ZITA MEDICAL MANAGEMENT

www.hsmc.gr/en/pharmakeftiki-journal/

PHARMAKEFTIKI, 37, 2S, 2025 | 416-419 RESEARCH

https://doi.org/10.60988/p.v37i2S.243

Development and assessment of rhodamine B-loaded nanostructured lipid carrier-based *in situ* gel for enhanced brain targeting

Salam Shanta Taher[1,*], Khalid Kadhem Al-Kinani[1]

[1]*Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq*

KEY WORDS:
BBB; *in situ* gel; *in vivo*; NLC; rhodamine B

ARTICLE INFO:
Received: January 08, 2025
Revised: February 06, 2025
Accepted: February 14, 2025
Available online: October 10, 2025

*** CORRESPONDING AUTHOR:**
Salam Shanta Taher, Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq; e-mail: sallam.hashem@copharm.uobaghdad.edu.iq

ABSTRACT

The development of effective drug delivery systems for brain targeting remains a significant challenge due to the restrictive nature of the blood-brain barrier. The intranasal route has emerged as a promising alternative, enabling direct drug transport to brain tissue via the nasal cavity. The primary objective of the present study was to investigate the incorporation of rhodamine B (a model fluorescent marker) into *in situ* gel-based nanostructured lipid carriers (NLCs) for potential brain targeting *via* the trigeminal or olfactory pathways. The formulated NLCs were characterized for particle size and polydispersity index (PDI). *In vivo* biodistribution studies were conducted in rats, which were divided into a control group and a treatment group receiving a single intranasal administration of rhodamine B-loaded NLC *in situ* gel. The distribution of the nanocarriers within brain tissues was assessed using fluorescence microscopy. The optimized formulation exhibited a mean particle size of 109.3 ± 9.8 nm and a PDI of 0.35 ± 0.07. Biodistribution analysis revealed a time-dependent accumulation of rhodamine B within brain tissues, with peak fluorescence intensity observed at 2 h post-administration. These findings suggest that rhodamine B-loaded NLC *in situ* gel enhances brain-targeting efficiency and may serve as a viable strategy for intranasal delivery of therapeutics intended for the management of central nervous system disorders.

1. Introduction

Delivering drugs effectively to the brain remains a major challenge in pharmaceutical research due to the selective permeability of the blood-brain barrier, which restricts the entry of most therapeutic agents. The

PHARMAKEFTIKI, 37, 2S, 2025 | 416-419

nose-to-brain delivery route has emerged as a promising strategy for the direct transport of various compounds from the nasal cavity to distinct brain regions *via* the olfactory or trigeminal pathways[1]. This approach necessitates the use of nanocarriers to facilitate the efficient transport of therapeutic agents across the nasal mucosa and into the brain. Nanocarriers enhance bioavailability, improve molecular stability, and enable targeted delivery, thereby optimizing navigation through the olfactory and trigeminal routes.

The development of robust nanosystems has become a priority in drug delivery research. Lipid-based nanocarriers – including microemulsions, spanlastics, and nanostructured lipid carriers (NLCs) – have been extensively investigated for their ability to deliver both lipophilic and hydrophilic drugs, while protecting them from enzymatic degradation and mucociliary clearance within the nasal cavity. Concurrently, the exploration of thermosensitive polymers with mucoadhesive properties for the formulation of *in situ* gel matrices is ongoing, with the aim of enhancing the therapeutic efficacy of lipid-based nanocarriers and minimizing adverse effects[2–4].

Rhodamine B, a fluorescent dye, is widely employed as a model compound for the assessment of the performance of novel drug delivery systems. Its incorporation into an NLC-based *in situ* gel enables real-time tracking of biodistribution and facilitates validation of brain-targeting potential[5]. The present study aimed at developing and evaluating a rhodamine B-incorporated NLC *in situ* gel for brain-targeted delivery in rats.

2. Methodology

Rhodamine B was procured from Central Drug House (CDH®). Glyceryl monostearate and glycerol trioleate were obtained from Shanghai Macklin Biochemical Co., Ltd. Tween 40 and other analytical-grade reagents were purchased from Sisco Research Laboratories (India). Soluplus® and Kolliphor® P 407 were sourced from D-BASF.

Glyceryl monostearate (used as the solid lipid) and glycerol trioleate (serving as the oil phase) were combined and melted at 85°C. During lipid phase preparation, 5 mg of rhodamine B were incorporated. A hot aqueous surfactant solution containing Tween 40 and Soluplus® was added dropwise to the lipid phase under continuous stirring for 30 min. The resulting emulsion underwent sonication in a water bath for 15 min, followed by probe sonication for 2.5 min using an ultra-probe sonicator (Q Sonica Q500, USA) at 30% amplitude with a 2-sec on/off pulse cycle, performed over an ice bath to achieve reduced particle size[6].

The optimized rhodamine B-loaded NLC was subsequently incorporated into a gel matrix containing 19% Kolliphor® P 407 and 0.1% carbopol. The final formulation was stored at 4°C for further analysis. Particle size and polydispersity index (PDI) were measured using a Malvern Zetasizer Ultra (Hungary); each sample was diluted with double-distilled water and analysed in triplicate.

In vivo studies were conducted on rats under approval from the Research Ethics Committee for Experimental Investigations at the College of Pharmacy of the University of Baghdad (protocol number: REC02202507A). To assess brain-targeting efficiency, a small-scale study was designed using ten adult female Wistar rats (190–220 g). The animals were divided into a control group (n=2), which received no treatment, and a treatment group (n=8), which received 40 µL of the rhodamine B-loaded NLC *in situ* gel intranasally *via* a micropipette. At designated time points (0, 0.5, 1, 2, and 5 h), two rats were euthanized using an overdose of diethyl ether followed by cervical dislocation.

Brain tissues were immediately harvested, rinsed with isotonic saline, and fixed in 10% formaldehyde solution for histopathological analysis. Tissue slide preparation involved three sequential steps: fixation, embedding, and sectioning. Fixation stabilized tissue architecture using formalin or alcohol-based fixatives. Embedding was performed in paraffin wax following dehydration and clearing, providing structural support for slicing. Sectioning was carried out using a microtome to produce slices of 3–5-µm thickness, which were mounted on slides for staining and microscopic evaluation.

Fluorescence imaging was conducted using a fluorescence microscope (Zeiss, Germany) equipped with rhodamine-specific filters (excitation wavelength: 530–570 nm). Images were captured using a digital

PHARMAKEFTIKI, 37, 2S, 2025 | 416-419

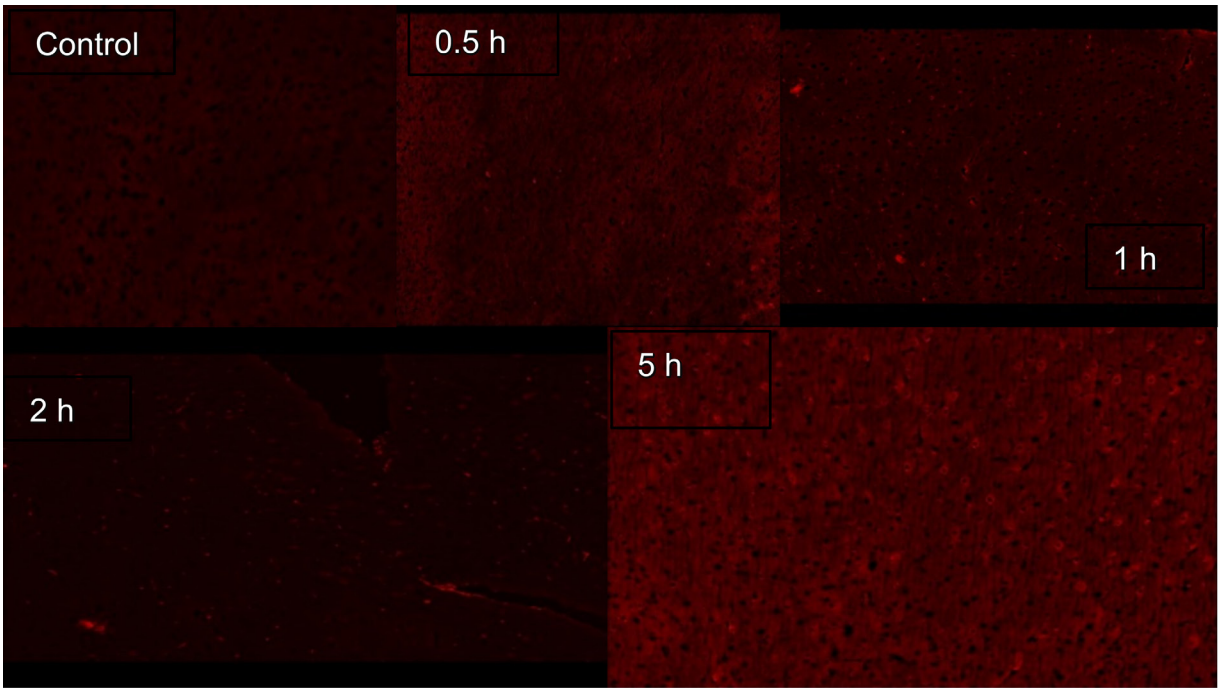

Figure 1. *Fluorescence images of brain tissues from the control group (no treatment) and from the groups administered rhodamine B-loaded nanostructured lipid carrier (NLC) in situ gel via the intranasal route.*

camera (Axiocam 202 mono) and processed in Adobe Photoshop to enhance contrast while preserving data integrity[7].

3. Results and Discussion

The rhodamine B-loaded NLC *in situ* gel exhibited a particle size of 109 nm and a PDI of 0.35. Numerous studies have identified particle size as a critical determinant for successful nose-to-brain delivery[8,9]. Smaller particles are known to traverse the mucosal layer more rapidly and are more readily absorbed by the olfactory epithelium compared to particles exceeding 200 nm. Additionally, PDI influences the *in vivo* behavior of lipid-based nanocarriers, affecting their circulation time, absorption, biodistribution, and clearance. A PDI below 0.3 is generally preferred for intranasal formulations, as lower values – combined with smaller particle sizes – support uniform drug absorption across the nasal mucosa[10].

The biodistribution study revealed a time-dependent accumulation of rhodamine B in brain tissues. As shown in Figure 1, weak fluorescence signals were detected at 30 and 60 min post-administration, indicating initial uptake. The signal was localized to the olfactory bulb, suggesting successful intranasal transport. Fluorescence intensity peaked at 2 h, reflecting maximal brain uptake. Although the signal diminished by the 5-h mark, it remained detectable, indicating sustained retention of the compound. Early detection of fluorescence in multiple brain regions following intranasal administration of the optimized *in situ* gel underscores the rapid transport of rhodamine B from the nasal cavity to the brain *via* the olfactory pathway, involving both neuronal and extraneuronal mechanisms.

4. Conclusion

This study demonstrates that rhodamine B-loaded NLC *in situ* gel achieves peak brain distribution at 2 h following intranasal administration, with gradual clearance thereafter. These findings highlight the potential of this deliv-

PHARMAKEFTIKI, 37, 2S, 2025 | 416-419

ery system for brain-targeted therapies, offering both rapid uptake and sustained release of therapeutic agents.

Acknowledgements

The authors would like to express their thanks to the College of Pharmacy of the University of Baghdad, for its support and the resources provided.

Conflicts of interest

None exist.

ORCIDs

0000-0003-1889-3073 (S.S. Taher); 0000-0002-1233-8944 (K.K. Al-Kinani)

References

1. Patharapankal E.J., Ajiboye A.L., Mattern C., Trivedi V. Nose-to-brain (N2B) delivery: an alternative route for the delivery of biologics in the management and treatment of central nervous system disorders. *Pharmaceutics* 16(1), 66, 2023. DOI: 10.3390/pharmaceutics16010066
2. Jaber S.A., Rajab N.A. Preparation and *in vitro* / *ex vivo* evaluation of nanoemulsion-based *in situ* gel for intranasal delivery of lasmiditan. *Iraqi J. Pharm. Sci.* 33(3), 128–141, 2024. DOI: 10.31351/vol33iss3pp128-141
3. Alkufi H.K., Kassab H.J. Soluplus-stabilized nimodipine-entrapped spanlastic formulations prepared with edge activator (Tween20): comparative physicochemical evaluation. *Pharm. Nanotechnol.* 13(3), 551–563, 2025. DOI: 10.2174/0122117385348551241028102256
4. Tamer M.A., Kassab H.J. The development of a brain targeted mucoadhesive amisulpride loaded nanostructured lipid carrier. *Farmacia* 71(5), 1032–1044, 2023. DOI: 10.31925/farmacia.2023.5.18
5. Sabry S.A., Abd El Razek A.M., Nabil M., Khedr S.M., El-Nahas H.M., Eissa N.G. Brain-targeted delivery of Valsartan using solid lipid nanoparticles labeled with rhodamine B; a promising technique for mitigating the negative effects of stroke. *Drug Deliv.* 30(1), 2179127, 2023. DOI: 10.1080/10717544.2023.2179127
6. Hashim A.A., Rajab N.A. Anastrozole loaded nanostructured lipid carriers: preparation and evaluation. *Iraqi J. Pharm. Sci.* 30(2), 185–195, 2021. DOI: 10.31351/vol30iss2pp185-195
7. Reimold I., Domke D., Bender J., Seyfried C.A., Radunz H.E., Fricker G. Delivery of nanoparticles to the brain detected by fluorescence microscopy. *Eur. J. Pharm. Biopharm.* 70(2), 627–632, 2008. DOI: 10.1016/j.ejpb.2008.05.007
8. Tamer M.A., Kassab H.J.. Optimizing intranasal amisulpride loaded nanostructured lipid carriers: formulation, development, and characterization parameters. *Pharm. Nanotechnol.* 13(2), 287–302, 2025. DOI: 10.2174/0122117385301604240226111533
9. Taher S.S., Al-Kinani K.K. Current nanotechnological strategies for delivery of anti-retroviral drugs: overview and future prospects. *Curr. Drug Ther.* (in press). DOI: 10.2174/0115748855331460241017100207
10. Bonaccorso A., Musumeci T., Serapide M.F., Pellitteri R., Uchegbu I.F., Puglisi G. Nose to brain delivery in rats: effect of surface charge of rhodamine B labeled nanocarriers on brain subregion localization. *Colloids Surf. B Biointerfaces* 154, 297–306, 2017. DOI: 10.1016/j.colsurfb.2017.03.035

HOW TO CITE:
Taher S.S., Al-Kinani K.K. Development and assessment of rhodamine B-loaded nanostructured lipid carrier-based *in situ* gel for enhanced brain targeting. *Pharmakeftiki* 37(2s), 416-419, 2025. https://doi.org/10.60988/p.v37i2S.243