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Design of a Reversible Cholinesterase Inhibitor Mamentine HCL Nanogel: Formulation and *In-vitro* Evaluation

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Memantine hydrochloride is a is a reversible cholinesterase inhibitor used in the treatment of Alzheimer's disease, low-moderate affinity, uncompetitive n-methyl-d-aspartate (NMDA) receptor antagonist, with strong voltage dependency and rapid blocking/unblocking kinetics. The present study was explore the potential of thermosensitive nanogel of mamentine loaded nanoparticle. In situ gel choosing due to restrict unwanted exposure in blood and other healthy tissues, thus eliminate hemolytic side effects of the drug and offer easy administration *in vivo.* Nanoparticle prepared by ionic gelation method and further the dried nanoparticle incorporates with *in situ* gel. The *in situ* gel prepared by cold method using the solutions of Poloxamer-188 and Carbopol-934. The Transmission electron microscopy showed the spherical particles with smooth surface which was in conformity with the SEM and Zetasizer data for particle size. The pH of the formulations was found to be satisfactory and was in the range of 6.8±0.039 -7.4±0.053 and also mucoadhesive strength was show in table. The mucoadhesive strength of all formulations was varies from 2398±0.0004 to 4945±0.0002 dynes/cm^2. *In-vitro* diffusion study of the in situ gel (N_1-N_8) was performed using modified Franz diffusion cell with dialysis membrane in phosphate buffer pH 6.5 for a period of 24 hours. The *in vitro* release study were fitted into various kinetic models viz zero

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order, first order, higuchi model and korsmeyer peppas equation. Stability studies for optimized formulations were carried out at 4.0 ± 0.5°C and 37 ± 0.5ºC for a period of four weeks. There was no significant variation found in physical appearance, average particle size and % drug content of the *in situ* nanogel N_2. No visible changes in the appearance of the gel formulation were observed at the end of the storage period.

Keywords: In situ gel of nanoparticles; Mamentine HCl; in vitro release; stability study.

1. INTRODUCTION

Alzheimer's disease (AD) is the most frequent cause of dementia among the elderly [1]. This disease is charac- terized by an insidious decline in cognitive and non- cognitive functions and is devastating for patients, their family and society. Many types of neurotrans-mitters are affected in this chronic and progressive neurode- generative disorder, and the relative importance of each in relation to clinical findings has not been fully elucidated. Today, no curative treatment exists [2]. The intranasal delivery enhances targeting and reduced systemic side effects [3]. The direct nose-to-brain transport can reduce drug distribution to non-targeted sites, minimizing adverse effects. Scientists started to look for different approaches for brain delivery of drugs, and nasal administration has recently gained special interest. There are various approaches to facilitate nose- to-brain drug delivery, and among them, one finds the use of getting formulation that inhibits the mucociliary clearance, and that of drug delivery nanosystems [4].

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Memantine HCl is a reversible cholinesterase inhibitor used in the treatment of Alzheimer's disease. This does not cross the blood brain barrier (BBB) owing to its hydrophilic nature. Further, a particle size below 200 nm is a very important prerequisite for crossing BBB [6]. So, it was chosen as the drug candidate in present work which was designed to overcome the problems of conventional dosage forms and can be used for brain targeting.

The object of present study was to formulated and evaluated mamentine loaded thermo sensitive in situ nanogel for nasal delivery. In present project a novel drug delivery system i.e. in situ polymeric gel is designed in this type of manner that the gel will load mamentine HCl in better concentration and it will also incorporate penetration enhancer as a way to enhance the absorption of release drug from gel to the systemic circulation. The prepared formulation will remain in liquid form before administration but on administering nasal path then it turns into gel due to its interaction with lachrymal fluid environments like pH, temperature, and ions. Its gel form will retain for maximum period of time and work as reservoir for mamentine. The in situ gel will release the drug in very sustained and controlled manner as well as it also increases the retention and contact time thus increase the bioavailability of entrapped mamentine by creating it bioavailable by raise contact time for longer period of time. This novel in situ gel will triumph over the disadvantages related to traditional dosage form drop and other like low retention time and instantaneous absorption. This novel in situ gel will be affected over the disadvantages related to conventional dosage form (drop and other) like low retention time and immediate absorption of drug that acts most effective for a minimum time. The in situ gel will protect mamentine, improve its retention inside the nasal cavity and release it in a controlled manner for an extended time period to reduce the dose and manipulate blood glucose stage effectively.

2. MATERIALS AND METHODS

2.1 Meterials

Mamentine HCl was obtained as a gift sample from Aurobindo Pharmaceutical Pvt. Ltd. Goa. Chitosan was obtained from Himedia Laboratories Pvt. Ltd. Poloxamer-188 was obtained from Sigma Aldrich, Mumbai. Hydroxypropyl methylcellulose (HPMC) and Carbopol from Central Drug House, Mumbai, India. All other chemicals and solvents were of

analytical grade and used as received. Distilled water was prepared in laboratory using all glass distillation apparatus.

2.2 Preparation of Chitosan Nanoparticle of Mamentine HCl

Nanoparticles (NP) were be prepared as indicated by Calvoet al., [7], utilizing ionotropic gelation method with slight modification in which chitosan (0.4% w/v) was dispersed in aqueous acetic acid solutions (1 % v/v) (pH 6.1), while TPP (0.1 % w/v) was dispersed in deionized water. Mamentine HCl solution was premixed with chitosan arrangement before the expansion of the TPP arrangement drop shrewd into the chitosan solution under magnetic stirring (600 rpm) at surrounding temperature for 2-4 hr. The acquired nanoparticles preparation was lyophilized and store in 4- 8ºC until further utilization.

2.3 Optimization of process Variable

The effect of formulation process variables such as stirring time, stirring speed, surfactant concentration on the particle size was studied. From the results obtained, optimum level of those variables was selected and kept constant in the subsequent evaluations.

2.4 Effect of Chitosan Quantity

The effect of chitosan quantity on the particle size was studied by varying one chitosan. Chitosan nanoparticles were prepared corresponding to varying concentrations of chitosan such as 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9% keeping the amount of Acetic acid (1% v/v), stirring time (4 hours) and stirring speed (600 rpm) constant.

2.5 Characterization of Nanoparticles

2.5.1 Determination of particle size

Particle size analyses were performed by Zetasizer 3000. The measurements were carried out at a fixed angle of 90°. The freeze dried powdered samples were suspended in Milli- Q water (1mg/ml) at room temperature (25˚C) and sonicated for 30 sec. in an ice bath before measurement to prevent clumping. The mean particle diameter and size distribution of the suspension were assessed. Analysis was carried out thrice for each batch of sample under identical conditions and mean values were reported. The same suspension was used for measuring the Zeta potential of drug loaded nanoparticles, by using the same equipment [8].

2.5.2 Preparation of mamentine hydrochloride in Situ nasal gel

Precisely weighted amount of the nanoparticle was dissolve in distilled Water. The solution of Poloxamer-188 and Carbopol-934 were prepared utilizing cold preparation. A specific volume of distilled water was cooled off to 4°C. Poloxamer-188 and Carbopol 934 was sprinkled over deionized cold water independently and was permitted to hydrate for 12 hours to create a clear solution. At that point both the polymer arrangements were blend legitimately with ceaseless mixing. The Benzalkonium chloride was added to the above polymer scattering. At that point put away in the fridge. The scatterings were then put away in an icebox until clear arrangements were acquired and polymer dispersion was gradually added to the drug solution under aseptic condition. Formulation of in situ nasal gel are given in Table 1 [9].

Table 1. Preparation of mamentine hydrochloride in Situ nasal gel

Formulation	N1	N2	N3	N4	N5	N6	N7	N8
Nanoparticles(mg)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Poloxamer-188	14	16	20	14	16	20	14	16
Carbopol	0.1	0.1	0.1	0.2	0.2	0.2	0.2	0.3
HPMC	0.1	0.2	0.3	0.2	0.3	0.4	0.4	0.1
Propylene Glycol	1	1	1	1	1	1	1	1
Benzalkonium Chloride (% w/v)	1	1	1	1	1	1	1	1
Triethanolamine	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Distilled water (ml)	100	100	100	100	100	100	100	100

2.6 Evaluation and Charecterization of *in Situ* Gel [10,11]

2.6.1 Determination of pH

Weighted of gel formulations were transfered in 10 ml of beaker and estimated it by using the advanced pH meter.

2.6.2 Measurement of viscosity

The viscosity of gels was determined by using a Brook Field viscometer DV-II model. T-Bar spindles in combination with a helipath stand were used to measure the viscosity and have accurate readings.

2.6.3 Mucoadhesive strength

Detachment Stress is the power required to detach the two surfaces of mucosa when a definition/gel is set in the middle of them". The detachment stress was measured by using a modified analytical balance [11].

2.6.4 *In-vitro* diffusion study

An in-vitro drug release study was performed utilizing altered Franz dissemination cell. Dialysis layer (Hi Media, Molecular weight 5000 Daltons) was put among receptor and donor compartments. In-situ gel proportional to 100 mg of memantine was set in the contributor compartment and the receptor compartment was loaded up with phosphate cushion, pH 5.5. The dispersion cells were kept up at 37±0.5°C with blending at 50 rpm all through the investigation [12].

The quantitative determination of the qualities acquired in disintegration/dissolution tests is simpler when scientific equations that express the disintegration results as an element of a portion of the measurement shapes attributes are utilized. The pharmacokinetic model to be applied for different method, like zero order, first order, higuchi and pappas model to be applied.

2.6.5 Stability studies

optimized preparation of In-situ gel were exposed to accelerated stability testing under storage condition at 4 ± 1^0C and at room temperature (37 ± 1^0C). both the preparation was put away in screw capped, amber colour little glass bottles at 4 ± 1^0C and 37 ± 1^0C. Examine of the samples were determination for vesicle size and mdrug content after a time of 15, 30, 45 and 60 days [13].

3. RESULTS AND DISCUSSION

The pH of the formulations was found to be satisfactory and was in the range of 6.8±0.039 - 7.4±0.053, as shown in Table 2. The preparations were fluid at room temperature and at the pH formulated. Terminal sterilization via autoclaving had no impact on the pH. as shown in Table 2 and Fig. 1. The viscosity of gels was determined by using a Brookfield viscometer DV-II model. T-Bar spindle in combination with a helipath stand was used to measure the viscosity and have accurate readings. The factors like temperature, pressure, sample size etc. which affect the rheological properties of gels were kept under control in the working area. The temperature, pressure, sample size etc were also kept constant. Some materials are quite sensitive to temperature a relatively small variation will result in a significant change in viscosity while others are insensitive. The temperature which alters the viscosity was maintained at 25ºC because the increase of temperature decreases the viscosity of gels and vice-versa. The shear rate (rpm) and time were variable factors. Before the measurement of viscosity the gels were removed from the freezer and were brought to normal by placing them at room temperature. This was necessary as there was a rise in viscosity in the gels which were not normalized.

The best method for the selection of spindle was trial and error starting from T91 spindle. Spindles in increasing number were used depending on the % torque and error. The goal is to obtain a viscometer dial or display (% torque) reading between 10 & 100, the relative error of measurement improves as the reading approaches 100. Spindle T 95 was found to be suitable and was used for the measurement of viscosity of all the gels. The Helipath T- Bar spindles were rotated up and down in the sample giving variable viscosities at a number of points programmed over the time. Five readings taken over a period of 60 seconds were averaged to obtain viscosity.

The results show that the viscosity of the gels increased with an increase in polymer concentration. The increase in viscosity with the polymer concentration may be due to increase in bonds between the polymer molecules which

lead to formation of a hard and dense compact mass. This may also be due to less amount of liquid in gels with high polymer concentration as compared to gels of low polymer concentration or in other words it can be said the higher the polymer concentration more shear stress if required to produce a specified rate of shear as shown in Table 2, graphical presentation shown in Fig. 2.

Mucoadhesive Strength Detachment Stress is the power required to detach the two surfaces of mucosa when a definition/gel is set in the middle of them". The detachment stress was measured by using a modified analytical balance The result of mucoadhesive strength was show in table. The mucoadhesive strength of all formulations was varies from 2398±0.0004 to 4945±0.0002dynes/cm^2.

Table 2. Results of mamentine HCl nasal *in Situ* gel formulations

Code	pH	Spreadability (gm.cm/sec.)	Viscosity (cps)	Drug content (%)
N_1	6.9±0.63	12.92±4.61	6852.13±1.94	98.73± 0.53
N_2	7.1±0.39	11.63±4.76	9867.15±0.84	96.84± 0.48
N_3	7.2±0.28	12.03±3.63	8763.57±2.73	99.27± 0.74
N_4	7.4±0.83	11.63±6.53	9984.65±1.83	97.85± 0.45
N_5	6.9±0.57	12.06±4.39	9854.64±0.73	98.74± 0.49
N_6	7.2±0.84	13.31±5.61	9469.74±1.73	97.38± 0.62
N_7	6.8±0.28	12.63±4.58	8649.74±1.82	99.68± 0.73
N_8	6.8±0.34	12.82±3.48	7483.68±0.84	98.83± 0.29

**The values are express as mean ±SD for n=3*

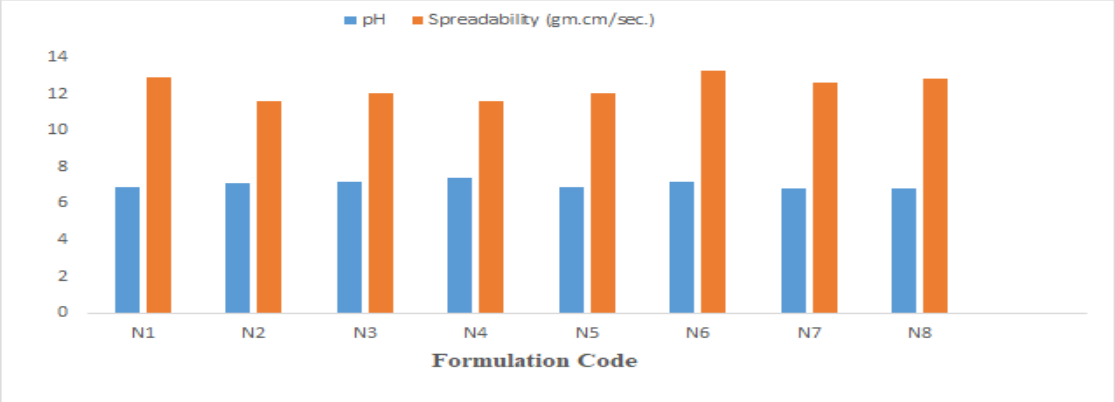

Fig. 1. Graphical Representation of Spreadability and pH

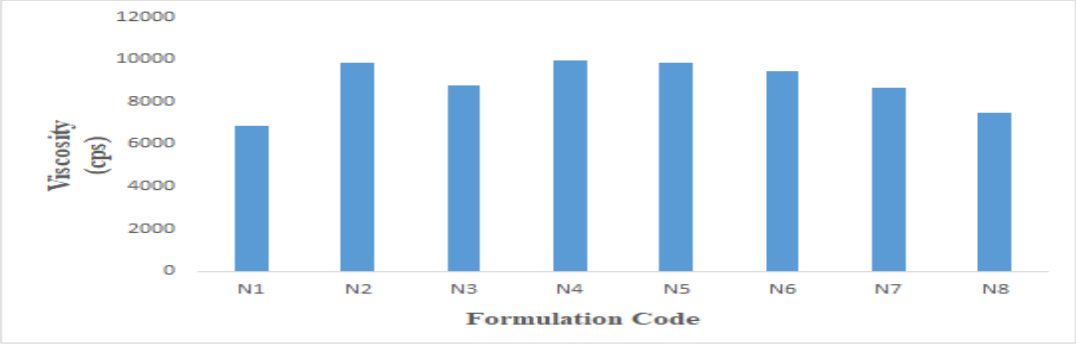

Fig. 2. Graphical representation of viscosity

Table 3. *In-vitro* drug release data of mamentine nanogel

Time	Formulation Code							
(h)	N_1	N_2	N_3	N_4	N_5	N_6	N_7	N_8
1	20.1 ± 0.39	19.8 ± 1.30	19.8 ± 1.30	21.5 ± 2.16	18.3 ± 1.48	21.2 ± 1.54	17.3 ± 2.48	21.4 ± 1.18
2	24.8 ± 1.47	25.3 ± 1.39	25.3 ± 1.39	27.3 ± 1.40	25.4 ± 1.34	28.3 ± 3.48	22.7 ± 1.38	26.4 ± 2.40
3	27.1 ± 0.48	28.4 ± 0.98	28.4 ± 0.98	31.3 ± 2.45	31.3 ± 2.45	33.8 ± 1.49	28.4 ± 3.43	31.2 ± 1.45
4	35.3 ± 1.83	35.3 ± 3.84	35.3 ± 3.84	38.5 ± 1.38	36.5 ± 2.49	37.8 ± 2.48	35.2 ± 1.38	37.1 ± 2.49
6	39.2 ± 0.48	41.5 ± 1.73	41.5 ± 1.73	43.4 ± 2.95	42.4 ± 1.48	44.6 ± 2.35	42.2 ± 3.45	45.2 ± 1.48
8	45.2 ± 1.59	47.5 ± 1.48	47.5 ± 1.48	49.5 ± 3.04	46.7 ± 3.28	49.5 ± 1.50	51.2 ± 1.48	49.2 ± 2.38
12	53.2 ± 1.49	52.6 ± 0.62	52.6 ± 0.62	56.7 ± 2.40	54.5 ± 2.45	57.4 ± 1.46	58.4 ± 2.83	54.3 ± 3.48
24	68.3 ± 0.58	67.5 ± 0.73	67.5 ± 0.73	69.4 ± 3.40	59.6 ± 1.10	61.3 ± 1.40	63.3 ± 2.48	65.5 ± 1.58

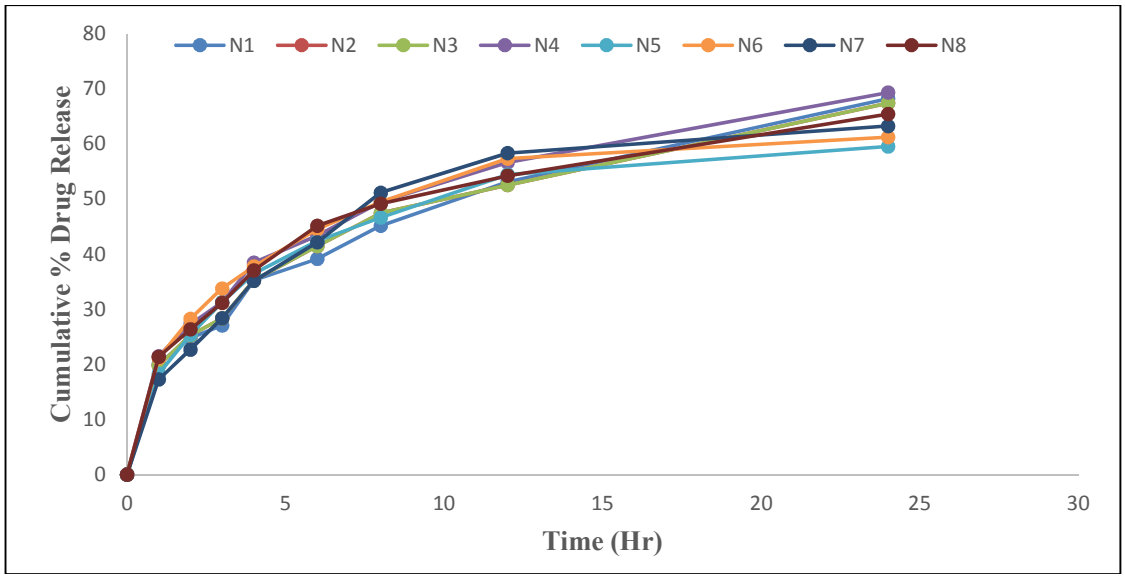

Fig. 3. *In-vitro* drug release data of mamentine nanogel

In-vitro diffusion study of the in situ gel (N1-N8) was performed using modified Franz diffusion cell with dialysis membrane in phosphate buffer pH 6.5for a period of 24 hours. The data obtained from diffusion studies are summarized in Table 3 and Fig. 3.The release rate of Mamentine HCl from in situ formulation over dialysis membrane was significantly higher than its transport across skin, indicating the barrier properties of skin for drugs.

Stability studies for optimized formulations were carried out at 4.0 ± 0.5°C and 37 ± 0.5ºC for a period of four weeks. There was no significant variation found in physical appearance, average particle size and % drug content of the *in situ* nanogel N_2. No visible changes in the appearance of the gel formulation were observed at the end of the storage period as shown in Table 4 and Table 5.

Table 4. Effect of storage temperature on the Particle size of drug loaded *in situ* nanogel N2.

Time (Days)	Average particle size (nm)	
	4.0 ± 0.5°C	**37 ± 0.5ºC**
0	52.2±0.73	62.2±2.73
15	51.93±0.36	65.09± 1.75
30	51.73±2.37	68.86±3.62
45	51.67±1.63	70.73±4.74
60	51.62±3.53	72.59±3.17

**Average of 03 readings*

Table 5. Effect of storage temperature on the % Drug content of loaded *in situ* nanogel N2

Time (Days)	Drug Content (%)	
	4.0 ±1°C	**37 ± 1ºC**
0	73.12±0.25	65.12±0.25
15	73.06± 0.57	60.03±0.48
30	72.86± 0.72	58.81± 0.37
45	71.35± 0.47	55.27± 0.74
60	71.20± 0.62	39.17± 0.52

4. CONCLUSION

The present work was taken up to use the gel forming solution of Poloxamer-188, together with the mucoadhesive polymer such as Carbopol in order to develop a nasal *in situ* gel of Mamentine HCl which can be expected to prove beneficial for overcoming the limitations of oral administration route like first pass metabolism of drug, side effects of drug after its oral administrations like fatigue, diarrhea, nausea, vomiting, etc. From this study, it is concluded that, among all formulation prepared MG8 was the best optimized formulation. Prepared gel can be use as promising nasal drug delivery system for the anti-Alzheimer drug Mamentine HCl, which would enhance nasal residence time owing to increased viscosity and mucoadhesive characteristics; furthermore, it also exhibited a permeation enhancing effect.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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