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Development and Evaluation of Nanosuspension Loaded Nanogel of Nortryptiline HCl for Brain Delivery †

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Abstract: Nanogel systems loaded with nanosuspension is a promising approach for nose-to-brain delivery in order to reduce dose and dosing frequency and also improve the bioavailability of the drug. In the present study, an attempt was made to develop the nanosuspension loaded insitu nasal nanogel of nortryptiline HCl (NTH) to get an effective administration through the intranasal route to reach the brain via the olfactory and trigeminal nerves to improve the therapeutic efficiency. The nanoprecipitation-ultrasonication method followed by high-pressure homogenization was elected for the preparation of nanosuspension, which was further incorporated into the in situ gelling polymer solution. Optimized nanosuspension loaded nanogels were prepared by using gellan gum. Optimized formulation show an average particle size of 10–100 nm, a good PDI value, an increase in solubility, a good gelation property, and the desired viscosity to adhere to the nasal mucosa after ionic interactions. In vitro drug release was found to be greater than drug solution over a period of 60 min. Spreadability and viscosity studies show better results for getting good residence time. Hence, it was proved that insitu nanogel is one of the best possible approaches for the targeting of drugs towards the brain in nanoform.

Keywords: Brain targeting; Intranasal delivery; Homogenization; Nanosuspension; Insitu gel

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1. Introduction

One of the most well-known fields of nanotechnology studies is nanomedicine [1]. It helps in the development of specialized pharmaceutical treatments for disease diagnosis, prevention, and treatment using nanotechnology [2]. The size of a nanoparticle ranges from 10-1000 nm. The active pharmaceutical ingredient (API) is entrapped, encapsulated, dissolved, or linked to the nanoparticle matrix [3]. Although nanoparticulate drug delivery systems have several uses, some recent studies have concentrated on the delivery of drugs and treatments across the blood-brain barrier (BBB) [4]. There are several kinds of nanomedicine; however, nanosuspension has a primary emphasis on providing opportunities for drugs that are poorly soluble. Gellan gum (GG) is produced commercially through microbial fermentation using the microorganism *sphingomonas elodea* or *pseudomonas elodea* [5]. Even at low concentrations, GG has the ability to form a firm gel and exhibits bioadhesive properties. Gellan gum is capable of forming gels with monovalent (H^+, K^+, Na^+) as well as divalent ions (Ca^{2+}, Mg^{2+}) [6].

The effectiveness of antidepressant therapy is based on drug concentrations and bioactivity; however, standard oral and parenteral medicines have limitations due to challenges in crossing the BBB [7]. Intranasal administration has the potential to bypass the BBB. This is due to the fact that the trigeminal nerves and olfactory region of the brain are directly connected to the nasal cavity [8,9].

Nortriptyline hydrochloride (NTH), a tricyclic antidepressant, is indicated in the treatment of major depression. Some of the NTH-related challenges, such as its poor oral bioavailability (around 50%) and fluctuating plasma levels, could prevent patients from complying with treatment and lead to failure of therapy.

The purpose of this research is to prepare and optimized an intranasal formulation by incorporating the nortryptiline HCl nanosuspension into the insitu gel for nose-to-brain delivery. The research focuses on the development of nasal gels loaded with poor water soluble drug formulations for intranasal delivery.

2. Materials and Methods

Nortriptyline HCl (NTH) was provided as a gift sample by Micro Labs Pvt. Ltd. Mumbai, India. The excipient that has been used in the research such as tween 80, poloxamers, HPMC K4M, gellan gum, paraben and solvent such as methanol, ethanol and DMSO (analytical grade) was purchase from Research-Lab Fine Chem. Industries, Mumbai. The water used for the experiments was distilled.

2.1. FTIR Analysis

FTIR studies are performed on the FTIR instrument (IR affinity 1S Shimadzu). In this sample was placed on the area of detection, and the FTIR spectrum and peak table were recorded.

2.2. Preparation of Nanosuspension Loaded with Nortryptiline HCl

Nanosuspension was prepared by the nanoprecipitation-ultrasonication method followed by a high pressure homogenizer (GEA Niro Soavi Italy, Panda Plus 2000 Homogenizer). During nanoprecipitation-ultrasonication, the organic phase consisting of NTH and varying concentrations of different surfactants (Tween 80, Poloxamer 407, and Poloxamer 188) in methanol was incorporated into the aqueous phase, i.e., 20 mL of distilled water containing stabilizer HPMC K4M with 10 min. of sonication by a probe sonicator (Athena technology). Prepared nanosuspension was further passed through a homogenizer to achieve the desired particle size by optimizing at 800 bar, suitable for nose-to-brain delivery (Table 1) [10,11].

Table 1. Formulation table of Nortryptiline HCl loaded Nanosuspension.

Batch Code.	Drug (mg)	Methanol (ml)	Ultrasonication Time (min)	Surfactant (mg)			HPMC K4M (mg)	D.W. (ml)
				Tween 80	P188	P407		
NS1	200	3	10	100			100	20
NS2	200	3	10	200			200	20
NS3	200	3	10	300			300	20
NS4	200	3	10		100		100	20
NS5	200	3	10		200		200	20
NS6	200	3	10		300		300	20
NS7	200	3	10			100	100	20
NS8	200	3	10			200	200	20
NS9	200	3	10			300	300	20

2.3. Characterization of Nanosuspension loaded with Nortryptiline HCl

2.3.1. Entrapment Efficiency

Nortriptyline HCl loaded nanoparticles were separated from the aqueous suspension by centrifugation using a cooling centrifuge (Remi C24 Plus) at 10,000 rpm and 4 °C for 20 min. The supernatant was collected, and drug content (free drug) in the supernatant and

with suitable dilutions and absorbance were determined by the UV spectrophotometric method at 240 nm amd calculated [12].

2.3.2. Solubility Studies

Saturation solubility was carried out by adding an excess amount of drug to each vial of a specific solvent and sealing it with a stopper. These vials were attached to an orbital shaker for 24 h at a speed of 50 rpm, and a temperature of around 37 ± 5 °C was maintained throughout the method. Each solution from the vials with suitable dilutions was scanned in a UV-visible spectrophotometer at 240 nm and its solubility calculated [11,12].

2.3.3. Particle Size, Polydispersibility index (PDI) and Zeta Potential

The particle size distribution during process optimization was measured using a laser diffractometer, Mastersizer 2000 (Malvern Instruments). The Zeta potential of optimized batches was determined by a Zetasizer (Malvern Instruments). Nanosuspension samples for analysis were prepared by diluting appropriately with distilled water [11,12].

2.3.4. TEM Analysis

Particle size and the optimization of nanosuspension on the basis of the morphology of nanoparticles were determined by transmission electron microscopy (TEM). The nanosuspension was placed on a carbon-coated grid that was placed on a paraffin sheet, and the sample was left over to allow the nanoparticles to adhere to the carbon substrate. Then the grid was stained with phosphotungstate for 20s. The prepared sample was dried under the IR lamp and then examined by TEM (TEM-FEI, Tecnai G2 spirit biotwin). The photographic images were captured at different magnifications by using Soft Imaging Viewer software [12].

2.4. Preparation of Nanosuspension Loaded Insitu Nasal Nanogel

Deacetylated gellan gum solution is prepared by using different concentrations of gellan gum (500, 1000, and 1500 mg) that are completely dissolved in distilled water using a magnetic stirrer at 300 rpm. On the other hand, PEG 400 (upto 4 mL) was added in optimized nanosuspension (calculated volume) to get a clear sol-gel and to minimize the interactions of ions with gellan gum solution before administration into the nasal cavity. Both solutions were mixed with the addition of methyl paraben (10 mg) as a preservative (Table 2). Finally, the formulation was stored in a properly capped glass container at room temperature and evaluated (Figure 1) [13].

Table 2. Formulation table of Insitu nasal gel.

Composition	NSG1	NSG2	NSG3
Gellan Gum (mg)	500	1000	1500
Optimized Nanosuspension (ml)	12.5	12.5	12.5
PEG 400 (ml)	4	4	4
Methyl Paraben (mg)	10	10	10
Water	q.s.	q.s.	q.s.

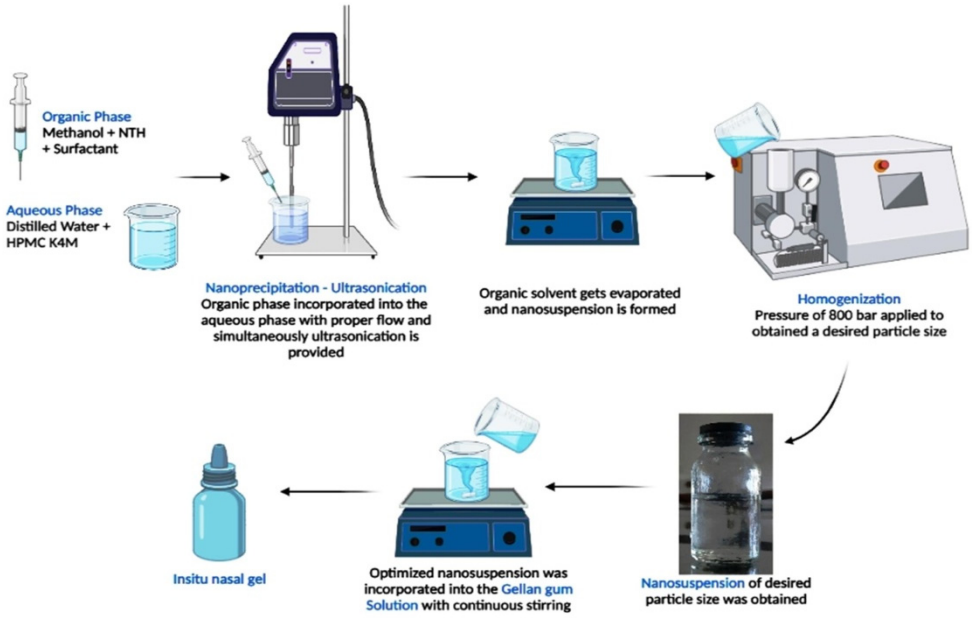


Figure 1. Schemtic representation of preparation of NTH nanosuspension loaded insitu nasal gel.

2.5. Characterization of Nanosuspension Loaded Insitu Nasal Nanogel

2.5.1. Clarity

The clarity of the formulated insitu gels was determined by visual inspection under a black and white background, and it was graded as follows: Turbid, +; Clear, ++; and Very clear (glassy), +++.

2.5.2. Drug Content

The appropriate amount of formulation was diluted in the appropriate solvent, and drug content was determined by using a UV-visible spectrophotometer at λ max 240 nm [13].

2.5.3. pH

Digital pH meter (Hanna Italy, Probe type) is used for the determination of pH. 20 mL of each formulation was taken in a beaker, and a glass electrode was sufficiently dipped into the samples of formulations. Then, the pH of the solution was recorded [13].

2.5.4. Spreadability

Spreadability is the area travelled per unit time (cm^2/min) by the solgel formulation. Whatmann's filter paper was used for the determination of the spreadability of solution formulations NSG1 to NSG3. A 1 mL graduated pipette with a rubber bulb was clamped vertically to the stand in such a way that the tip of the pipette was 2 cm above the horizontal surface of round-shape filter paper. A 0.1 mL sol formulation was dropped at center of the filter paper. At a fixed time interval of 20s, the surface area covered by the formulation was measured [13].

2.5.5. In Vitro Gelation Study

In vitro gelling capacity was determined by placing a freshly prepared solution of in situ gels in a vial containing freshly prepared simulated nasal fluid (pH 5.5–6.5) in a ratio of 1:1 and equilibrating at 37 °C. A visual assessment of gel formation was carried out. The time required for gelation to form gel was noted [13].

2.5.6. In vitro Release

In vitro studies of prepared formulations were carried out using Franz diffusion cells. The diffusion membrane was placed between donor and receiver compartment. Donor compartment contains prepared formulations and the receiver compartment containing simulated nasal fluid (SNF) was maintained at 37 ± 0.5 °C. At specific time intervals, 1 mL of sample was withdrawn from the receptor compartment and replaced with fresh SNF to maintain the sink condition. The samples withdrawn were filtered, and the amount of drug permeated was determined using a UV-visible spectrophotometer at λ max 240 nm [13]. The comparison between the drug solution, nanosuspension and optimized insitu gel was studied. Kinetic models were applied using DD Solver 1.0 (software) to study the drug release.

2.5.7. Viscosity

Brookfield digital viscometer Model DV2T, spindle no. 64, with Rheocalc T 1.2.19 software was used for the determination of viscosity of prepared formulation. Viscosity measurements were done before (at neutral pH) and after gelling (at SNF pH 5.5–6.5) [14].

3. Results and Discussion

3.1. FTIR Analysis

Preformulation study was performed by using FTIR to determine the compatability between the nortriptyline HCl and formulation excipients.

FTIR spectrum of nortriptyline HCl, the N-H stretching was observed at 2932.45 cm^{-1}. C=C stretching was found at 1591.74 cm^{-1}. The C-N stretching was found at 1055.45 and 1158.15 cm^{-1}. The peaks attributed to C-H stretchings were observed at 753.08 cm^{-1} and 804.43 cm^{-1}. Observed peaks confirm that the drug is in pure form and the observed peaks also match the standard reported peaks.

In the FTIR spectrum of the Optimized formulation, the peak found at 2939.52 cm^{-1} and 1589.34 cm^{-1} resembles the N-H stretching and C=C stretching, respectively. The peaks observed at 1056.99 cm^{-1} and 1157 cm^{-1} are attributed to C-N stretching, while 748.12 cm^{-1} shows C-H stretching. All of the above peaks resemble the confirmation of the drug in the optimized formulation. Therefore, FTIR studies illustrate that the drug does not interact with any of the excipients and has compatibility within the formulation.

3.2. Characterization of Nanosuspension Loaded with Nortryptiline HCl

3.2.1. Entrapment Efficiency

During the studies, it was found that the 1:2 (Drug: Polymer) ratio gives an increase in entrapment but not significant as compared to 1:1 and also reported studies state that an increase in polymer concentration causes an increase in particle size. Therefore, the 1:1 ratio is found to be optimized and gives an entrapment efficiency of 93.24 ± 1.23% for P407 (NS8).

3.2.2. Solubility Studies

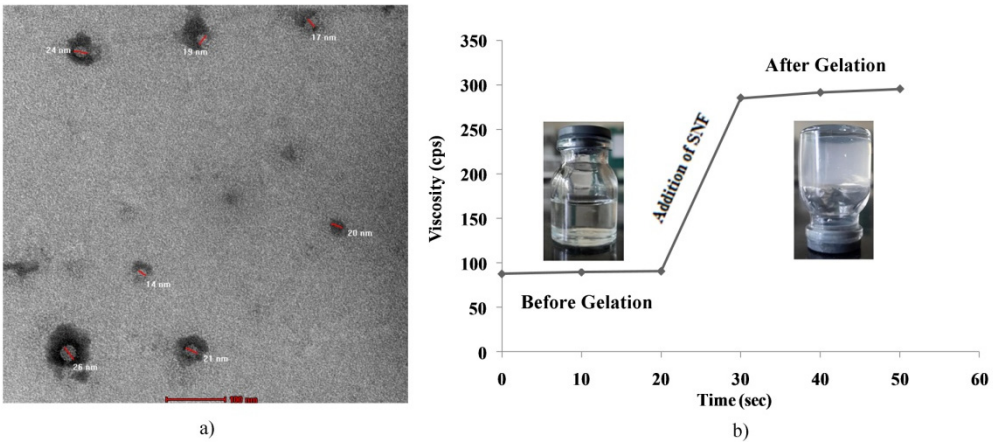
Solubility of the NTH was increase at the nasal pH by preparing the nanosuspension by 4 folds for P407 (NS8).

3.2.3. Particle Size, Polydispersibility index (PDI) and Zeta Potential

The average particle size of the optimized formulation (NS8) was found to be 96.6 ± 16.24 nm, and the PDI was found to be 0.252, which demonstrates that the prepared nanoparticles are able to penetrate for brain delivery and are also homogenous. The zeta potential of the optimized formulation (NS8) was found to be −23.8 ± 5.64 mV. The zeta potential study states that the particles are stable in the optimized nanosuspension (NS8).

3.2.4. TEM Analysis

In the TEM image, it is clearly observed that the prepared nanoparticles are in the range of 10–100 nm (Figure 2a) of optimized nanosuspension (NS8). Thus, the particle size studied by Zeta and TEM states that the desired particle size is achieved.



a) b)

Figure 2. (**a**) TEM microscopy of optimized nanosuspension (**b**) Gelation study at nasal pH of insitu nasal gel.

According to performed studies, poloxamer 407 batch exhibits optimum results. Hence, NS8 is considering for the preparation of nanosuspension loaded insitu nasal nanogel.

3.2. Characterization of Nanosuspension Loaded Insitu Nasal Nanogel

3.2.1. Clarity

The clarity of the formulated insitu gels was determined by visual inspection and founds to be very clear for NSG1 as compared to NSG2 and NSG3 (Table 3).

3.2.2. Drug Content

The % drug content of the prepared insitu gels was found to be in the range of 97.14 ± 1.8% to 98.21 ± 1.5% (Table 3). NSG1 shows highest percentage of drug content.

3.2.3. pH

The pH of all the prepared formulations was found to be 5.5 to 5.9 within the nasal pH range, which proved that the all insitu gels are compatible with nasal pH to reduce irritation during administration (Table 3).

3.2.4. Spreadability

It is very important for insitu gel to have suitable spreadability to administer easily and to spread easily on nasal mucosa without leakage after administration. From the prepared insitu gels, NSG1 shows good spreadability as compared to NSG2 and NSG3 (Table 3).

3.2.5. In Vitro Gelation Study

Gelation studies were carried out using simulated nasal fluid with formulation in 1:1 ratio. All the prepared formulations show gelation within 50s (Table 3) (Figure 2b). NSG1 consumes less time as compared to NSG2 and NSG3. Thus, it states that insitu formed gel preserves its integrity without dissolving or eroding so as to localize the drug at the absorption site for an extended duration or to overcome mucociliary clearance.

Table 3. Different parameters studies of prepared insitu gel.

Formulation Code	Clarity	Drug Content (%)	pH	Gelation Time (s)	Spreadability (cm^2)
NSG1	+++	98.21 ± 1.5%	5.5	40 ± 1.8	1.727 ± 0.5
NSG2	++	97.61 ± 2.1%	5.6	41 ± 1.4	1.413 ± 0.4
NSG3	++	97.14 ± 1.8%	5.9	43 ± 1.9	1.350 ± 0.3

From the above studies, it is revealed that NSG1 was found to be superior formulation. Hence, NSG1 is taking into consideration for the in vitro release and viscosity study.

3.2.6. In Vitro Release

The release of drug solution was found to be 49.04 ± 2.11% and the release of nanosuspension was found to be more about 80.55 ± 2.14%, and furthermore, the final formulation shows the maximum release of 89.93 ± 2.08% in 60 min. This comparison of the drug release states that the permeation of the drug increases with a decrease in the particle size of the drug by preparing a nanoform. The kinetic studies (DD Solver 1.0 software) state that the formulation follows Korsmeyer's Peppas model.

3.2.7. Viscosity

Viscosity increases with an increase in the concentration of gelling agent, as was observed in the prepared formulations. To instill the formulation into the nasal cavity, viscosity should be optimum and also undergo a rapid sol-gel transition due to ionic interaction. Hence, the NSG1 fulfilled the properties of viscosity as compared to the others.

4. Conclusions

In the current research work, we explained how gellan gum, an anionic polymer that forms gel through ionic interactions, was used to develop Nortryptiline HCl nanosuspension loaded insitu gel for nasal administration. This formulation demonstrates an effective drug release of about 89.93%, which might increase bioavailability and therapeutic effectiveness. Particle size was less than 100 nm, and the sol-gel transformation took time less than 50s, which exhibits superior outcomes for intranasal delivery. This suggests that NTH loaded nanosuspension based insitu gel is able to deliver drug directly to the brain through the nose. The present article is expected to help scientists in developing an insitu gelling system for brain and lung delivery to improve the efficacy of treatments for brain and lung related disorders as well as helps to enhance the bioavailability of drugs through the intranasal route.

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