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FORMULATION, CHARACTERISATION AND *IN-VITRO* EVALUATION OF NOVEL IONICALLY CROSS LINKED CASEIN NANOPARTICLES FOR MEMANTINE HYDRO-CHLORIDE DELIVERY

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Keywords:

Alzheimer's disease, Protein nanoparticles and Ionically crosslinked method

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ABSTRACT: The objective of the study was to fabricate 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. The particle size varied from 148 to 317 nm and zeta potential was in negative and its value found to be - 46.4 mV. The drug content for the Memantine HCl loaded casein nanoparticles varied from 69.5 ± 7.2% to 87.9 ± 1.2%. The entrapment efficiencies were found to be minimum and maximum of 55.50 ± 2.4% and 86.30 ± 3.6%. The percentage yields of all formulations were in the range of 48.24 ± 1.24 to 86.13 ± 1.37%. *In-vitro* release of drug follows zero order and showed sustained release behaviour for a period of 24 hr. The optimized formulation contained STTP and casein in 1:3 ratio and could demonstrate the sustained release sucessfully. Memantine HCl loaded casein nanoparticle is a potential new delivery system for treatment of Alzheimer's disease.

INTRODUCTION: Nanoparticles as a delivery system are to control particle size, surface properties and to release of pharmacologically active agents in order to achieve the site specific action of the drug at the therapeutically optimal rate and dose regimen. These are defined as particulate dispersions or solid particles with a size in the range of 10 - 1000 nm. Due to heir large surface to volume ratio these nanoscale structures possess unique properties and dissolution behaviour which are expected to avoid the unwanted side effects.

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Sustained release of the drug from the nano-particles maintains the therapeutic concentration for long durations. Nanoparticles are prepared by various techniques like polymerization, solvent precipitation, nanoprecipitation and ionic gelation methods. The choice of a particular approach depends mainly on the nature of incorporated active pharmaceutical ingredient (*i.e.* hydro-phobicity / hydrophilicity of the drug and its sensitivity to the solvent) and the physiochemical properties of the matrix substance (*i.e.* solubility and molecular weight) [1].

Over the past few decades there has been considerable interest in developing protein nanoparticles as drug delivery vehicles. The underlying rationale is their exceptional characteristics, namely biodegradability, non-antigenicity, abundant renewable sources,

extraordinary binding capacity with various drugs, and the ease of scaling up during manufacture. Casein, recognized as safe protein, is the major milk protein that forms an integral part of the daily diet. It possesses a number of interesting properties that make it a good candidate for conventional and novel drug delivery systems [2]. Casein may be advantageous as an alternative to albumin as a matrix for drug delivery, because it is inexpensive and has better amphiphilicity, good dispersibility, and rapid reconstitution in aqueous systems.

Neurodegenerative diseases represent a crucial and exponentially increasing challenge to the health care systems all over the world. Alzheimer's disease (AD) is the most common form of dementia and currently affected 35 million patients across the world which is expected to be double in the next 20 years. Many hydrophilic drugs and neuropeptides fail to cross the blood–brain barrier (BBB). Consequently, the complete therapy for Alzheimer's disease (AD) can't be achieved. Though many approaches have been developed to overcome this problem nanoparticles are found advantageous as they provide sustained and targeting release with better stability during storage [3].

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 [4]. 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.

Hence, the present study was aimed to formulate Memantine HCl loaded casein – sodium tripolyphosphate (STPP) nanoparticles using ionically crosslinked method. These nanoparticles were characterized for their physiochemical properties and *in vitro* release studies.

MATERIALS AND METHODS: Memantine Hydrochloride was purchased from A. S. Joshi and Company, Mumbai. Casein was obtained from Rolex chemical Industries, STPP was obtained from Sisco research laboratory Pvt. Ltd., Mumbai and all other ingredients used were of analytical grade.

Preformulation Studies:

Identification of Pure Drug: FTIR spectroscopy was used for identification of pure drug.

Determination of λ_{max}: Accurately weighed 10 mg of Memantine HCl was transferred in a 100 ml volumetric flask to which phosphate buffer was added in small proportions to dissolve Memantine HCl. 20 µg/ml solution of Memantine HCl was prepared and transferred into a 10 ml volumetric flask to complete the volume to 7 ml followed by addition of 1.4 ml eosin reagent and 1.2 ml of 0.2 M acetate buffer pH 3.6. The resulting solution was scanned in UV - Vis spectrophotometer from 600 - 400 nm to determine the λ_{max} [5].

Compatibility Studies: A successful formulation of a stable and effective dosage form depends on selection of excipients that are added to promote the consistent release and bioavailability of the drug and protect it from degradation. If the excipients are new and have not been used in formulation containing the active substance so far, the compatibility studies are mandatory.

Drug - Polymer Compatibility Studies by FTIR: This was confirmed by infrared light absorption scanning spectroscopy (IR) studies. Infra red spectra of pure drug and mixture of formulations were recorded by dispersion of drug and mixture of formulations in suitable solvent (KBr) using Fourier Transform Infrared Spectrophotometer [6].

Drug-polymer Compatibility Studies by DSC: The differential scanning calorimetry analysis was performed for the compatibility studies between the drug and the polymer. Each sample was sealed in Aluminium disc and purged with air at a flow rate of 40 ml / min and maintain the temperature at 25ºC - 200ºC. The DSC spectrum of the pure Memantine HCl was compared with mixture of the Memantine HCl and the chitosan [6].

Calibration of Standard Curve: Accurately weighed 100 mg of Memantine Hydrochloride was dissolved in 100 ml of pH 6.8 phosphate buffer solution. The resultant solutions were having concentration of 1000 µg/ml (1 mg/ml). 10 ml of this solution was further diluted up to 100 ml with 6.8 pH phosphate buffer to give a solution of 100 µg/ml concentration. This resultant solution was used as working stock solution and further dilutions

were prepared from the same solution. Aliquots of 0.1 mg/ml Memantine HCl standard working solution were transferred into a set of 10 ml volumetric flasks to produce solutions within the concentration range of 10, 20, 40, 60, 80 and 100 µg/ml. To each flask, 6.8 pH phosphate buffer was added up to the volume 7 ml followed by 1.4 ml ESN reagent and the solutions were mixed well before adding 1.2 ml of 0.2 M acetate buffer pH 3.6. The volumes were finally completed with buffer and the colored solutions were measured for absorbance at 557 nm [5]. A calibration curve of absorbance against concentration was plotted and the drug followed the Beer's and Lambert's law in the concentration range of 10 - 100 µg/ml. The regression equation and correlation coefficient were determined.

Preparation of NP's: Aqueous casein solution (1% w/v) was adjusted to pH 2.0 with 1 N hydrochloric acid. Tween 80 (2% v/v) was added as a surfactant to casein solution under magnetic stirring. Dichloromethane solution of Memantine as the oil phase was mixed with aqueous casein phase by homogenization at a speed of 13,000 rpm for 20 minutes to obtain an oil-in-water emulsion. The ratio of oil and aqueous phase was 1:10 v/v. STPP solution (0.5% w/v) was added dropwise to the oil-in-water emulsion under gentle magnetic stirring. After 2 hours of crosslinking, nanoparticles were isolated by centrifugation at 20,000 RPM and 10°C for 30 minutes, and subsequently washed several times with water [2, 7]. The particles were lyophilized and stored in dry conditions at 25 °C.

Characterisation of Prepared NP's: The obtained formulations of Memantine hydrochloride loaded casein nanoparticles were characterized for the following parameters.

Particle Size Analysis and Surface Morphology: Measurement of particle size was performed by Photon Correlation Spectroscopy (PCS) known as Dynamic Light Scattering using a Zetasizer® 3000 (Malvern Instruments, NIPER, Mohali). All samples were diluted with ultra purified water and measured at 25° and 90° scattering angle, recorded for 180 s. The mean diameter for each sample was generated by cumulative analysis in triplicate. The morphological examination of nanoparticles was performed using scanning electron microscopy (SEM) (Tecnai 20 G2 S TWIN at Punjab University, Chandigarh; set at 200 kV). At structural point of view, the arrangement of components and orientation of molecules within the nanoparticle can determine its behaviour and stability [8]. For this purpose scanning electron microscopy (SEM) was employed.

Surface Charge Determination: Nanoparticles were characterized with Zeta potential (ζ) using a Zeta Sizer (Suralabs., Hyderabad) [8]. The measurements were performed using an aqueous dip cell in an automatic mode by placing diluted samples (with ultra-purified water) in the capillary measurement cell and cell position was adjusted.

Entrapment Efficiency: The Entrapment Efficiency (EE%) is also known as Association Efficiency. The drug loaded nanoparticles were centrifuged at a high speed of 3500 - 4000 rpm for 30 min and the supernatant was assayed for non-bound drug concentration by using spectrophotometer. Entrapment efficiency was then calculated as follows [8]:

EE% = (Total amount of drug added – Non bound drug / Total amount of drug added) × 100

Drug Content: Drug content was determined by centrifugation method [9]. The nanoparticles suspension was centrifuged at 15,000 rpm for 40 min at 25 °C to separate the free drug in the supernatant. Concentration of Memantine HCl in the supernatant was determined by UV - Vis spectrophotometer at 557 nm after suitable dilution.

Percentage Yield: It is determined by the equation

Percentage yield = (Weight of dried nanoparticles recovered / Sum of initial dry weight of starting material) × 100

***In vitro* Release Studies:** *In vitro* drug release studies were conducted by means of incubator orbital shaker [10]. 50 mg of each accurately weighed formulation was transferred into 250 ml conical flask containing 100 ml pH 6.8 phosphate buffer solution. They were kept in an orbital shaker at 50 rpm maintained at 37 °C. Aliquots of 5 ml buffer were withdrawn at predefined time intervals and the medium was replaced with same volume of buffer. The withdrawn samples were centrifuged at

4000 rpm for 15 min. The supernatant was collected. This study was carried out for 24 h, and the concentration of drug release was estimated by determining the absorbance at 557 nm using UV spectrophotometer [11].

Kinetic Modelling: In order to understand the kinetics and mechanism of drug release from optimized formulation F3, the result of *in vitro* drug release study of nanoparticles were fitted with various kinetic equations like zero order (cumulative % release *vs*. time), first order (log % drug remaining *vs*. time), Higuchi's model (cumulative % drug release *vs*. square root of time). R^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots [12].

Stability Study: The stability study was carried using batch F3. The stability of drug loaded nanoparticles was evaluated in terms of its drug content, entrapment efficiency and *in vitro* drug release [13]. The stability of nanoparticles was evaluated in PBS (pH 6.8). Nanoparticles formulation was incubated at 37 ± 1 °C for a period of 90 d. After specified time intervals, the suspension was centrifuged at 4,000 rpm for 1 h, supernatant was removed and the amount of drug was detected by UV - Vis spectrophotometrically method at 557 nm.

RESULTS AND DISCUSSION

Preformulation Studies:

Identification of Pure Drug: FT - IR spectroscopy used to determine the functional group present in the pure drug sample Memantine HCl has shown the characteristic peaks at 2978.73, 2941.58, 2859.39, 2896.81, 2838.91, 1511.78, 1455.27, 1355.83, 436.30 and 448.78 cm^{-1}. The absorption bands between 2800 and 3200 cm^{-1} indicates the presence of –NH stretching of amine or amide groups. The wave numbers observed at 1511 and 1455 may be assigned to the C = O and C - N bonds respectively and the sharp peak occurred at 1511 indicates presence of C = O group attached to –NH. The IR spectrum of Memantine HCl is as follows:

Determination of λ_{max}: The Memantine HCl with ESN in acidic medium forming an orange-red ion-pair complex was scanned using UV - Vis spectrophotometer from 800 - 400 nm to determine the λ_{max}. The λ_{max} was found to be at 557 nm, where the calibration curve of Memantine HCl was developed.

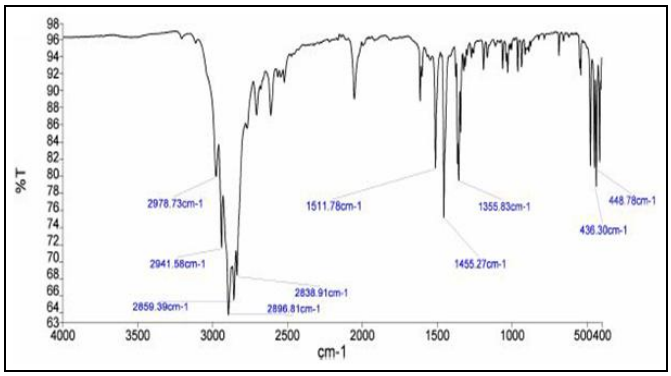


FIG. 1: IR SPECTRA OF MEMANTINE HCl

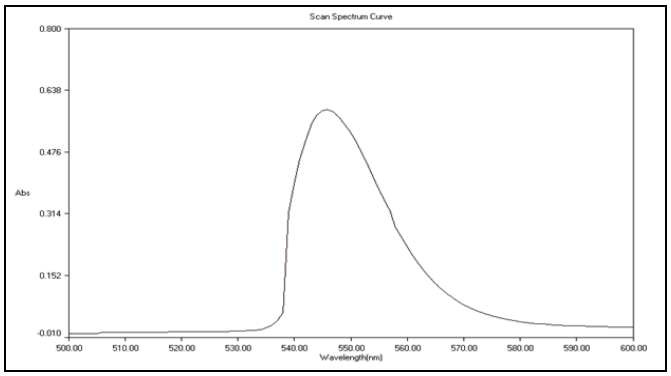


FIG. 2: λ_{max} OF MEMANTINE HCl

Drug - Excipient Compatibility Studies:

Fourier Transform Infra Red Spectroscopy (FTIR): The interaction studies were carried out to ascertain any kind of interaction of drug with the excipients used in the preparation of protein nanoparticles. Physical mixture of memantine and each selected excipients were prepared in the 1:1 w/w ratio by gently blending with spatula to get homogeneous mixture for IR analysis. The FTIR spectra of Memantine HCl were recorded on a FTIR multiscope spectrophotometer (Brooker) equipped with spectrum 11.0.0.0449 software using KBr pellet method. The spectrum for each sample was recorded over than 400 - 4000 cm^{-1}. The FTIR spectra of the pure drug and formulations were shown in **Fig. 3 - 7**.

Inference: The FTIR spectrum of formulations showed characteristic absorption bands which were comparable with absorption bands of individual sample. The results illustrated that, there were no chemical instabilities in drug-excipient combinations.

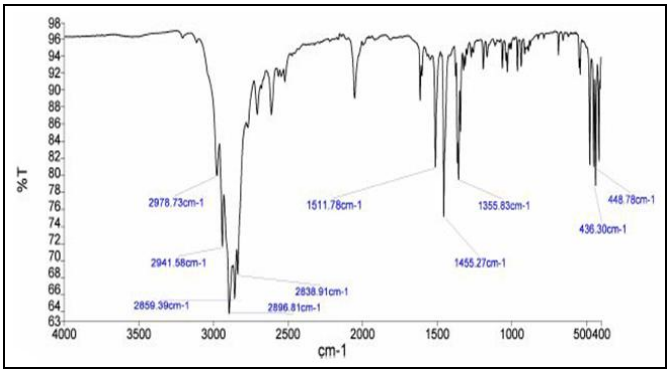


FIG. 3: IR SPECTRA OF MEMANTINE HCl

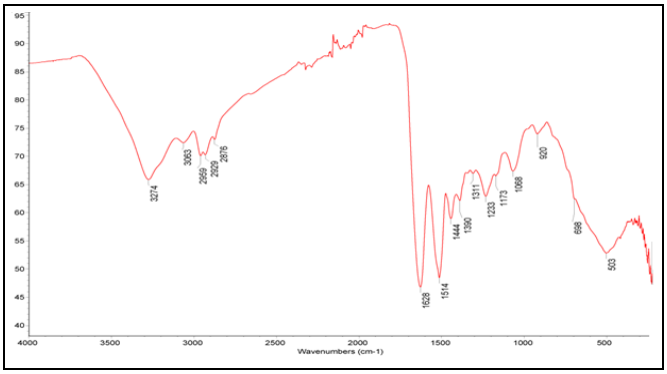


FIG. 4: IR SPECTRA OF CASEIN

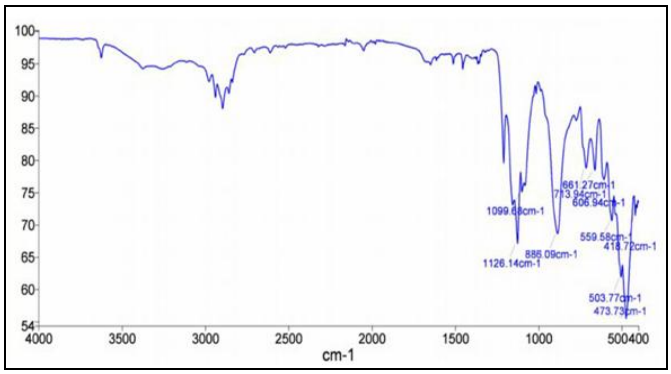


FIG. 5: IR SPECTRA OF STPP

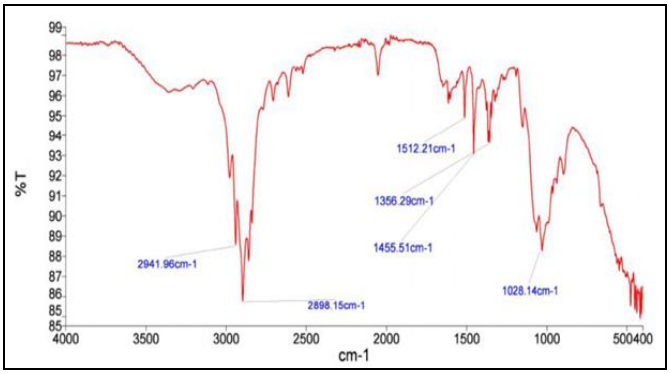


FIG. 6: IR SPECTRA OF MEMANTINE HCl + CASEIN

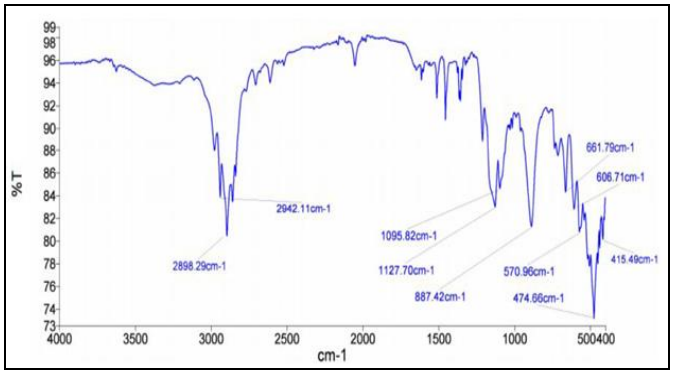
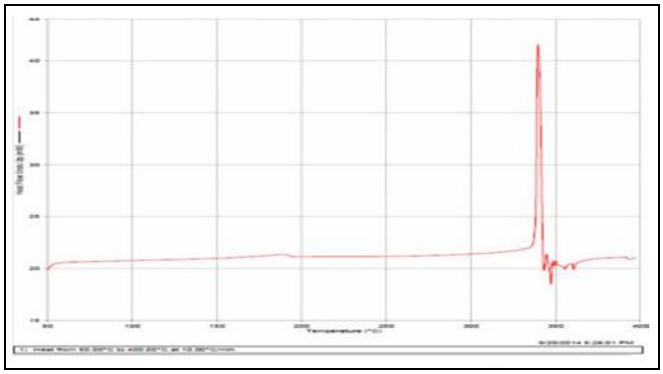


FIG. 7: IR SPECTRA OF MEMANTINE HCl + CASEIN + STPP

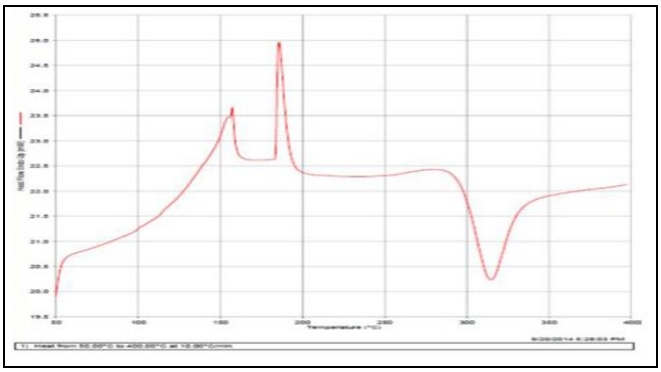
Differential Scanning Calorimetry (DSC): Drug excipient interactions play a vital role with respect to release of drug from the formulation amongst others. DSC has been used here to study the physical and chemical interaction between the drug and excipients used. The DSC thermo grams obtained are displayed in **Fig. 8.** It shows that the decomposition temperature of drug was 341 ºC and formulation was 342 ºC. It indicates there is no chemical interaction between Memantine HCl and the protein Casein used.

Inference: The DSC thermogram revealed that the formulation showed superimposition of drug, however mild shift was observed. The DSC results revealed that there was no interaction between the drug and additives used in the formulation.

Standard Graph of Memantine HCl: The standard curve of Memantine HCl was done by using pH 6.8 PBS as the medium and the maximum absorbance was found at 557 nm. The standard graph was constructed by making the concentrations of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml solutions. The absorbance of solutions was examined under UV - visible spectrophotometer at an absorption maximum of 557 nm. The standard graph was constructed by taking the absorbance on Y - axis and concentrations on X - axis. The standard calibration curve of Memantine HCl in pH 6.8 PBS was shown in **Fig. 9.** Drug concentration and absorbance followed linear relationship the curve obeyed Beer - Lambert's law and the correlation coefficient value (R^2) is 0.997.



A



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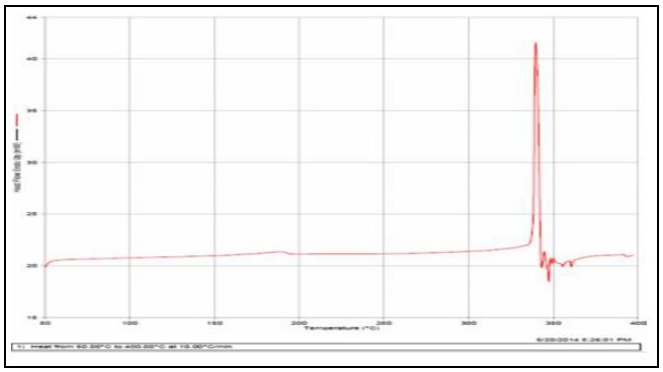


C

FIG. 8: DSC THERMO GRAMS OF (A) PURE MEMANTINE HCl (B) CASEIN AND (C) MEMANTINE HCl + CASEIN

Preparation of Nanoparticles: Nanoparticles of Memantine HCl were prepared by Ionotropic gelation technique using Casein and STPP in varying concentration ratio like 1:1 to 6:1. The casein nanoparticles were prepared based on the ionic interaction of a positively charged casein solution and negatively charged STPP solution. The charge density of both casein and STPP solution has a great effect on the ionic interaction. Total six batches were formulated (F1, F2, F3, F4, F5 and F6) and all the formulations were investigated for various parameters like Scanning electron microscopy (SEM), particle size, Zeta potential, Drug content, Entrapment efficiency, Percentage yield, *in vitro* drug release and drug release kinetics.

TABLE 1: STANDARD CALIBRATION CURVE OF MEMANTINE HCl

S. no.	Conc. (µg/ml)	Absorbance in pH 6.8 PBS (nm)
1	0	0
2	20	0.014
3	40	0.023
4	60	0.033
5	80	0.044
6	100	0.055

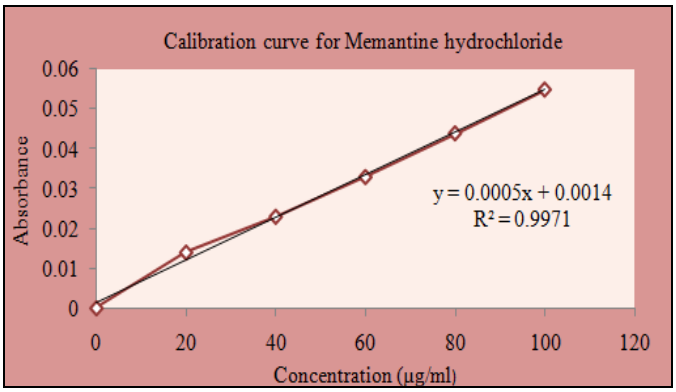
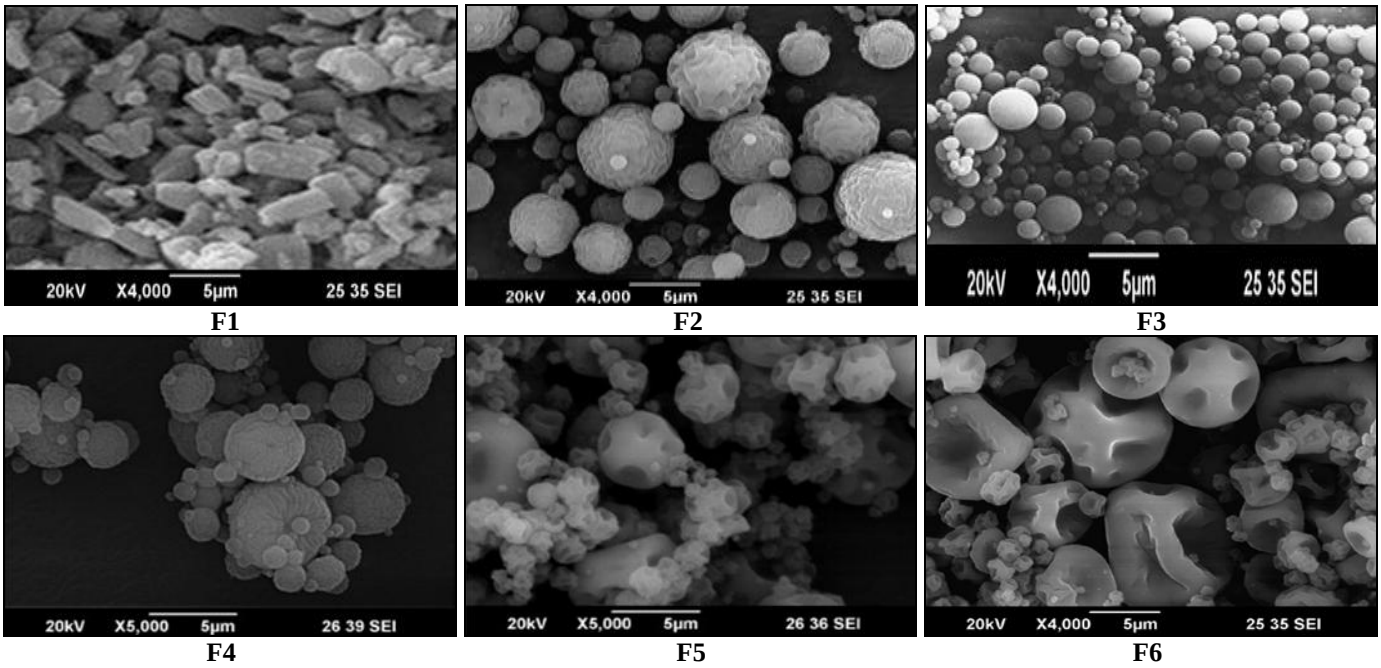


FIG. 9: THE STANDARD CALIBRATION CURVE OF MEMANTINE HCl

Characterisation of Prepared NPs:

Scanning Electron Microscopy (SEM): SEM analysis of the F1 showed that the nanoparticles has irregular structure and the view of F2 - F6 showed that the nanoparticles are hollow spherical structure with a large central cavity in which Memantine HCl was loaded.

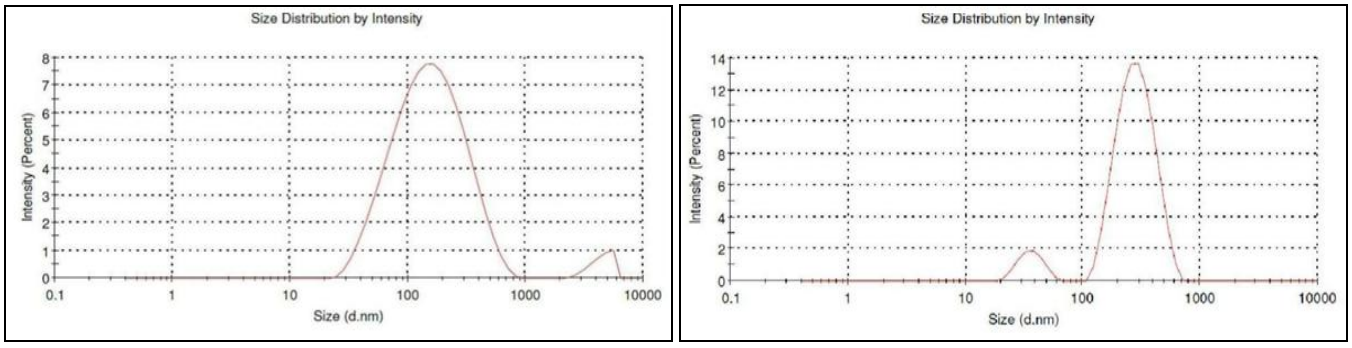
The outer surface of the nanoparticles was smooth and shell of the nanoparticles also showed some porous structure. SEM analysis of formulations F1 - F6 is shown in **Fig. 10.**



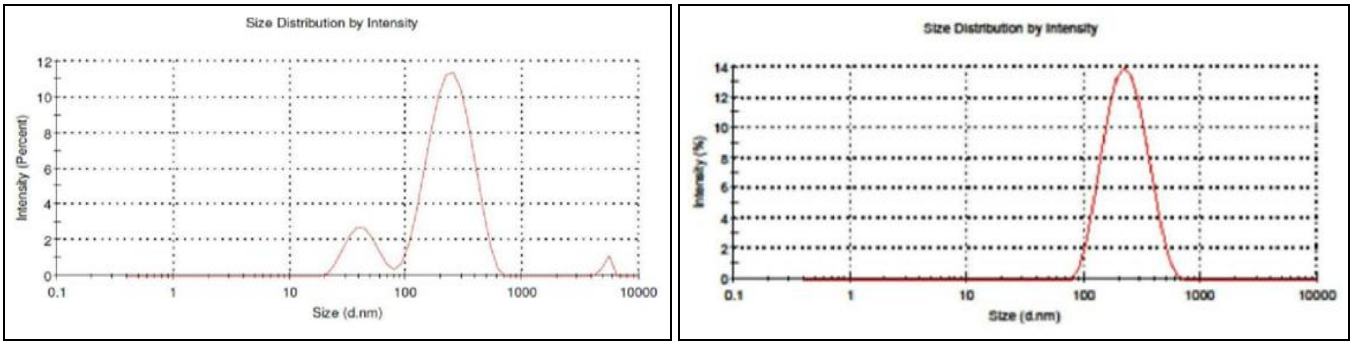
F1 **F2** **F3**

F4 **F5** **F6**

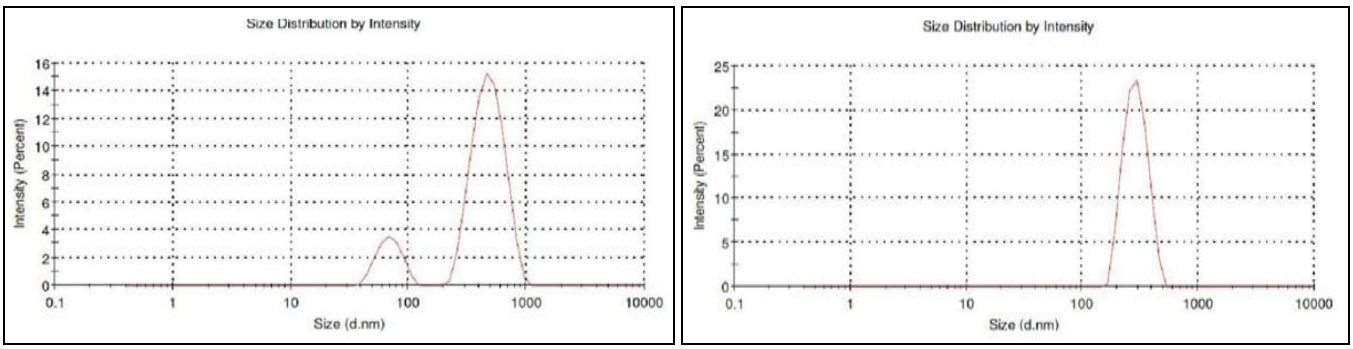
FIG. 10: SCANNING ELECTRON MICROGRAPHS OF NANOPARTICLES FORMULATIONS F1 – F6



F1 (Particle size 190 nm) **F2 (Particle size 195 nm)**



F3 (Particle size 148 nm) **F4 (Particle size 220 nm)**



F5 (Particle size 284 nm) **F6 (Particle size 317 nm)**

FIG. 11: SIZE ANALYSIS OF NANOPARTICLES FORMULATIONS F1 – F6

TABLE 2: SIZE ANALYSIS

S. no.	Formulation	Mean particle Size (nm)
1	F1	190 nm
2	F2	195 nm
3	F3	148 nm
4	F4	220 nm
5	F5	298 nm
6	F6	317 nm

Surface Charge (Zeta Potential): The electrostatic repulsion between particles with the same electric charge prevents the aggregation of the particles. The zeta potential values of formulation F3 were negative which demonstrate that the anionic surface of drug delivery system would provide improved targeting ability as compared to the cationic carrier. The zeta potential of the formulation F3 with a concentration of casein 3:1 was found to be - 46.4, which implies that it is having good stability.

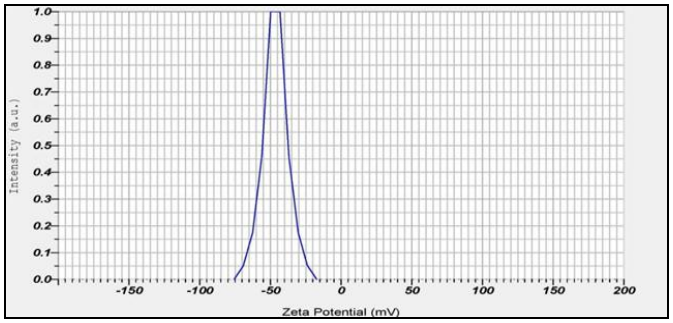


FIG. 12: ZETA POTENTIAL OF NANOPARTICLES IN FORMULATION F3

Drug Content: The drug content was evaluated for all the formulations and it was observed that the nanoparticles obtained from F1 formulation showed maximum drug content (87.9 ± 1.2%) and F6 showed minimum drug content (69.5 ± 7.2%). The drug content was decreased with increase in casein concentration. This may be due to loss of drug during manufacturing stage or increase in entrapment efficiency, so that drug is not available for estimation. This result indicated that there was no drug loss by manufacturing process or by excipients used in the formulation.

TABLE 3: DRUG CONTENT

S. no.	Formulation	Drug content (%)
1	F1	87.9 ± 1.2
2	F2	86.7 ± 3.2
3	F3	84.3 ± 2.6
4	F4	80.4 ± 3.2
5	F5	75.8 ± 6.8
6	F6	69.5 ± 7.2

Mean ± S.D. of three determinations

Entrapment Efficiency: Prepared nano-formulations were estimated for entrapment efficiency and the results are shown in the **Table 4**. Drug entrapment efficiency varied from 55.50 ± 2.4 to 86.30 ± 3.6%. This result indicated that drug entrapment efficiency increased with increasing concentration of casein up to 0.3% (F3). After that, there was no significant increase in entrapment efficiency. This may be due to unavailability of drug for entrapment.

This can be attributed to fact that higher extent of casein resulted in formation of a more rigid network structure which prevent the leaching out of drug during preparation of nanoparticles. The optimum efficiency was based on the drug content and casein usage. From drug content and entrapment efficiency results casein nanoparticles F3 were considered as optimum trials.

TABLE 4: DRUG ENTRAPMENT EFFICIENCY

S. no.	Formulation	Entrapment Efficiency (%)
1	F1	55.50 ± 2.4
2	F2	62.71 ± 3.7
3	F3	85.62 ± 0.8
4	F4	84.40 ± 2.9
5	F5	85.40 ± 1.4
6	F6	86.30 ± 3.6

Mean ± S. D. of three determinations

Percentage Yield: The percentage yield of nanoparticles prepared by ionotropic gelation method was recorded in **Table 5** which was determined by weighing the collected nano-particles. The measured weight was divided by the initial dry weight of starting materials, which were used for the preparation of the nanoparticles. The percentage yields of nanoparticles of all formulations were in the range of 48.24 ± 1.24 to 86.13 ± 1.37%. It was found that when concen-tration of casein increased, the percentage yields also increased. The formulation F6 showed maximum percentage yield of 86.13 ± 1.37% compared to other formulations.

TABLE 5: PERCENTAGE YIELD

S. no.	Formulation	Percentage Yield
1	F1	48.24 ± 1.24
2	F2	52.78 ± 1.56
3	F3	68.88 ± 1.31
4	F4	76.25 ± 1.21
5	F5	84.29 ± 1.30
6	F6	86.13 ± 1.37

Mean ± S. D. of three determinations

***In vitro* release studies:** From the *in-vitro* release studies of Memantine HCl loaded casein nano-particles (F1 - F6), it was observed that release profiles in intestinal medium (pH 6.8 phosphate buffer) were found to have very good controlled efficacy. The drug release depends upon concentration of the casein and increase in casein concentration produced much more time for release of drug for all formulations. High casein concentration (≥ 0.4% casein - F4 to F6) showed slow drug release for more than 24 h. Low casein concentration (≤ 0.2% casein - F1 to F2) showed quick drug release within short period. Hence the formulations (F1 to F2, F4 to F6) were considered to be not satisfactory for controlled delivery of Memantine HCl either by quick release or over retarding. Memantine HCl nanoparticles prepared with 0.3% casein (F3) showed controlled and sustained drug release for a period of 24 h. The percentage cumulative drug release of F3 at the end of 24 h was found to be 95.85 ± 0.54%. *In vitro* drug release of Memantine HCl loaded casein nanoparticles is shown in **Fig. 13**.

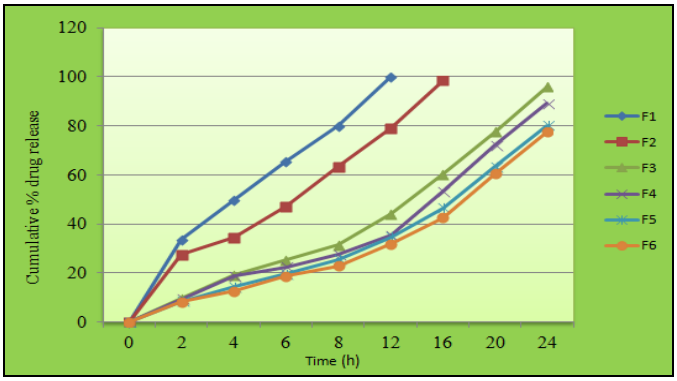


FIG. 13: *IN VITRO* DRUG RELEASE STUDIES

TABLE 6: PERCENTAGE CUMULATIVE DRUG RELEASE

Time (h)	% Cumulative Drug Release					
	F1	F2	F3	F4	F5	F6
2	33.77 ± 0.14	27.72 ± 0.44	09.56 ± 0.36	09.42 ± 0.32	08.41 ± 0.31	08.33 ± 0.14
4	49.95 ± 0.65	34.61 ± 0.68	19.25 ± 0.28	18.65 ± 0.65	14.42 ± 0.32	12.74 ± 0.55
6	65.45 ± 0.68	47.32 ± 0.14	25.43 ± 0.19	22.52 ± 0.75	19.65 ± 0.65	18.85 ± 0.72
8	80.10 ± 0.25	63.31 ± 0.47	31.34 ± 0.37	27.71 ± 0.22	25.52 ± 0.75	22.85 ± 0.34
12	99.94 ± 0.66	79.20 ± 0.45	44.11 ± 0.87	35.49 ± 0.64	34.71 ± 0.22	31.90 ± 0.48
16	---	98.52 ± 0.26	60.23 ± 0.29	53.21 ± 0.25	46.49 ± 0.64	42.63 ± 0.22
20	---		77.56 ± 0.55	72.10 ± 0.63	63.21 ± 0.25	60.42 ± 0.26
24	---		95.85 ± 0.54	88.99 ± 0.42	80.10 ± 0.63	77.42 ± 0.26

Kinetic Modelling of Drug Release: Dissolution data of the optimized formulation F3 was subjected to regression analysis and were fitted to kinetic models **Table 7**. The R^2 value of zero order and first order was found as 0.9623 and 0.7106 respectively. This result suggests that the drug released by zero order kinetics. Further to ascertain the exact mechanism of drug release the dissolution data of the optimized formulation was subjected to Peppas and Higuchi's diffusion equation. The R^2 value of Higuchi's and peppas diffusion equation was obtained as 0.9623 and 0.801 respectively. This result suggests that the drug released followed diffusion mechanism.

TABLE 7: KINETIC MODELLING OF DRUG RELEASE FOR F3 FORMULATION

S. no.	Time (min)	Log T	Square root of Time	% CR	% Drug Remaining	Log % CR	Log % Drug Remaining	Square root of % Drug Remaining
1	0	0	0	0	100	0	2	4.6410
2	2	0.3010	1.4142	9.56	90.44	0.9804	1.9563	4.4886
3	4	0.6020	2.0	19.25	80.75	1.2844	1.9074	4.3222
4	6	0.7781	2.4494	25.43	74.57	1.4053	1.8725	4.2090
5	8	0.9030	2.8284	31.34	68.66	1.4960	1.8367	4.0948
6	12	1.0791	3.4241	44.11	55.89	1.6445	1.7473	3.8233
7	16	1.2041	4.0	60.23	39.77	1.7798	1.5995	3.4133
8	20	1.3010	4.4721	77.56	22.44	1.8896	1.3510	2.8205
9	24	1.3802	4.8989	95.85	4.15	1.9815	0.6180	1.6070

Accelerated Stability Studies: The optimized formulations F3 was wrapped and sealed in an aluminum foil. Results of stability studies are shown in **Table 8**. The % drug content, entrapment efficiency and *in vitro* release study of most satisfactory formulation was determined and result showed that there was no significant changes occurs during storage after 90 days.

TABLE 8: ACCELERATED STABILITY STUDIES OF OPTIMIZED FORMULATIONS F3

Evaluation Parameter	Formulation Code	0 days	30 days	60 days	90 days
Drug content	F3	84.3 ± 2.6	83.6 ± 3.2	82.7 ± 4.2	82.1 ± 1.2
Drug entrapment efficiency (%)	F3	86.30 ± 3.62	85.85 ± 0.09	85.35 ± 0.15	84.80 ± 0.67
In vitro drug release (%)	F3	95.85.± 0.41	94.62 ± 0.32	94.13 ± 0.16	93.72 ± 0.25

CONCLUSION: Various trials with change in the concentration ratio of STPP and casein had proven that, the best suitable concentration was 1:3. The particle size and zeta potential of optimized formulation (F3) was found to be 148 nm and –46.4, which indicates that the formulation was having good stability. The nanoformulations were designed for sustained release of the drug for a period of 24 h and this may reduce the frequency of dosing, thereby minimizing the occurrence of side effects.

From this results, it was concluded that F3 formulation was considered to be the best formulation and serves as a potential formulation for the treatment of Alzheimer's disease. But, more animal studies and extensive clinical studies are needed to examine and justify the efficacy of the prepared drug delivery system.

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