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Preparation and Evaluation of Donepezil Loaded Nanoparticles

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ABSTRACT

The purpose of the present study was to formulate and evaluate donepezill loaded nanoparticles for effective treatment of Alzheimer's disease. Drug loaded nanoparticles was prepared by using modified nanoprecipitation technique, for this Ethyl Cellulose was selected as polymer in different ration with drug. The prepared nanoparticles were then subjected to several evaluation parameters like measurement of particles size, zeta potential, surface morphology, surface entrapment and drug content, in vitro dissolution study and stability study. Experimental results revealed that % yield was in range of 33.5% to 40.83% and drug entrapment was 70.003% to 86.8%. Particle size evaluation clearly state that prepared formulation within nano range (61.21 to 265.6 nm). Zeta potential value was find in the range of -32.9 to -17.4 mv and surface morphology study indicate smooth surface without fracture. In vitro release study shows that drug was released at a control rate and release rate depends on polymeric concentration. By kinekic study it was observed that zero order model is dominant for all five formulations and in all cases a super case II transport mechanism is dominant. After comparing all the evaluation parameter, formulation F4 was selected as optimized formulation and subjected for stability study which indicate that optimized formulation was stable. From this experiment it was clearly concluded that Donepezil Hydrochloride (model drug) can be successfully delivered to brain by preparing the nanoformulation at a control rate.

Keywords: Nanoparticles, Donepezil, Modified nanoprecipitation method, Stability study

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INTRODUCTION

AD is a brain disorder of the elderly age (above 60 age), first discovered by Dr. Alois Alzheimer in 1907, which is described as degenerative disease of the central nervous system. AD patients shows symptoms like decline in cognitive ability and severe behavioral abnormalities such as irritability, anxiety, depression, disorientation and restless. 1-4% of total population affected by AD after age of 65-70 years and 4% of total population affected by AD after age of 85 years. Actual cause of AD is not known, but it is clear that it develops because of a complex series of events that take place in the brain for long period of time. Some research shown connection between AD and head injury, genetic, environmental and life style factors. In case of AD protein beta amyloid accumulated outside neurons (called beta-amyloid plaques or neuritic plaques) and an abnormal form of the protein tau inside neurons (called tau tangles or neurofibrillary tangles) thus information transfer fails which cause impairment in memory and other symptoms.[1] Due to the tight junction adjacent endothelial cells in blood brain barrier (BBB), it is major obstacle for development of the drug formulation for brain disease. It is estimated that 98% of central nervous system (CNS) active drug are not able to cross the BBB. Only some small molecules having lipophilicity (more lipophilic better transport), molecular weight (>500 daltons like barbiturate, nicotine, ethanol) and charge (low hydrogen bonding capability) cross the blood brain barrier and reaches into the CNS. Majority of small molecules like protein and peptide do not crosses the BBB. In this present study donepezil loaded nanoparticles were prepared by modified nanoprecipitation method may effectively cross BBB and increase drug concentration at its site of action and serve as an effective tool to treat AD.[2]

MATERIALS AND METHOD

Materials required

Donepezil was a gift sample from Alkem laboratories (Haridwar). All other chemicals used were of analytical grade.

Preparation of nanoparticles by modified nanoprecipitation method:

Drug was dissolved in acetone and then water added into this solution and then sonicate for 5min. Polymer (ethylcellulose) and 150 mg of propylene glycol were dissolved in chloroform this solution sonicate for 5min, and this solution was added drop by drop to the drug solution to form dispersion then sonicate for 5 min. the dispersion was added slowly to 10 ml of aqueous ethanol solution (70%). After 5 minutes of mixing, the organic solvent were removed by evaporation at 35°under normal pressure, nanoparticles were separated by using cooling

centrifuge (10000 rpm for 20 min), supernatant were removed and nanoparticles washed with water and dried at room temperature in desicator for 24 hours.[3] Five different batches of nanoparticle were prepared by varying the amount of nanoparticle. Different composition batches were shown in Table 1.

Table: 1 Formulation of nanoparticle in different drug polymer ration

S. No	Ingredients	Formulation				
		F1	F2	F3	F4	F5
1.	Drug (Donepezil. HCl)	100 mg	100 mg	100 mg	100 mg	100 mg
2.	Ethyl cellulose	100 mg	200 mg	300 mg	400 mg	500 mg
3.	Acetone	3 ml	3 ml	3 ml	3 ml	3 ml
4.	Water	5 ml	5 ml	5 ml	5 ml	5 ml
5.	Propylene glycole	1 ml	1 ml	1 ml	1 ml	1 ml
6.	Chloroform	15 ml	15 ml	15 ml	15 ml	15 ml
7.	Ethanol	100 ml	100 ml	100 ml	100 ml	100 ml

Characterization of nanoparticles:

%Yield:

The yield of microspheres was determined by comparing the whole weight of microspheres formed against the combined weight of the copolymer and drug.[4]

$$\%\ yield = \frac{\text{amount of nanoparticle}}{\text{amount of drug} + \text{polymer}} \times 100$$

Particle size distribution and zeta potential:

Value of Particle size and Zeta Potential prepared nanoparticles determined by using Malvern Zetasizer (Ver. 6.11).

Surface Morphology:

Surface morphology study carried out by Scanning Electron Microscopy (SEM) of prepared nanoparticle.[5]

Drug Content / Surface entrapment / Drug entrapment:

After centrifugation amount of drug present in supernatant (w) determined by UV spectrophotometery. After that standard calibration curve plotted. Then amount of drug present in supernatant subtracted from the total amount used in the preparation of nanoparticles (W). (W-w) is the amount of drug entrapped. % drug entrapment calculated by[6]

$$\%\ drug\ entrapment = \frac{\mathrm{W} - \mathrm{w}}{\mathrm{W}} \times 100$$

Polydispersity index:

Polydispersity index of prepared nanoparticles was carried out by using Malvern Zetasizer.[7]

In-vitro release Study:

In-vitro drug release studies were performed in USP Type II dissolution apparatus at rotation speed of 50 rpm. The prepared immersed in 900ml of phosphate buffer solution in a vessel, and temperature was maintained at 37±0.20°C. Required quantity 5ml of the medium was withdrawn at specific time periods and the same volume of dissolution medium was replaced in the flask to maintain a constant volume. The withdrawn samples were analyzed using UV spectrophotometer. [8]

Kinetic Study:

For estimation of the kinetic and mechanism of drug release, the result of *in vitro* drug release study of nanoparticles were fitted with various kinetic equation 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.[3]

Stability of Optimized Formulation:

Stability studies of prepared nanoparticles determined by storing optimized formulation at 4°C ±1°C and 30°C ± 2°C in stability chamber for 90 days. The samples were analyzed after a time period like at 0, 1, 2, and 3 months for their drug content, drug release rate ($t_{50\%}$) as well as any changes in their physical appearance (ICH Q1A (*R*2) 2003). [9]

RESULTS AND DISCUSSION

% yield:

Table: 2 % yield of prepared formulation

Formulation	%yield
F1	33.5 %
F2	30.66 %
F3	37.5 %
F4	39.00 %
F5	40.83 %

Donepezil loaded Nanoparticles were prepared successfully by modified nanoprecipitation method. % yield of prepared nanoparticles was determined. The yield of formulated nanoparticles was observed between 33.5 to 40.83% as shown in Table 2. And observed data shows that yield of nanoparticles increase with amount of polymer increase.

Particle size distribution and zeta potential:

Table: 3 Particle size distribution of Donepezil HCl loaded nanoparticles

Formulation code	Particle size Size (RNM)	Mean intensity (%)
F1	95.07	3.3
	110.1	11.9
	127.5	21.6
	147.7	26.3
	171.0	22.5
	198.0	12.1
	229.3	2.3
F2	61.21	0.4
	70.89	3.5
	82.09	8.7
	95.07	14.0
	110.1	17.7
	127.5	18.5
	147.7	16.2
	171.0	11.8
	198.0	6.6
	229.3	2.3
	265.6	0.1
F3	82.06	17.6
	95.07	38.4
	110.1	34.4
	127.5	9.6
F4	61.21	98.4
	70.89	1.6
F5	95.07	11.0
	110.1	56.4
	127.5	32.6

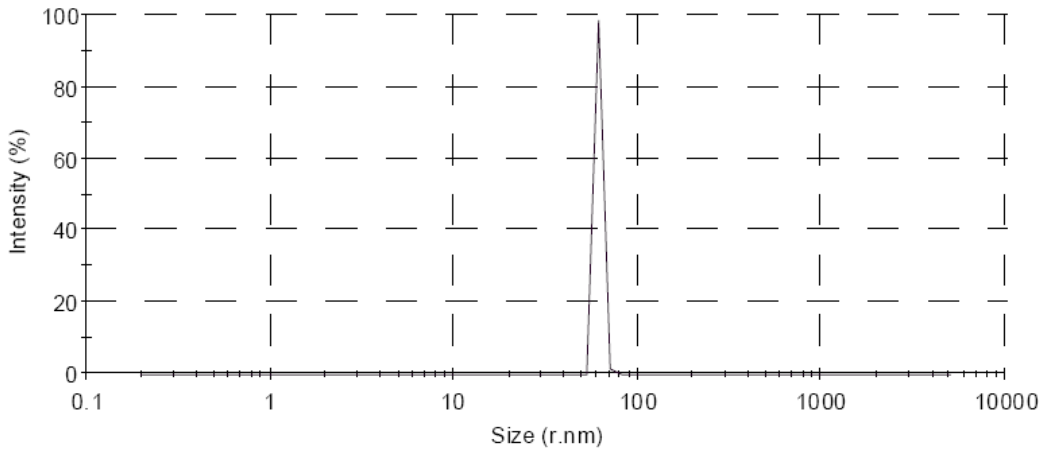

Figure: 1 Size distribution by volume curve of formulation F4

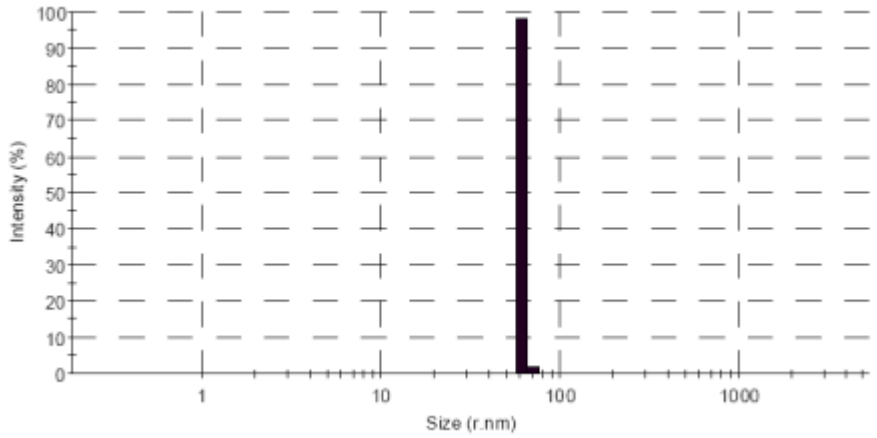

Figure: 2 Statics graph of formulation F4.

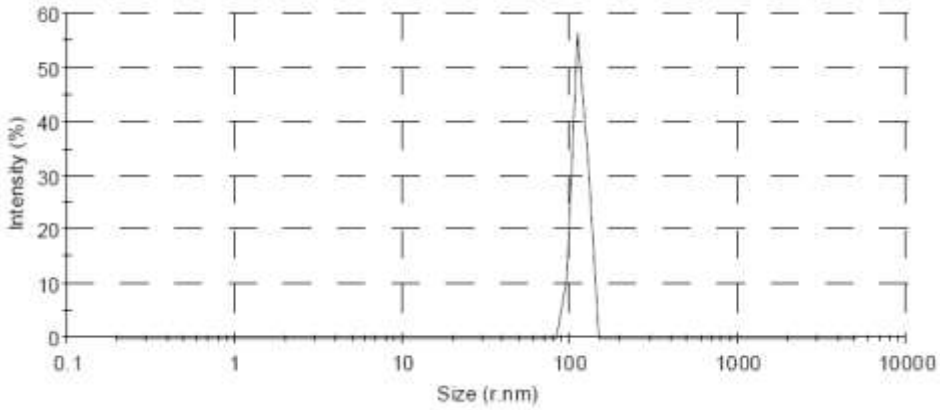

Figure: 3 Size distribution by volume curve of formulation F5.

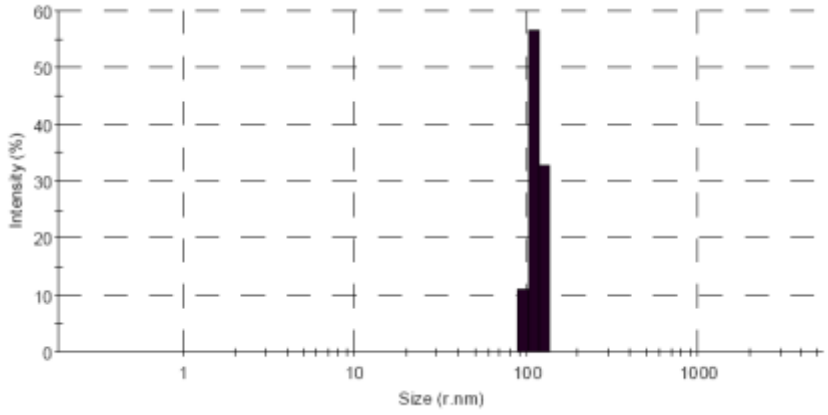

Figure: 4 Statics graph of formulation F5

Malvern Zetasizer (Ver. 6.11) used for particle size evaluation and zeta potential. All the prepared nanoparticles were in nanosized. The minimum particle size distribution found in formulation F4 and then F5 shown in Figure 1-4. Particle size range 61.21 to 265.6 nm was observed as shown in Table 3. There was no relationship between polymeric concentration and particle size within 200nm favorable for effective targeting.

Table: 4 Zeta potential value of Donepezil HCl loaded nanoparticles

S.No	Formulation code	Zeta Potential (mv)
1.	F1	-27.6
2.	F2	-19.1
3.	F3	-17.4
4.	F4	-32.9
5.	F5	-19.8

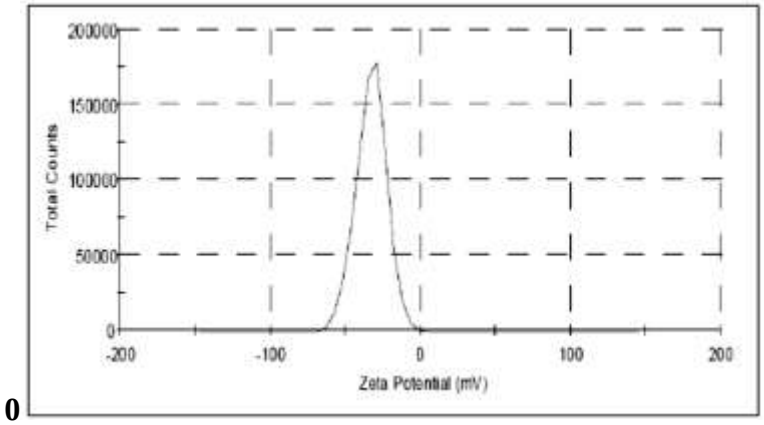

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Figure: 5 Zeta potential distribution of formulation F4.

It is observed that prepared nanoparticles zeta potential was between -32.9 to -17.4 mv shown in Table no: 4. Zeta potential value of nanoparticle between -30 to +30mv indicated more stability. Value of zeta potential generally depends on polymer that used in the preparation. Formulation F4 zeta potential was -32.9 mv observed which signify more stability of formulation F4 shown in Figure 5.

Surface Morphology:

Figure: 6 SEM image of formulation F4

Donepezil loaded Nanoparticles subjected for scanning electron microscopy for determination of surface morphology. As experimental result revealed all prepared Nanoparticles having smooth surface without fracture (Figure 4.).

Drug Content / Surface entrapment / Drug entrapment:

Table: 5 Surface entrapment and drug content of Donepezil HCl loaded nanoparticles

S.No.	Formulation	Abs.	Surface Entrapment (%)	Drug content (%)
1.	F1	0.027	2.727	72.73
2.	F2	0.021	2.307	76.93
3.	F3	0.045	2.00	80.00
4.	F4	0.021	1.667	83.33
5.	F5	0.012	1.200	88.00

Observed value of surface entrapment of nanoparticle is low (1.2 to 2.727 as shown in Table: 5). This surface entrapment lower value shows that higher drug was entrapped (70.003% to 86.8%). The highest value of drug entrapment was 86.8% of formulation F5 (Table: 5). It was observed that as polymer ratio increases drug entrapment increases. From the result it may be concluded that lower surface entrapment and better drug entrapment provide greater control release efficiency of prepared nanoformulation.

Polydispersity index:

Table: 6 Data of polydispersity index

S No.	Formulation Code	Polydispersity Index
1.	F1	0.222
2.	F2	0.808
3.	F3	0.359
4.	F4	0.383
5.	F5	0.281

Polydispersity was estimated by using Malvern zetasizer. Observed data of polydispersity index shows that formulation F1, F3, F4, F5 follows mid range polydispersity index and formulation F2 was very polydispersity observed result shown in Table: 6.

In-vitro release Study:

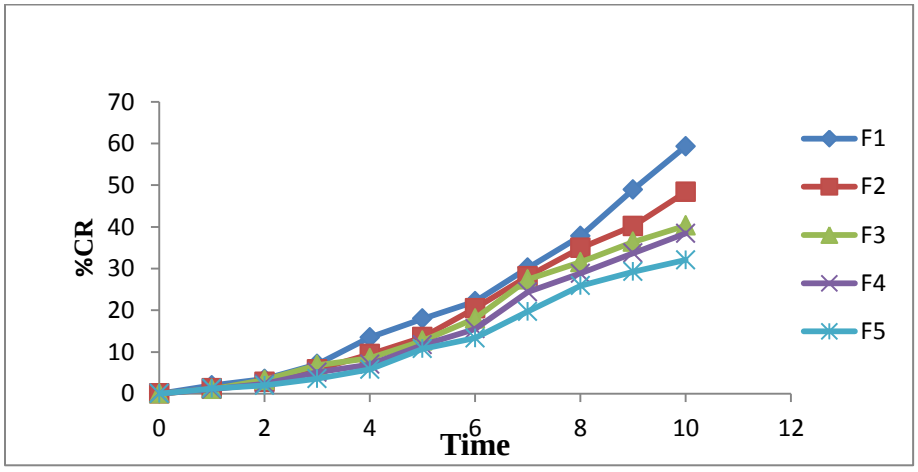

Figure 7: *In-vitro* Zero order release profile of formulation F1 to F5

Table: 7 Kinetic study of formulation F1 to F5

Formulation Code	r^2				n*	Best fit model	Mechanism of release
	Zero order	First order	Higuchi	Hixon crow			
F1	0.9434	0.8796	0.9245	0.9039	1.5389	Zero order	Supercase II transport
F2	0.9527	0.9197	0.9331	0.9321	1.6615	Zero order	Supercase II transport
F3	0.9604	0.9411	0.9328	0.9482	1.6031	Zero order	Supercase II transport
F4	0.9506	0.9299	0.9349	0.9374	1.647	Zero order	Supercase II transport
F5	0.9481	0.9326	0.9377	0.9381	1.5588	Zero order	Supercase II transport

In vitro release study of Donepezil HCl loaded nanoparticles was studied using USP dissolution apparatus type II. The release rate of drug in a particular solvent medium is largely depends on the nature and amount of polymer that used for the preparation of that particular formulation. In this project five different formulation was prepared using different ratio of drug and polymer. From the observed release data of the different formulation it was suggested that released rate and cumulative percentage drug release were directly related to the amount of polymer. As the polymeric concentration increases the release rate of drug decrease significantly. the drug release profile of nanoparticle shown on Figure: 7. Kinetic models are used to describe drug release. Thus the model fitting analysis (Zero Order, Higuchi, Hixon Crowell, First Order) were done by comparing the coefficient of regression (r^2) values and corresponding *n* value of all the kinetic equation. It was observed that the individual formulation having different r^2 value for different model. On the basis of higher value of r^2 we select the best fit model for all five formulation. From experimental result it was observed that zera order model is dominant for all five formulation. This type of release achieved may be due to fact when the drug is surrounded with a polymeric membrane that is permeable for both drug and water. Saturation or solution conditions reaches after swallowing the core becomes hydrated and the drug dissolves. The core serves as a saturated reservoir of drug. Drug release proceeds by partitioning from the reservoir into the membrane followed by diffusion across the membrane into the systemic circulation. So long as saturation is maintained in the core, there will be a stationary concentration gradient across the membranes and release will proceed at constant rate. To predict the mechanism of release following equation $\boldsymbol{M_t/M_\infty = kt^n}$ was used to analyze data. n = 0.5 means Fickian diffusion. 0.5< n < 1.0 Nonfickian diffusion and n = 1.0 case II diffusion. n value calculated for the prepared

nanoparticles in all cases a super case II transport mechanism is dominant. On the basis of experimental result formulation F4 was selected as optimized formulation and subjected for stability study.

Stability study:

In stability study as experimental result shows there is no significant change in physical appearance, drug content and t_{50} value during the 90 day study period. It can be concluded that optimized Donepezil Hydrochloride loaded polymeric nanoparticles are physically as well as chemically stable (Table: 8).

Table: 8 Stability study

Temperature	Evaluation Parameters	Observation (Months)			
		0	1	2	3
30°C ± 2°C	Physical appearance	_ _ _	No change	No change	No change
	%Drug content	83.33	82.73	82.65	82.12
	$t_{50\%}$ (hrs)	9.62	8.57	8.43	8.28
4°C ±1°C	Physical appearance	_ _ _	No change	No change	No change
	%Drug content	83.33	82.89	82.71	82.65
	$t_{50\%}$ (hrs)	9.62	9.54	8.74	8.45

CONCLUSION

Donepezil loaded nanoparticles were successfully prepared by modified nanoprecipitation method. Different formulation using different concentration of polymer efficacy of entrapment of donepezil. Prepared donepezil loaded nanoparticle were in nanosize. *In-vitro* release study revealed that donepezil loaded nanoparticles were capable of releasing the drug in a controlled manner. This study clearly demonstrated that the Donepezil Hydrochloride (model drug) can be successfully delivered to brain by preparing the nanoformulation. Drug delivery to the Central Nervous System (CNS) for diagnosis and treatment of neurodegenerative disorder like Alzheimer's disease is restricted due to blood brain barrier (BBB). Use of nanoparticles is among the most promising strategies to successful increase the CNS penetration of several therapeutic moieties. By effective targeting nanoparticles may enhance bioavailability of drug, reduce dosage regimen, maintain therapeutic concentration of drug, and reduces toxic effects to other organs, reduce hepatic and renal toxicity of drug. Donepezil hydrochloride shows first pass metabolism, by reducing the particle size (nanoparticle), drug directly circulated to blood stream, which reduce its first pass metabolism hence improve the patient compliance.

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