could be because the top layer of the lipid nanoparticles breaks down at high temperatures and high relative humidity, causing them to stick together. Over time, both the drug loading (%) and trapping effectiveness (%) went down, but there was no change that could be seen.

In vivo evaluation:

Pharmacokinetic and brain targeting experiments were conducted *in vivo*.

Brain targeting Investigation:

These measurements were used to figure out how much delivery went from nose to brain. The brain/blood ratios for the donepezil-loaded solid lipid nanoparticles (i.n.), the donepezil solution (i.n.), and the donepezil solution (i.v.) were 1.50 for the solid lipid nanoparticles, 0.164 for the solution, and 0.03 for the solution (Table 4). The fact that the donepezil-loaded particles had a much higher brain-to-blood ratio showed that the solid lipid nanoparticles could target the brain. The amount of solubility of the solid lipid nanoparticles loaded with donepezil that were given through the nose was 196.89±13.11 per cent in the brain and 108.12±10.10 per cent in the blood, compared to the solution. The results showed that putting solid lipid nanoparticles containing donepezil into the nose increased its solubility in the brain by a significant amount ($P<0.05$). These results were 3.11 for DTI, 289.71% for DTE, and 66.44% for DTP for the solid lipid nanoparticles loaded with donepezil and 1.61 for DTP, 157.20% for DTE, and 37.13%

for DTP. DTI readings above 1 may show that the path from the nose to the brain is straight. In the end, it was found that using donepezil-loaded solid lipid nanoparticles for brain targeting was more effective than using donepezil solution because the values of DTI, DTE (%), and DTP (%) were higher. with and without donepezil. IV.

Conclusion

We successfully made solid lipid nanoparticles that were loaded with donepezil and tested them for a number of *in vitro* and *in vivo* factors. It was found that all of the parameters were within a good range. According to the results of an *in vitro* release study, the solid lipid nanoparticles that were loaded with donepezil showed a steady release pattern. Stability studies showed that particle size, zeta potential, capture efficiency, and drug loading did not change much over six months at 4.0±2.0 °C (refrigerator) and 25±2.0 °C /60 ±5.0% RH. The better solid lipid nanoparticles that were filled with donepezil were found to last for 2.61 years. Researchers who studied the pharmacokinetics and brain targeting of rats found that when solid lipid nanoparticles containing donepezil were injected intracere-moniously, there was a lot more drug in the brain than when the drug was in solution. So, this study showed that solid lipid nanoparticles can be used to deliver donepezil to the brain through the ventricles.

References

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 Sci. 20(6): 5209-5209.

Ahire ED, Surana KR, Patil CD, Shah HS, Sonwane GB and Talele SG. (2020) Role of omega-3 fatty acids in different neurodegenerative disorders. In: Applied Pharmaceutical Science and Microbiology, Apple Academic Press, pp. 173-194.

Arora D, Bhatt S, Kumar M, Verma R, Taneja Y, Kaushal N, Tiwari A, Tiwari V, Alexiou A, Albogami S, Alotaibi SS, Mittal V, Singla RK, Kaushik D and Batiha GE. (2022) QbD-based rivastigmine tartrate-loaded solid lipid nanoparticles for enhanced intranasal delivery to the brain for Alzheimer's therapeutics. Front Aging Neurosci. 14: 960246.

Behera J, Keservani RK, Yadav A, Tripathi M and Chadoker A. (2010) Methoxsalen loaded chitosan coated microemulsion for effective treatment of psoriasis. Int J Drug Delivery, 2: 159-167.

Bhandari M, Rasool N and Singh Y. (2022) Polymeric lipid nanoparticles for donepezil delivery. In: Polymeric Biomaterials and Bioengineering: Select Proc APA Bioforum 2021, Singapore, Springer Nature Singapore, pp. 51-63.

Butani S. (2018) Fabrication of an ion-sensitive in situ gel loaded with nanostructured lipid carrier for nose to brain delivery of donepezil. Asian J Pharmaceut. 12(4). https://doi.org/10.22377/ajp.v12i04.2838

Espinoza LC, Silva-Abreu M, Clares B, Rodríguez-Lagunas MJ, Halbaut L, Cañas MA and Calpena AC. (2019) Formulation strategies to improve nose-to-brain delivery of donepezil. Pharmaceutics 11(2): 64.

Keservani RK, Kesharwani RK, Sharma AK, Gautam SP and Verma SK. (2017) Nutraceutical formulations and challenges. In: Developing new functional food and nutraceutical products, Academic Press, pp. 161-177.

Keservani RK, Sharma AK and Kesharwani RK. (2016) Medicinal effect of nutraceutical fruits for the cognition and brain health. Scientifica (Cairo) 2016: 3109254.

Keservani RK, Tung BT, Singh S and Kesharwani RK. (2023) Nutraceuticals in Cancer Prevention, Management, and Treatment. CRC Press.

Kopparam M, Kulkarni SV and Shivu SN. (2020) Development, characterization and in vitro evaluation of donepezil solid lipid nanoparticles. Res J Pharma Technol. 13(7): 3113-3121.

Nguyen TTL and Maeng HJ. (2022) Pharmacokinetics and pharmacodynamics of intranasal solid lipid nanoparticles and nanostructured lipid carriers for nose-to-brain delivery. Pharmaceutics 14(3): 572.

Pardeshi VN, Lokhande T, Mahajan SK and Surana KR. (2024) Novel applications of functional foods in brain health. In: Applications of Functional Foods in Disease Prevention, Apple Academic Press, pp. 71-88.

Patil SJ, Surana KR and Mahajan SK. (2023) In vitro antimicrobial and antifungal activity of Mentha piperita active phytoconstituents. Res J Agril Sci. 14(6): 1875-1877.

Singh P, Kesharwani RK and Keservani RK. (2017) Antioxidants and vitamins: Roles in cellular function and metabolism. In: Sustained energy for enhanced

human functions and activity, Academic Press, pp. 385-407.

Sonawane VN, Suryawanshi KG, Wagh KH, Sonawane SL, Sonar AD, Sakle SJ., Surana KR, Patil DM and Sonawane DD. (2023) A comparative study of dissolution profiles on various brands of diclofenac sodium prolonged release tablet formulation. Prog Chem Biochem Res. 6(4): 341-355.

Surana K, Ahire ED, Pawar R, Khairnar R, Mahajan S, Kshirsagar S and Keservani RK. (2022) Oral health and prebiotics. In: Prebiotics and probiotics in disease regulation and management, (eds.) Kesharwani R.K., Rao T.J.M. and Keservani R.K., Scrivener Publishing LLC, pp. 291-309.

Tekade AR, Suryavanshi MR, Shewale AB and Patil VS. (2023) Design and development of donepezil hydrochloride loaded nanostructured lipid carriers for efficient management of Alzheimer's disease. Drug Develop Indust Pharma. 49(9): 590-600.

Topal GR, Mészáros M, Porkoláb G, Szecskó A, Polgár TF, Siklós L and Bozkir A. (2020) ApoE-targeting increases the transfer of solid lipid nanoparticles with donepezil cargo across a culture model of the blood–brain barrier. Pharmaceutics 13(1): 38.

Yasir M, Sara UVS, Chauhan I, Gaur PK, Singh AP, Puri D and Ameeduzzafar. (2018) Solid lipid nanoparticles for nose to brain delivery of donepezil: Formulation, optimization by Box–Behnken design, in vitro and in vivo evaluation. Artificial Cells Nanomed Biotechnol. 46(8): 1838-1851.

Yasir M, Zafar A, Noorulla KM, Tura AJ, Sara UVS, Panjwani D, Khalid M, Haji MJ, Gobena WG, Gebissa T and Dalecha DD. (2022) Nose to brain delivery of donepezil through surface modified NLCs: Formulation development, optimization, and brain targeting study. J Drug Deliv Sci Technol. 75: 103631.