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Reversible Cholinesterase Inhibitor Loaed Chitosan Based Nanoparticle

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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 kineticsThe present study was explore the potential of mamentine loaded nanoparticle.with varying quantity of chitosan byionotropic gelation method.The effect of chitosan quantity on the particle size was studied by varying stirring time and stirring speed. The particle morphology can be modulated by selecting the agitation speed as well as drug polymer ratio In the present study.The evaluation parameters like Zeta potential, Entrapment efficiency and Poly dispersity index,of optimized formulation CN15 was found to be 364.2±3.37, -8.46, 79.9±0.2 sand 0.283± 0.048 respectively Determination of percentage yield and loading efficiency,in vitro drug release was also found optimum en 99-100%.

ARTICLE DETAILS

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KEYWORDS: Ionotropic gelation method; chitosan based nasal drug delivery system; in situ gel systemevaluation; in-situ polymeric gel formulation, Mamentine HCl

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INTRODUCTION

Alzheimer's Disease is a neuropathological disorder that causes dementia by progressively degenerating the neurons that are responsible for learning and memory processes. Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects over 24 million people worldwide; representing an immense medical, social and economic burden. 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 [1]. 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.

Chitosan contains abundant amino and hydroxyl groups, which enable nanoparticle formulation via both physical and chemical cross-linking [2]. Ionic cross-linking of Chitosan is a typical non-covalent interaction, which can be realized by association with negatively charged multivalent ions such as tripolyphosphate (TPP) [3,4]. For pharmaceutical applications, physical cross-linking is more promising since the cross-linking is reversible and may largely avoid the potential toxicity of the reagents. Although diverse efforts have been made to obtain the chitosan nanoparticles via TPP cross-linking following the pioneering work of Calvo et al.The Chitosan nanoparticle further incorporated with cabopol gel applied by nasal route of administration has gained substantial interest for obtaining brain uptake of polar or hydrophilic drugs. The olfactory region connected to nasal cavity is the only site of the body where the CNS is in direct contact with the external environment. So, develop a new formulation of memantine hydrochloride loaded chitosan nanoparticles for possible targeted delivery to the brain. **Rao *et al.*, (2018)** fabricated protein nanoparticles for better controlled and targeting action of drug, which can also overcome the problems like multidose therapy, poor patient compliance and high cost associated with conventional formulations. Memantine HCl loaded casein nanoparticles (F1 to F6) were prepared by ionically cross-linked method. The formulated nanoparticles were evaluated for external morphological characters, determination of particle size analysis, zeta potential, drug content, entrapment efficiency and in-vitro release studies [5]. **Ionotropic gelation method,** mechanism of chitosan NP formation is based onelectrostatic interaction between amine group of chitosan andnegatively charge group of polyanion such as tripolyphosphate. Thistechnique offers a simple and mild preparation method in theaqueous environment. First, chitosan can be dissolved in acetic acid in the absence or presence of stabilizing agent, such as poloxamer,which can be added in the

chitosan solution before or after theaddition of polyanion. Polyanion or anionic polymers was thenadded and nanoparticles were spontaneously formed undermechanical stirring at room temperature. The size and surfacecharge of particles can be modified by varying the ratio of chitosanand stabilizer.

MATERIALS AND METHODS

Material

Mamentine HCl was obtained as a gift sample from Aurobindo Pharmaceutical Pvt. Ltd. Goa. Chitosan was obtained from HimediaLaboratories Pvt. Ltd. Poloxamer-188 wasobtained from Sigma Aldrich, Mumbai. Hydroxypropyl methylcellulose (HPMC) andCarbopol 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.

Methods

Preparation of Chitosan Nanoparticleof Mamentine HCl

Nanoparticles (NP) were be prepared as indicated by Calvo et 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 withchitosan 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.

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.

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 **(Table 1).**

Characterization of Nanoparticles

i.Determination of Particle Size

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

ii.Determination of percentage yield and loading efficiancy

The percentage yield of the nanoparticles was determined by calculating accurately the initial weight of the raw materials and the last weightof the nanoparticles obtained [9].The drug loading efficiency (%) and Drug entrapment efficiency (%) of the nanoparticles can be calculated according to the following equation:

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

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

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)	4	4	4	4	4	4	4	4

Table 5.8: Composition of chitosan nanoparticle by varying Stirring time

Components	Formulation code							
	CN_9	CN_{10}	CN_{11}	CN_{12}	CN_{13}	CN_{14}	CN_{15}	CN_{16}
Mamentine HCl	10	10	10	10	10	10	10	10
Chitosan	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%
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)	1	2	3	4	5	6	7	8

Table 5.9: Composition of chitosan nanoparticle by varying Stirring speed

Components	**Formulation code**							
	CN_9	**CN_{10}**	**CN_{11}**	**CN_{12}**	**CN_{13}**	**CN_{14}**	**CN_{15}**	**CN_{16}**
Mamentine HCl	10	10	10	10	10	10	10	10
Chitosan	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%
Acetic acid	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%
Stirring speed (rpm)	100	200	300	400	500	600	700	800
Stirring time (hrs)	4	4	4	4	4	4	4	4

Compatibility study by FTIR

Identification and authentication of drug sample was done by *infrared spectroscopy*. The IR spectra showed the presence of principal groups 2978.73, 2941.58,2859.59,2846.81,2152.36, 1511.78, 1455.27, 1355.83. The principal groups of infrared spectroscopies showed that the drug sample was authenticated.

UV Spectropy

Identification and authentication of drug sample was done by ultraviolet spectroscopy and it was scanned in the range of 200-400 nm. Drug absorption maximum λ_{max} was found to be at 254 nm. Absorption maximum showed that drug sample was authenticated.

Effect of capacity temperature on drug content

After storage for a predefined time of 15, 30, 45 and 60 days, the drug content of both the preparation was determined. Medication content In nanoparticle was resolved spectrophotometrically to by indirectly the measure of drug content.

Mathematical treatment of *in-vitro* release data[10-12]

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.

Stability studies:

optimized preparation of nanoparticle were exposed to accelerated stability testing under storage condition at 4 ± 1^0C and at room temperature (37 ± 1^0C). both the preparation were 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[12].

RESULTS AND DISCUSSION

Determination of particle size

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.

Table 6.1: Evaluations of Nanoparticle formulations by OVAT

Formulation	**Particle Size (nm)**	**Entrapment efficiency (%)**	**Drug content (%)**	**Poly- dispersity index***
CN_1	337.2±4.84	76.7±0.2	64.63±0.78	0.234 ± 0.006
CN_2	358.6±5.38	62.2±0.6	69.73±0.83	0.345 ± 0.012
CN_3	382.8±3.85	78.6±0.8	72.56±0.63	0.380 ± 0.074
CN_4	448.7±6.78	83.1±0.3	63.52±0.45	0.342 ± 0.098
CN_5	455.6±8.27	86.3±0.5	69.48±0.54	0.245 ± 0.009
CN_6	372.6±4.73	82.2±0.7	63.53±0.32	0.454 ± 0.004
CN_7	411.5±6.83	79.2±0.9	72.12±0.25	0.319 ± 0.010
CN_8	342.3±4.89	77.5±0.7	67.58±0.42	0.254 ± 0.098
CN_9	368.4±2.48	83.8±0.4	71.12±0.38	0.482± 0.027
CN_{10}	448.5±5.39	86.3±0.8	69.57±0.44	0.642± 0.074
CN_{11}	353.6±6.39	81.3±0.5	67.98±0.58	0.371± 0.056
CN_{12}	358.4±4.73	83.4±0.6	71.12±0.39	0.493± 0.084
CN_{13}	362.8±5.75	78.9±0.8	72.59±0.45	0.353± 0.074
CN_{14}	352.6±4.38	73.3±0.7	73.45±0.78	0.348± 0.084

CN_{15}	364.2±3.37	79.9±0.2	74.57±0.69	0.283± 0.048
CN_{16}	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**

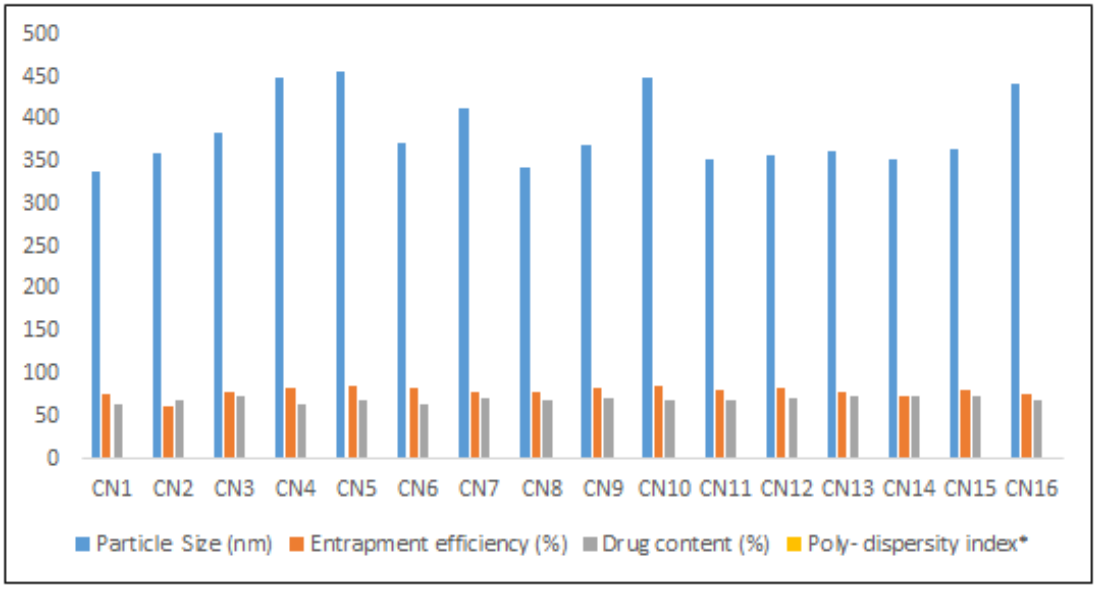

Figure 6.1: Evaluation of Nanoparticle Formulations by OVAT

6.2. 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**

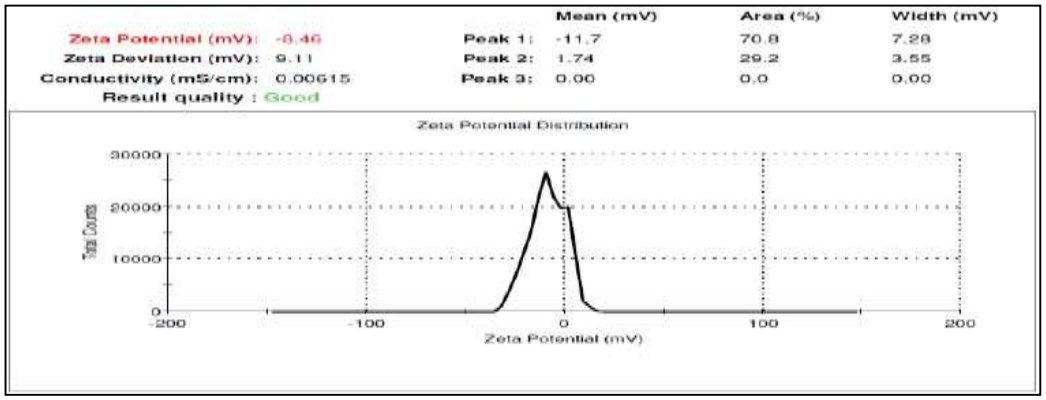

Fig.6.2. Zeta potential of Formulation CN15.

Surface morphological properties of Mamentine HCl loaded nanoparticlesCN15).

The surface morphology and shape of the mamentine HCl loaded nanoparticles (**CN_{15}**) was measured using scanning electron microscopy. The SEM image of nanoparticles revealed that the particles are of spherical in shape with relative smooth surface.

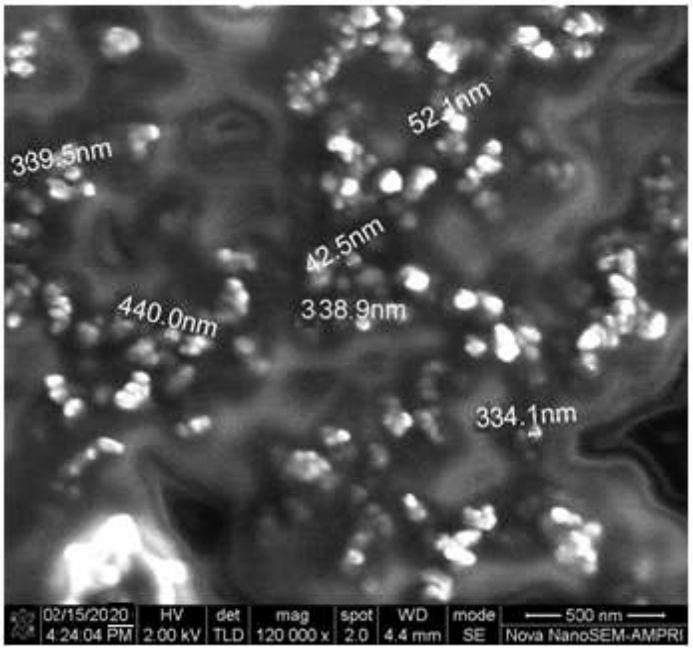

Fig.6.3. SEM image of the Mamentine HCl nanoparticle of or mutation(CN15. Transmission electron microscopy

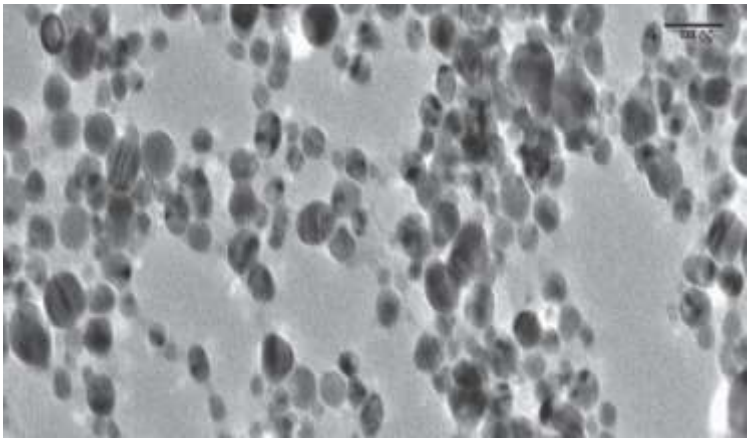

Fig.6.4. TEM image of the Mamentine HCl nano particle formulation(CN15).

The Transmission electron microscopyshowed the spherical particles with smooth surface which was in conformity with the SEM and Zetasizer data for particle size. Magnification of single particle showed the internal core drug inside the polymer and also confirmed the spherical particles with smooth surface.

Drug entrapment efficiency and drug loading

The entrapment efficiency of nanoparticles is the function of the characteristics of the polymer, drug, surfactant, process parameters etc. The high entrapment efficiency is observed when both drug and polymer have the high affinity to the same solvent. The amount of drug incorporation in the formulation and drug entrapment efficiency has direct effect on the drug release profile from the formulations. In the present study the drug loading and entrapment efficiency were affected by the drug and polymer ratio in the formulation. (**CN_{15}**) possess the optimum efficiency 79.9±0.2, given in Table:

Table 6.21: Effect of storage temperature on the Particle size of drug loaded *In situ*nanogel (.CN15).

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 6.22: Effect of storage temperature on the % Drug content of loaded *In situ*nanogel (CN15).

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

*Average of 03 readings

Table 6.23: Effect of storage temperature on the drug content of *insitu*nanogel (CN15).).

Time (Days)	Drug Content (%)	
	4.0 ±0.5°C	**37 ± 0.5ºC**
0	98.120 ± 0.021	88.120 ± 0.021
15	97.917± 0.575	85.083± 0.158
30	97.265 ±0.279	82.692 ± 0.573
45	97.119 ± 0.265	80.096 ± 0.875
60	97.086 ± 0.887	75.852 ± 0.745

*Average of 03 readings

Table 6.13: *In-vitro* drug release data for MG10

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 readings

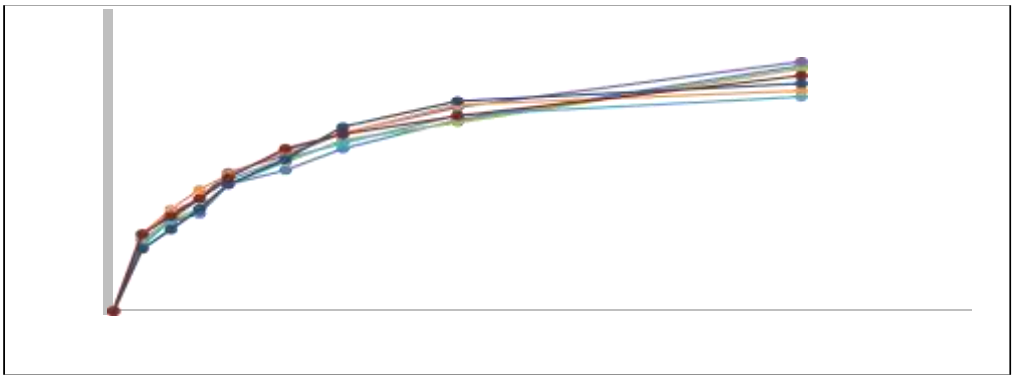

Fig. 3. In-vitro drug release data of mamentine nanoparticle CN9-CN16

CONCLUSION

The main objective of the study is to formulatehydrophilic drug loaded nanoparticles with thenanometer size and to increase the encapsulation efficiency of the drug. The nanoparticles were prepared by simple ionic gelation method using various concentrations of chitosan and an optimum concentration of TPP and further taken to formulate 08 number of nasal gels with poloxamer and carbopol. The prepared formulations were evaluated for particle size, shape, encapsulation efficiency, in vitro drug release and in vitro cytotoxicity. The optimized drug loaded nanoparticles showed the size of 330 to 651nm (364.2±3.37), with PDI below 0 to 1 (0.234 to 0.642),zeta potential -8.46 mv encapsulation efficiency of 79.9±0.2, and the drug content of 72.56 ± 0.25% without an initial burst effect up to one hour followed by sustained release up to 24 hrs,.The surface morphology and shape of the mamentine HCl loaded nanoparticles (**CN_{15}**) was measured using scanning electron microscopy. The Transmission electron microscopy showed the spherical particles with smooth surface which was in conformity with the SEM and Zetasizer data for particle size. 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 nanoparticles (**CN_{15}**) formulation. shown in Table 6.21 to Table 6.24.

AUTHORS' CONTRIBUTIONS

This authourhas contributed to designed and performed the analysis, collected the data and wrote the paper.She has made a substantial contribution for interpretation of data to write in the paper to make the final manuscript.

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CURRENT STATUS AND FUTURE PERSPECTIVE OF NANOGELS

The recombinant murine interleukin – 12 (IL – 12) encapsulated in CHP nanogels, via incubation at room temperature and injected in mice with subcutaneous fibrosarcoma leads delayed release & retardation the growth of tumor[85].

Nanogels have been primarily used for cancer therapy. Cholesteryl pullulan nanogel has shown in clinical trials for delivery of peptidase. The cholesteryl – HER – 2 vaccine was administered to nine patients with 300 µg with booster doses twice a week. From this shown that skin sensitivity at the site of S.C injection & CD4+ & CD8+ T- cell shows the better therapeutic efficacy. cholesterol pullulan nanogels show the reduce the cytoxicity to the nervous system cells and increase the binding capacity to AB oligomer in treating Alzheimer's disease.

Recently the new development of controlled diabetes by optical sensitive mamentine loaded silver nanoparticle nanogel of poly (4 – vinyl phenyl boronic acid – co – 2 – (dimethylamino) ethyl acrylate) have been designed [86].

Now a days nanogel is conjugated with antibiotics for the specific drug delivery and conducted at the single cell level.

In future the mechanism of blood brain barrier and cytosolic destination over and endosomal or nuclear are necessary to study for the specific and targeting drug delivery.