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Research Article

FORMULATION DEVELOPMENT AND EVALUATION OF MAMENTINE HCL LOADED THERMO SENSITIVE *IN SITU* NANOGEL FOR NASAL DELIVERY

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ABSTRACT

The object of present study was to formulate and evaluate mamentine HCl loaded thermo sensitive *in situ* nanogel for nasal delivery. In present project a novel drug delivery system *i.e. in situ* polymeric gel was designed in the manner that the gel load mamentine HCl in better concentration and it also incorporates penetration enhancer as a way to enhance the absorption of release drug from gel to the systemic circulation. In this research work Different Nanoparticles were (NP) prepared, using ionotropic gelation method with slight medication in which chitosan (0.4 % w/v) was dissolved in aqueous acetic acid solutions (1 % v/v) (pH 6.1), while TPP (0.1 % w/v) was dissolved in deionized water. Then the dried nanoparticle incorporated with *in situ* gel. *In situ* was gel was prepared by cold method using the solutions of Poloxamer-188 and Carbopol-934. From this study, it is concluded that, among all formulations prepared, MG_8 was the best optimized formulation. Prepared gel can be used as promising nasal drug delivery system for the anti-Alzheimer drug Mamentine HCl, which enhance nasal residence time owing to increased viscosity and mucoadhesive characteristics; furthermore, it also exhibited a permeation enhancing effect.

Keywords: Mamentine HCl, Ionotropic gelation method, Chitosan, In situ gel, Evaluation.

1. INTRODUCTION

Alzheimer's disease (AD) is the most frequent cause of dementia among the elderly [1]. This disease is characterized 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 neurodegenerative 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].

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 [5]. 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 formulate and evaluate 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 a manner that the gel loads Mamentine HCl in better concentration and 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.

2. MATERIAL AND METHODS

2.1. Material

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., 1997 [6], 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 4-8°C 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 (3 hours) and stirring speed (600 rpm) constant (table 1).

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 [7].

Table 1: Composition of SLN by varying quantity of Chitosan

Components	Formulation code							
	CN1	CN2	CN3	CN4	CN5	CN6	CN7	CN8
Mamentine HCl	10	10	10	10	10	10	10	10
Chitosan	0.1%	0.2%	0.3%	0.4%	0.5%	0.6%	0.7%	0.8%
Acetic acid	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Stirring speed (rpm)	600	600	600	600	600	600	600	600
Stirring time (hrs)	3	3	3	3	3	3	3	3

2.5.2. Determination of Percentage Yield and Loading Efficiency

The percentage yield of the nanoparticles was determined by calculating accurately the initial weight of the raw materials and the last weight of the nanoparticles obtained [8].

The drug loading efficiency (%) and Drug entrapment efficiency (%) of the nanoparticles can be calculated according to the following equation:

$$EE\ (\%w/w) = \frac{\text{Weight of the drug in nanoparticles}}{\text{Weight of the drug added}} \times 100$$

$$DL\ (\%w/w) = \frac{\text{Weight of the drug in nanoparticle}}{\text{Weight of the polymer and drug added}} \times 100$$

2.5.3. Preparation of Mamentine hydrochloride in Situ nasal gel

Precisely weighted amount of the nanoparticle was dissolved 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 deionised 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 table 2 [9].

Table 2: Formulation Development of *in Situ* Nanogel (F1-F-9)

Formulation	MG1	MG2	MG3	MG4	MG5	MG6	MG7	MG8
Nanoparticles	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	-	-	-	-	-	-	-	-
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.
Purified water (ml)	100	100	100	100	100	100	100	100

Table 3: Formulation Development of *in Situ* Nanogel (F9-F-16)

Formulation	MG9	MG10	MG11	MG12	MG13	MG14	MG15	MG16
Nanoparticles	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.
Purified water (ml)	100	100	100	100	100	100	100	100

2.6. Characterization of Nanoparticulate gel

2.6.1. Determination of pH

Weighed amount of gel formulations were transferred in 10 ml of beaker and estimated by using the advanced pH meter [10].

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 [11].

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 [12].

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 [13].

2.7. Mathematical treatment of *in-vitro* release data

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.

3. RESULTS AND DISCUSSION

The particle size is an important parameter as it has a direct effect on the stability, cellular uptake, drug release and biodistribution. The mean particle sizes of the prepared nanoparticles as measured by the Malvern zetasizer were in size range of 330 to 651 nm and the distribution of particle sizes are found to be monodispersed as the polydispersity index lies below 0 to 1 (0.234 to 0.642) in all the formulations. There were no noticeable differences between the sizes of nanoparticles obtained with different drug polymer ratio. The particle morphology can be modulated by selecting the agitation speed as well as drug polymer ratio. In the present study, the decrease in size of the particles has been reported (table 4). The Particle size, Zeta potential, Entrapment efficiency and Poly dispersity index of optimized formulation CN_{15} was found to be 364.2±3.37, -8.46, 79.9±0.2 and 0.283± 0.048 respectively (table 5).

3.1. Evaluation of *In situ* gel

The pH of the formulations was found to be satisfactory and was in the range of 6.8±0.28 to 7.4±0.83, as shown in table 6. The preparations were fluid at room temperature and at the pH formulated. Terminal sterilization via autoclaving had no impact on the pH. 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. *In-vitro* diffusion study of optimized formulation *in situ* gel (MG_8) was performed using modified Franz diffusion cell with dialysis membrane in phosphate buffer pH 6.5 for a period of 24 hours. The data obtained from diffusion studies are summarized in table 7. The *in vitro* release study were fitted into various kinetic models viz zero order, first order, higuchi model and korsmeyer peppas equation. When the regression coefficient values of were compared, it was observed that 'r' values of Higuchi model was maximum *i.e.* 0.978 hence indicating drug release from formulations was found to follow Higuchi release kinetics (table 8 and Fig. 1-4).

Table 4: Evaluations of Nanoparticle formulations by OVAT

Formulation	Particle Size* (nm)	Entrapment efficiency* (%)	Drug content* (%)	Polydispersity index*
CN1	337.2±4.84	76.7±0.2	64.63±0.78	0.234 ± 0.006
CN2	358.6±5.38	62.2±0.6	69.73±0.83	0.345 ± 0.012
CN3	382.8±3.85	78.6±0.8	72.56±0.63	0.380 ± 0.074
CN4	448.7±6.78	83.1±0.3	63.52±0.45	0.342 ± 0.098
CN5	455.6±8.27	86.3±0.5	69.48±0.54	0.245 ± 0.009
CN6	372.6±4.73	82.2±0.7	63.53±0.32	0.454 ± 0.004
CN7	411.5±6.83	79.2±0.9	72.12±0.25	0.319 ± 0.010
CN8	342.3±4.89	77.5±0.7	67.58±0.42	0.254 ± 0.098
CN9	368.4±2.48	83.8±0.4	71.12±0.38	0.482± 0.027
CN10	448.5±5.39	86.3±0.8	69.57±0.44	0.642± 0.074
CN11	353.6±6.39	81.3±0.5	67.98±0.58	0.371± 0.056
CN12	358.4±4.73	83.4±0.6	71.12±0.39	0.493± 0.084
CN13	362.8±5.75	78.9±0.8	72.59±0.45	0.353± 0.074
CN14	352.6±4.38	73.3±0.7	73.45±0.78	0.348± 0.084
CN15	364.2±3.37	79.9±0.2	74.57±0.69	0.283± 0.048
CN16	442.3±5.71	75.4±0.6	68.69±0.67	0.381± 0.093

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

Table 5: Particle size and zeta potential drug content of optimized formulation

Code	Particle size* (nm)	Zeta potential* (mv)	Entrapment efficiency* (%)	Poly dispersity index*
CN_{15}	364.2±3.37	-8.46	79.9±0.2	0.283±0.048

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

Table 6: Results of Mamentine HCl Nasal *in Situ* Gel Formulations

Code	pH	Spreadability (gm.cm/sec.)	Viscosity (cps)	Drug content (%)
MG1	7.1±0.85	12.53±2.73	7643.68±0.96	98.74 ±0.53
MG2	7.3±0.58	12.08±4.42	9874.03±1.73	98.85 ± 0.63
MG3	7.3±0.69	12.75±3.59	6539.06±1.74	97.51 ± 0.74
MG4	6.9±0.65	13.63±5.69	9743.37±1.26	97.85± 0.37
MG5	6.8±0.47	12.83±4.58	8864.86±2.74	98.58± 0.85
MG6	7.1±0.28	11.53±6.46	9763.11±1.92	98.84± 0.73
MG7	6.8±0.63	11.29±3.52	7963.49±0.74	97.39± 0.62
MG8	7.3±0.48	13.89±3.51	9045.37±0.84	98.85± 0.63
MG9	6.9±0.63	12.92±4.61	6852.13±1.94	98.73± 0.53
MG10	7.1±0.39	11.63±4.76	9867.15±0.84	96.84± 0.48
MG11	7.2±0.28	12.03±3.63	8763.57±2.73	99.27± 0.74
MG12	7.4±0.83	11.63±6.53	9984.65±1.83	97.85± 0.45
MG13	6.9±0.57	12.06±4.39	9854.64±0.73	98.74± 0.49
MG14	7.2±0.84	13.31±5.61	9469.74±1.73	97.38± 0.62
MG15	6.8±0.28	12.63±4.58	8649.74±1.82	99.68± 0.73
MG16	6.8±0.34	12.82±3.48	7483.68±0.84	98.83± 0.29

Table 7: Results of in vitro drug release study of optimized formulation MG_{10}

Time (h)	Square Root of Time(h)1/2	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
1	1	0	19.8±1.30	1.296	80.2	1.904
2	1.414	0.301	25.3±1.39	1.403	74.7	1.873
3	1.732	0.477	28.4±0.98	1.453	71.6	1.854
4	2.000	0.602	35.3±3.84	1.547	64.7	1.810
6	2.449	0.778	41.5±1.73	1.617	58.5	1.767
8	2.828	0.903	47.5±1.48	1.676	52.5	1.720
12	3.464	1.079	52.6±0.62	1.720	47.4	1.675
24	3.742	1.146	67.5±0.73	1.829	32.5	1.511

**Average of three reading*

Table 8: Release Kinetics of Optimized formulation MG_{10}

Zero order	First Order	Higuchi	Korsmeyer-peppas
	r^2		
0.895	0.964	0.978	0.911

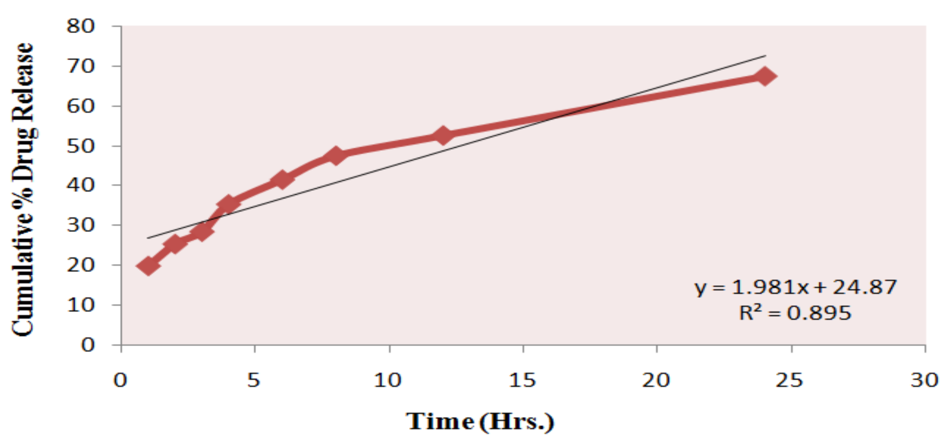

Fig. 1: Zero order release Kinetics of optimized formulation MG_{10}

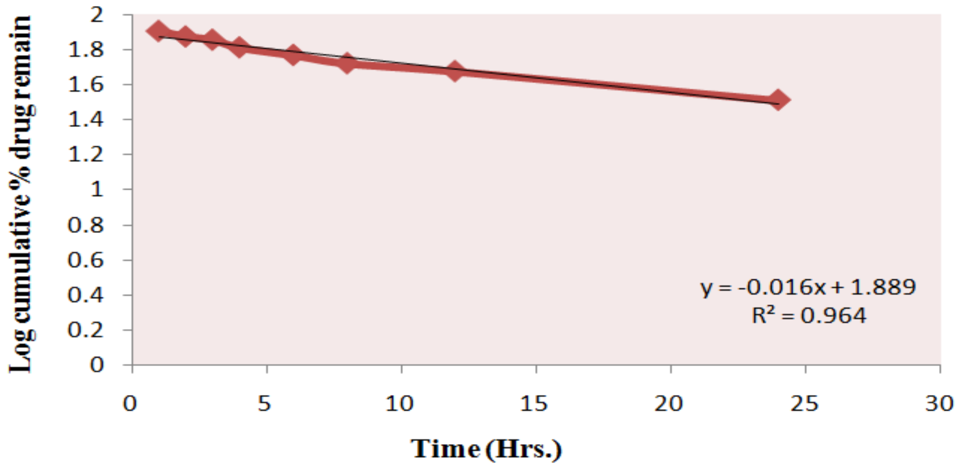

Fig. 2: First order release Kinetics of optimized formulation MG_{10}

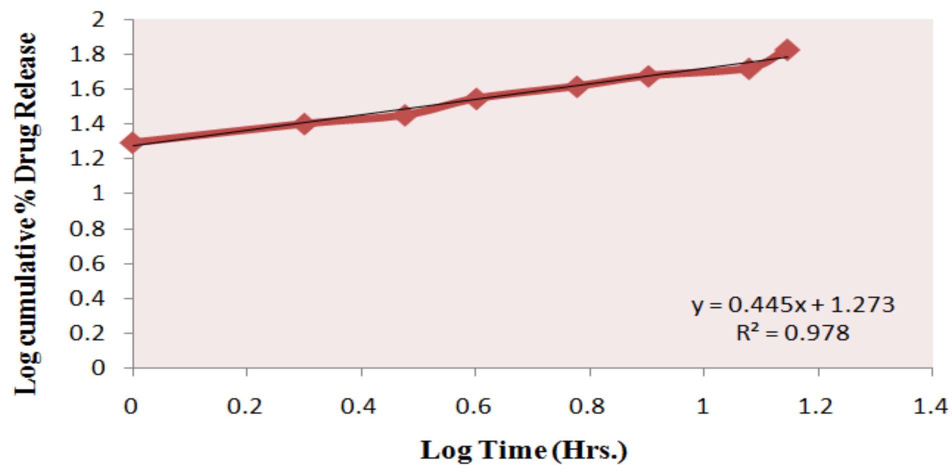

Fig. 3: Higuchi release Kinetics of optimized formulation MG_{10}

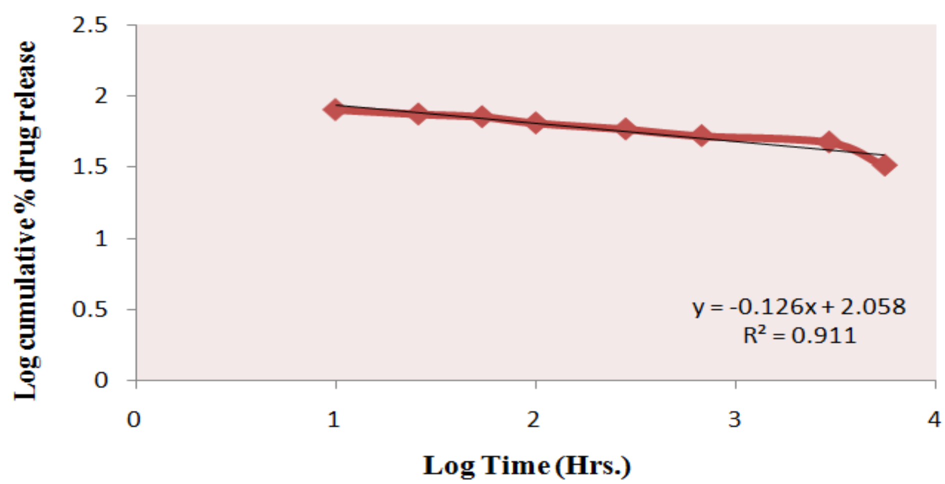

Fig. 4: Korsmeyer-peppas release Kinetics of optimized formulation MG_{10}

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 MG_{10} 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.

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