Development and Evaluation of Polymeric Nanoparticles of Donepezil Hydrochloride for Efficient Management of Alzheimer's disease

Mukhopadhyay Sayantan*, Bhatt Jyotsana[1], Kothiyal Preeti[2]
*, [2] Shri Guru Ram Rai University, Dehradun, Uttarakhand, India
[1] Department of Pharmaceutics, Shri Guru Ram Rai Institute of Technology and Science, Uttarakhand Technical University, Uttarakhand, India
***Corresponding author** - Sayantan Mukhopadhyay, Department of Pharmaceutics, Shri Guru Ram Rai Institute of Technology and Science, Shri Guru Ram Rai University, Uttarakhand, India.

***Abstract** – Alzheimer's disease is a progressive neurodegenerative disorder affecting about a large number of population worldwide. Increasing prevalence of alzheimer's lead to new innovations in the field of pharmaceutics for better delivery of drug to the target site i.e. brain. The aim of the present study is development and evaluation of donepezil hydrochloride loaded nanoparticles for efficient management of Alzheimer's disease. Modified solvent evaporation technique was utilized for the preparation of polymeric nanoparticles. Drug (Donepezil hydrochloride) & polymers (Eudragit S100 and Eudragit RS100) were taken in different ratios for preparing six formulations. The prepared nanoformulations were subjected to different evaluation parameters. After characterization these formulations were added to carbopol gel to prepare nanoparticulated gel for further study. On the basis of various evaluation parameters formulation FS1 was selected as the optimized formulation with particle size 195.9nm, percentage yield 47%, zeta potential -19.2, PDI as 0.642, % and percentage release as 84.41% in 24 hrs. The formulation FS1 followed zero order kinetic with supercase II diffusion respectively. Thus, concluding that the prepared donepezil hydrochloride loaded polymeric nanoparticles can be used efficiently to deliver drug to the brain for effective management of Alzheimer's.*

***Keywords:** Alzheimer's disease, Polymeric nanoparticles, modified solvent evaporation technique, spreadability study.*

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1. Introduction –

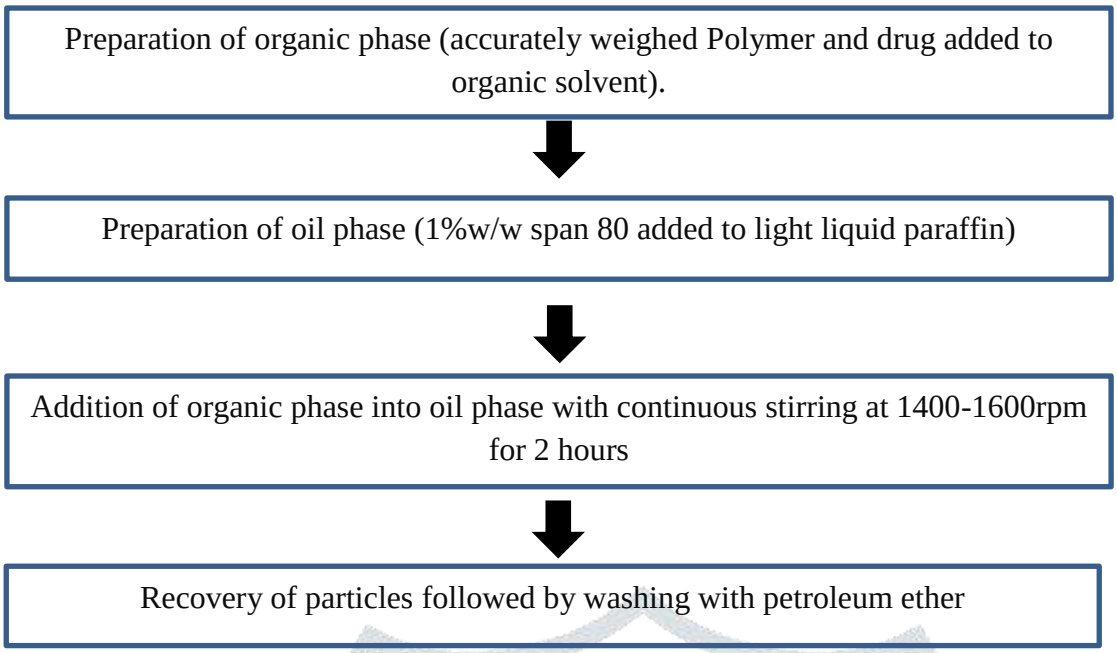
The brain is a control center of the body. It has developed a series of central nervous system barriers to protect itself from invading external molecules, pathogens and toxic substances. The barriers including Blood brain barrier (BBB), blood-cerebrospinal fluid barrier, blood retinal barrier and blood spinal cord barrier act as an interface between brain and the peripheral circulation. Each of the barriers has a different degree of permeability [1]. Among all the barriers, BBB is considered as the most selective barrier due to the presence of glial cells, astrocytes and various proteins contributing to the linkage between the endothelial cells [2, 3]. Thus, making it difficult to delivery various therapeutic agent into the brain for the treatment of different CNS disorders like Alzheimer's disease, Parkinson, brain etc. So, to overcome this problem and deliver therapeutic agent to the brain in optimum amount various strategies have been developed such as intracerebral implants, receptor mediated transport, intranasal drug delivery, nanoparticle drug delivery etc. [4]

Polymeric nanoparticles are solid drug carrier, having particle size ranging from 10-1000nm. These are known to be versatile drug carriers for targeted drug delivery. Owing to their very small size polymeric nanoparticles are immensely used in the medical field specifically in targeting drug to a specific organ or bypassing various biological barriers [5]. Polymeric nanoparticles can be defined as drug carriers in which drug is dissolved, encapsulated and entrapped in a polymer matrix. Known to be the most promising and advanced drug delivery system, nanoparticles have the ability to deliver drug to the target site in an effective manner [6]. In the present study donepezil hydrochloride loaded polymeric nanoparticles are prepared using modified solvent evaporation method for effective management of Alzheimer's disease.

2. Material and Method –

2.1 Preparation of Polymeric nanoparticles using modified solvent evaporation technique –

Donepezil hydrochloride loaded polymeric nanoparticles were prepared using modified solvent evaporation technique given below



Flowchart 1 Modified solvent evaporation technique

Formulation table for preparation of Donepezil hydrochloride loaded polymeric nanoparticles given in **table no.** i.

2.2 Characterization of prepared polymeric nanoparticles –

2.2.1 Percentage yield –

The percentage yield of the prepared polymeric nanoparticles was calculated using following equation [7]

$$\text{Percentage yield} = \frac{\text{Total mass of the polymeric nanoparticles}}{\text{Total mass of drug and polymer}} \times 100$$

2.2.2 Surface entrapment and Drug Entrapment efficiency –

The surface entrapment and entrapment efficiency of the drug was calculated using the centrifugation method. 10mg drug equivalent to formulation was added into a 10ml solvent. The solvent chosen for this method should be able to dissolve the free drug. This 10ml solution is then transferred into centrifuge tubes and centrifugation was carried out for 30 min at 10,000rpm. The supernatant was collected and subjected to UV spectroscopy for determining the free drug content. The entrapment efficiency of the drug was further calculated by using following equation [8]

$$\text{Drug Entrapment efficiency} = \frac{\text{Initial drug} - \text{Free drug}}{\text{Initial drug}} \times 100$$

2.2.3 Particle size and zeta potential –

Particle size and zeta potential of the nanoformulation was determined by using Differential light scattering with a zetasizer (Malvern Instrument, UK). The polymeric nanoparticles were dispersed into milique water and diluted accordingly. This solution was then analyzed using zetasizer for particle size and zeta potential. [9]

2.2.4 Surface morphology –

Surface morphology study of the prepared polymeric nanoparticles was conducted using scanning electron microscopy. The method was carried out by adding the nanoformulation into the holder cell and further coating it with gold using a spatter coater. Then the sample was analyzed in a beam of electron for surface morphology. [10]

2.3 Preparation of Nanoparticulated gel -

The prepared polymeric nanoparticles were added into the gelling base for the better delivery of the drug through topical route. The Nanoparticulated gel was prepared using carbopol 940 (2%w/w) as gelling base. Carbopol is hydrophilic in nature. The nanoparticles were added into the solvent containing specified amount of carbopol 940 this suspension is then stirred at 800 rpm till the formation of uniform gel. Triethanolamine was added accordingly to maintain the pH of the prepared formulation. [11]

2.4 Evaluation of Nanoparticulated gel –

2.4.1 Determination of pH –

The pH of the prepared gel was determined using calibrated digital pH meter. In this method 1gm of gel was added and dissolved in 100ml of distilled water and kept for 2 hours. Then, the pH of the following medium was measured using digital pH meter. [12]

2.4.2 Viscosity –

Viscosity of the prepared Nanoparticulated gel was evaluated using Brookfield viscometer. The prepared gel was subjected to different spindle at specific rpm. The data was collected during the procedure and a graph was prepared to determine the viscosity. [13]

2.4.3 Spreadability study –

Spreadability defines the extent of the gel spread onto the skin when applied. The method includes the use of two glass plates (20cmx20cm). Specific amount of prepared gel (1g) was placed in one of the glass slide and then second glass was placed above it. Then a weight (125g) was placed over the plates for proper spreading. Finally the increase in diameter was noted in cm to determine the spreadability. [12]

2.4.4 Determination of drug content –

For determination of drug content of the formulation 50 mg drug equivalent to formulation was added into a 50ml volumetric flask containing 50 ml of solvent in which both drug and polymer are soluble. The solution was shaken for 30 minutes and then kept for 24 hrs. After 24 hrs the dispersion was further shaken and then filtered. The resultant solution was then subjected to UV spectrophotometer at 265nm. [12]

2.5 In vitro diffusion studies-

In vitro diffusion study was conducted to determine the release of drug from the formulation. In this technique a two sided open cylindrical tube was used. One side of the cylindrical tube was mounted with dialysis membrane of suitable molecular weight and coated with 2gm of gel equivalent to 5mg of donepezil hydrochloride from one side. The dialysis membrane used was soaked in phosphate buffer pH 7.4 for 2 hours before experiment. The setup was made in a way that the end of cylindrical tube containing gel gets in contact with the diffusion medium i.e. phosphate buffer pH 7.4 (25ml). The assembly was then placed on a magnetic stirrer and the content was stirred at 700rpm. The samples were withdrawn every half hour for 3 hours and then every 1 hour for 24 hours. [14]

2.6 Drug release kinetic study –

The drug release kinetic specifies the release mechanism of drug from Nanoparticulated gel. The obtained release data was treated using following equation-

Zero order equation – The Graph plotted between amounts of drug release versus time.

First order equation – Graph between log cumulative percent of drug remaining versus time.

Higuchi equation – Graph between cumulative percent of drug release versus square root of time.

Korsmeyer –Peppas equation – Graph between log cumulative percent of drug release versus log time. [14]

3. Result and discussion –

3.1 Percentage yield –

Discussion - Percentage yield of the prepared nanoformulations FS1-FS3 and FR1-FR3 were calculated and was found between 47 % to 61.75% and 29% to 47.25% respectively **(Table no. ii)**. A conclusion may be drawn from above data that with increase in polymer concentration, the percentage yield of the nanoformulation increases.

3.2 Particle size distribution –

Discussion – The particle size of prepared formulations was evaluated using zetasizer. The particle size of the prepared formulations FS1-FR3 lied in a range 2.207nm to 4922nm **(Table no. iii)**. Formulation FS1 and FS2 were found to have the smallest particle size 195.9nm and 232.2nm with 100% intensity **(Fig no. i&ii)**. From the above data it was observed that with increase in ratio of polymer, the particle size of the formulation increase.

3.3 Zeta potential Study –

Discussion - Zeta potential of the formulated nanoparticles was found between -8.06mV to -20.3mV **(Table no. iv)**. As per the reports, nanoformulations with zeta potential more than +25mV and less than -25mV are known to have high degree of stability and from the given

data it was observed that the prepared nanoformulations show moderate stability. Zeta potential graph of formulation FS1 is given in **Fig no. iii**.

3.4 Polydispersity index of prepared Polymeric nanoparticles –

Discussion - The Polydispersity index data of the prepared formulation was given in **Table no. v**. It was observed that all the formulations are mid-range polydisperse. This may be due to wide-ranging size distribution of the particles. There was no co-relation established between the ratio of polymer and PDI value of the prepared nanoformulation.

3.5 Surface morphology study –

Discussion - From the obtained result it was observed that the prepared nanoformulation FS1 **(Fig no. iv)** showed the best surface morphology with smooth surface and uniform distribution of particles whereas in the formulation FS2 **(Fig no. v)** slight aggregation of particles was observed.

3.6 Evaluation of Surface entrapment and percentage entrapment efficiency –

Discussion - The surface entrapment and % entrapment efficiency of the prepared formulations was reported in **Table no.vi**. From the given data it was observed that less than 4.21% drug was present in the surface thus concluding the high percentage of drug entrapment within the polymer matrix. FS3 has the highest % entrapment efficiency 99.32%. It may also be concluded that the surface entrapment of drug decreases with increase in polymer concentration.

3.7 Evaluation of Donepezil hydrochloride loaded Nanoparticulated gel –

3.7.1 Viscosity –

Discussion - The viscosity of the prepared Nanoparticulated gel was evaluated using Brookfield viscometer **(Table no. vii)**. The graph was plotted between rpm and viscosity **(Fig no.vi)**. It was observed that with an increase in rate of shear, the viscosity decreases. Thus, concluding shear-thinning or pseudoplastic flow.

3.7.2 Spreadability and pH study of prepared gel

Discussion - pH of the prepared nanoparticulated gel was found to be 7.0±0.98 **(Table no. viii).** The observed value of pH lies within the range of physiological pH of the skin i.e., pH 6.8. Thus, suggesting no skin irritancy.

Spreadability of a gel is an important characteristic parameter. The spreadability of the prepared donepezil HCl loaded nanoparticulated gel was calculated as 13.08±5.14 which indicate easy spreadability of gel on application of small amount of shear.

3.7.3 Evaluation of Drug content of prepared nanoparticles –

Discussion - The drug content of the prepared formulation was reported in **Table no. ix**. From experimental result it was observed that in maximum number of result formulation percentage drug content was greater than 86.4%, which confirmed good drug loading capacity of prepared polymeric nanoparticles and from the study it may be concluded that % drug content largely depends on variables of preparation technology.

3.8 *In –vitro* diffusion study of donepezil loaded Nanoparticulated gel –

Discussion - The release data of the drug loaded nanoparticulated gel formulations was shown in **Fig. vii.** From the release data it was observed that more than 68.34% of drug was released in 24 hours study period by all the formulations. It was also clearly observed that release rate and % CR are inversely proportional to polymer concentration. With an increase in polymer concentration the release rate of the drug was retarded. Formulation FS1-FS3 and FR1-FR3 were prepared using polymer Eudragit S100 and Eudragit RS100 respectively but no significant difference was observed in release pattern of these formulation. The % cumulative release of the prepared formulations is given as FS1 84.41% (1:3 drug polymer ratio) > FS2 76.32 (1:5) > FS3 68.34 (1:7) and FR1 79.45 (1:3) > FR2 74.38 (1:5) > FR3 (1:7).

3.9 Drug release kinetic study-

Discussion - Kinetic models study is conducted to determine the release pattern of the drug from different dosage forms. By comparing the value of regression coefficient (r2) and n of all the kinetic equation, the best fit model and release mechanism for the formulations was determined. Korsmeyer-peppas model is used to determine the release mechanism of the drug from the formulation. The n- value of korsmeyer peppas equation ($Mt/M\infty = ktn$) is used to predict the type of diffusion. The value of n is used to evaluate the type of diffusion, as $n=0.5$ means fickian diffusion, $0.5<n<1$ means non-fickian diffusion and $n=1$ means supercase II diffusion.

Kinetic data for the formulation FS1-FS3 and FR1-FR3 was reported in **Table x**. By comparing the value of r2 of all kinetic models of different formulations, zero order was selected as best fit model which shows that the release rate of the drug from dosage form is dependent on time. On observing the n- value of korsmeyer peppas equation ($Mt/M\infty = ktn$) for all the formulations it was concluded that Supercase II transport is dominant which shows that the release of drug from dosage depends on the erosion of the polymer chain. In this type of diffusion

the drug is surrounded by a polymeric membrane from which it is easily permeable. The core of the formulation acts as a reservoir for the drug. The drug first release to membrane from reservoir and then diffuses to systemic circulation.

4 Conclusion -

The aim of the present study was development and evaluation of polymeric nanoparticles of donepezil hydrochloride for efficient management of Alzheimer's disease. Donepezil hydrochloride loaded nanoparticles were prepared by using modified solvent evaporation method. Six formulations were prepared by using different ratios of polymer (Eudragit S100 & Eudragit RS100) and drug. Prepared formulations were subjected to various evaluation studies before incorporating it into gel for the preparation of nanoparticulated gel. On the basis of various evaluation parameters like percentage yield, particle size, zeta potential, drug content, % entrapment efficiency, in vitro diffusion, surface morphology FS1 was selected as best optimized formulation and was considered suitable for further studies. Formulation FS1 has particles size 195.9nm with 100% intensity, percentage yield 47%, zeta potential -19.2, PDI as 0.642, % entrapment efficiency 96.57% and percentage release as 84.41% in 24 hrs. The formulation FS1 followed zero order kinetic with supercase II diffusion respectively. The vivo pharmacological screening was conducted using two models elevated plus maze and radial arm maze. The result obtained from the pharmacological study indicated that the prepared donepezil hydrochloride loaded nanoparticulated gel can be efficiently used in the treatment of Alzheimer's disease.

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Table (i) Formulation table for preparation of Donepezil hydrochloride loaded polymeric nanoparticles

S.no.	Ingredients	Formulations					
		FS1	FS2	FS3	FR1	FR2	FR3
1	Donepezil Hydrochloride	50(mg)	50(mg)	50(mg)	50(mg)	50(mg)	50(mg)
2	Eudragit S100	150(mg)	250(mg)	350(mg)	-	-	-
3	Eudragit RS100	-	-	-	150(mg)	250(mg)	350(mg)
4	Acetone	30(ml)	30(ml)	40(ml)	30(ml)	30(ml)	40(ml)
5	Light liquid paraffin	30(ml)	30(ml)	40(ml)	30(ml)	30(ml)	40(ml)
6	Span 80	1(%w/v)	1(%w/v)	1(%w/v)	1(%w/v)	1(%w/v)	1(%w/v)

Table (ii) Percentage yield of donepezil hydrochloride loaded nanoparticles

S.no.	Formulation code	Percentage yield (n=3) Mean ± S.D
1	FS1	47±1.24
2	FS2	48.35±1.65
3	FS3	61.75±0.99
4	FR1	29±1.79
5	FR2	42±2.01
6	FR3	47.25±0.76

Table no. (iii) Particle size distribution of prepared nanoparticles

S.NO.	Formulation code	Particle size (nm)	
		Diameter (nm)	% Intensity
1.	**FS1**	195.9	100
2	**FS2**	232.2	100
3	**FS3**	270.7	92.4
		4922	7.6
4	**FR1**	277.7	96.7
		5292	3.3
5	**FR2**	287.3	93.9
		3.490	6.1
6	**FR3**	319.9	89.8
		2.207	10.2

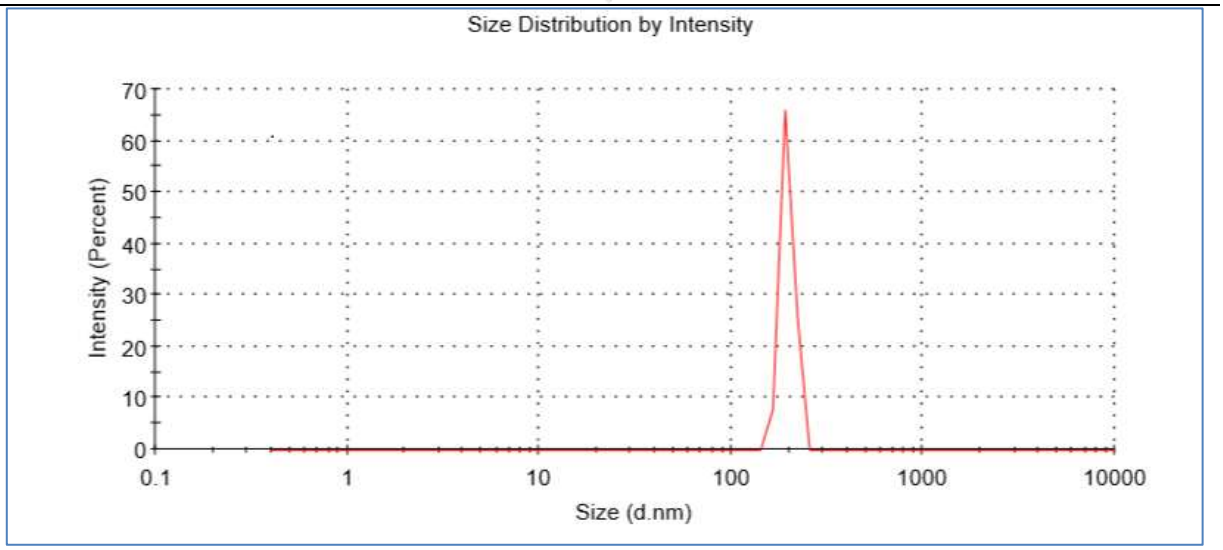

Fig. i Particle size distribution graph of formulation FS1

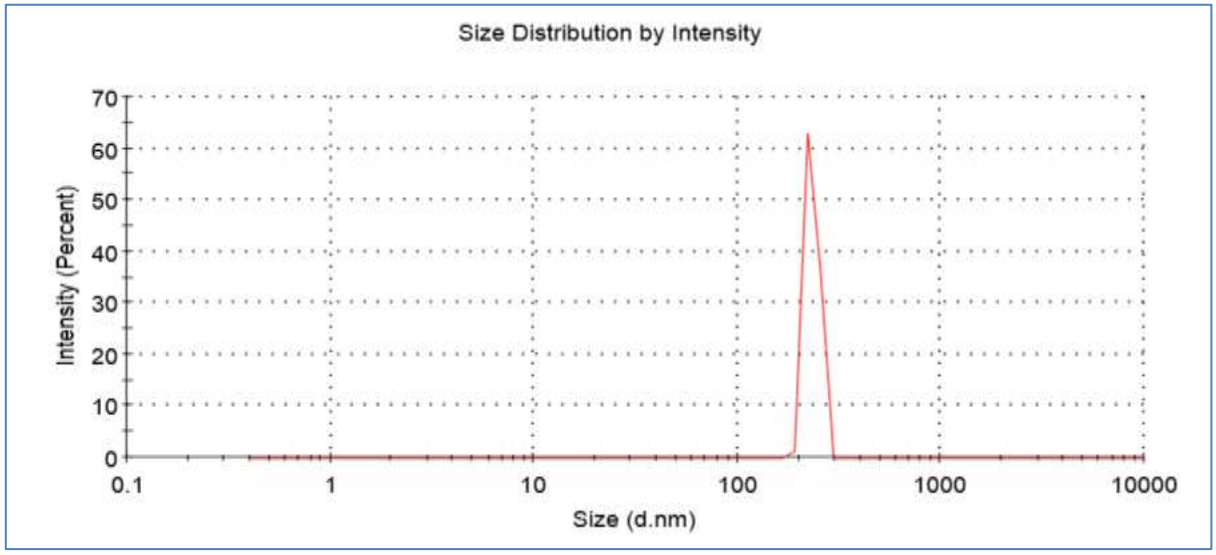

Fig. ii Particle size distribution graph of formulation FS2

Table (iv) Zeta potential of the prepared polymeric nanoparticles

S.no.	Formulation code	Zeta potential
1	FS1	-19.2
2	FS2	-20.1
3	FS3	-9.11
4	FR1	-20.3
5	FR2	-19.1
6	FR3	-8.06

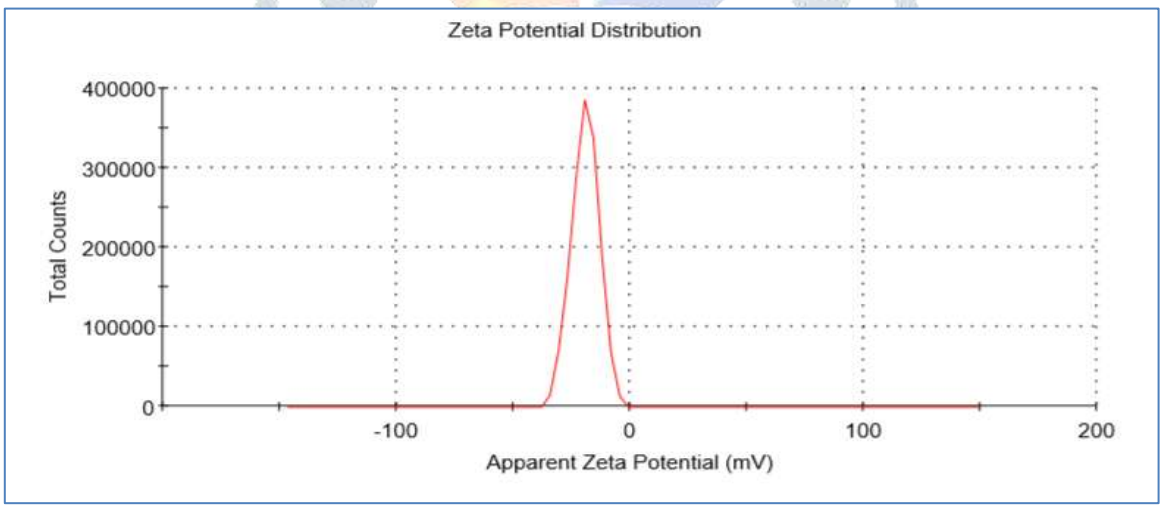

Fig. iii Zeta potential of prepared polymeric nanoparticles FS1

Table (v) Polydispersity index data of prepared donepezil loaded nanoparticles

S.no.	Formulation code	PDI	Report
1	FS1	0.642	Mid-range polydisperse
2	FS2	0.511	Mid-range polydisperse
3	FS3	0.443	Mid-range polydisperse
4	FR1	0.403	Mid-range polydisperse
5	FR2	0.376	Mid-range polydisperse
6	FR3	0.481	Mid-range polydisperse

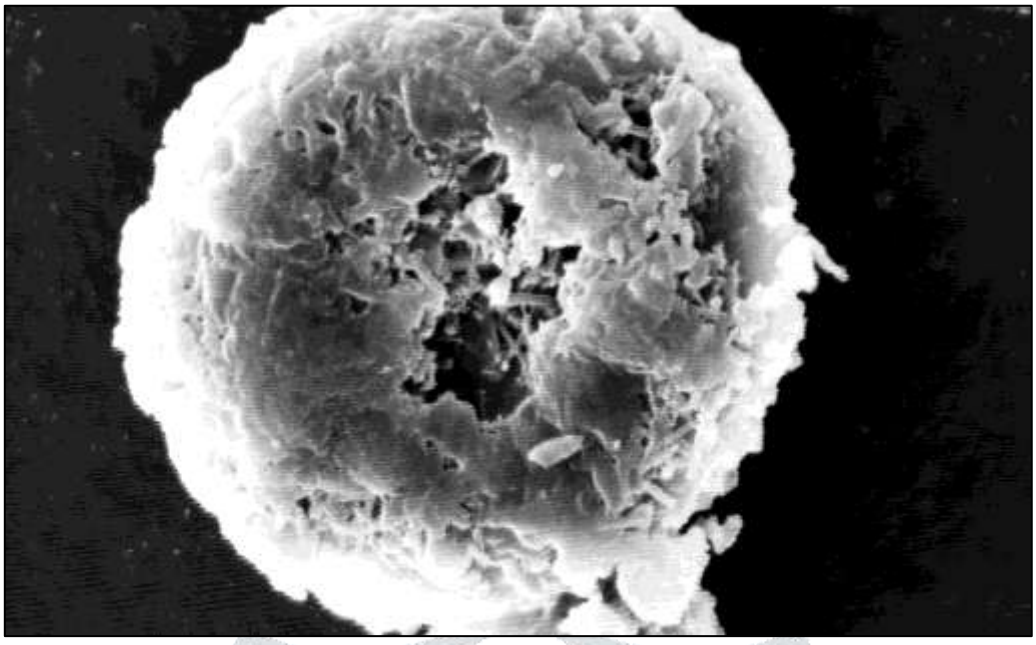

Fig iv Surface morphology study of formulation FS1

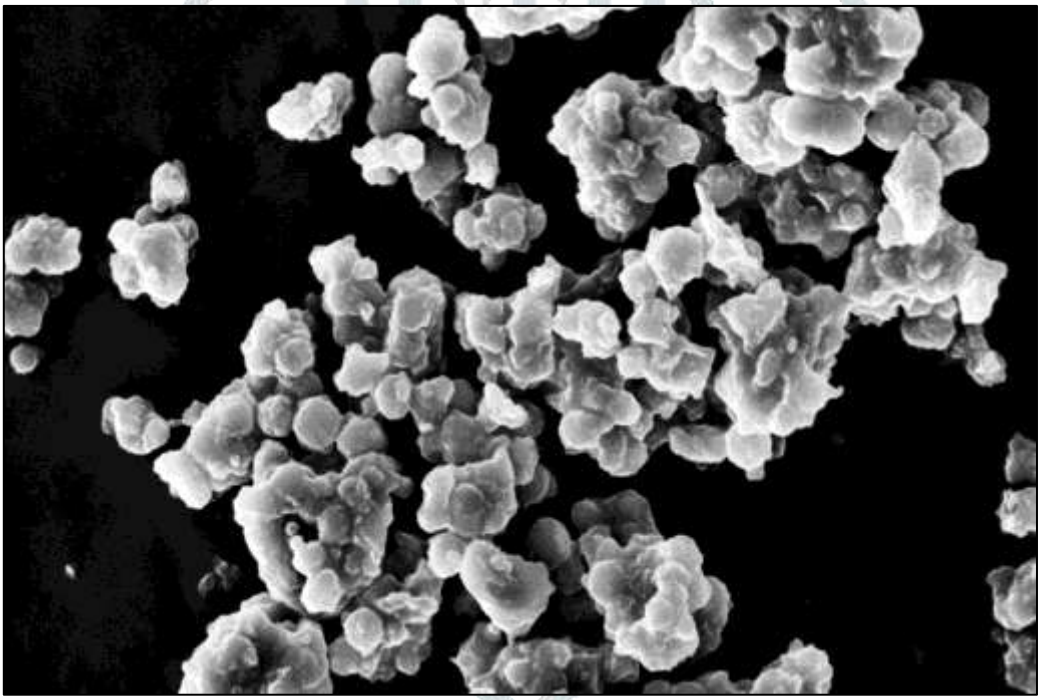

Fig v Surface morphology study of formulation FS2

Table (vi) Surface entrapment and % entrapment efficiency of prepared nanoparticles

S.no.	Formulation code	Surface entrapment (%) (n=3) Mean ± S.D	% Entrapment efficiency (n=3) Mean ± S.D
1	FS1	3.49±0.32	96.51±1.21
2	FS2	2.51±0.78	97.49±1.54
3	FS3	0.98±0.45	99.32 ±2.12
4	FR1	4.21±0.43	95.79±1.45
5	FR2	3.27±1.32	96.73±2.02
6	FR3	1.62±0.94	98.38±2.34

Table (vii) Viscosity of prepared gel

Spindle	RPM	% Torque	Viscosity (cps)	Avg. viscosity
64s p.f	2	1.3	3900	1804.667
	4	1.7	3750	
	6	1.9	1800	
	10	2.8	1620	
	20	3.4	1290	
	30	5.7	1140	
	50	8.1	972	
	60	9	900	
	100	14.4	870	

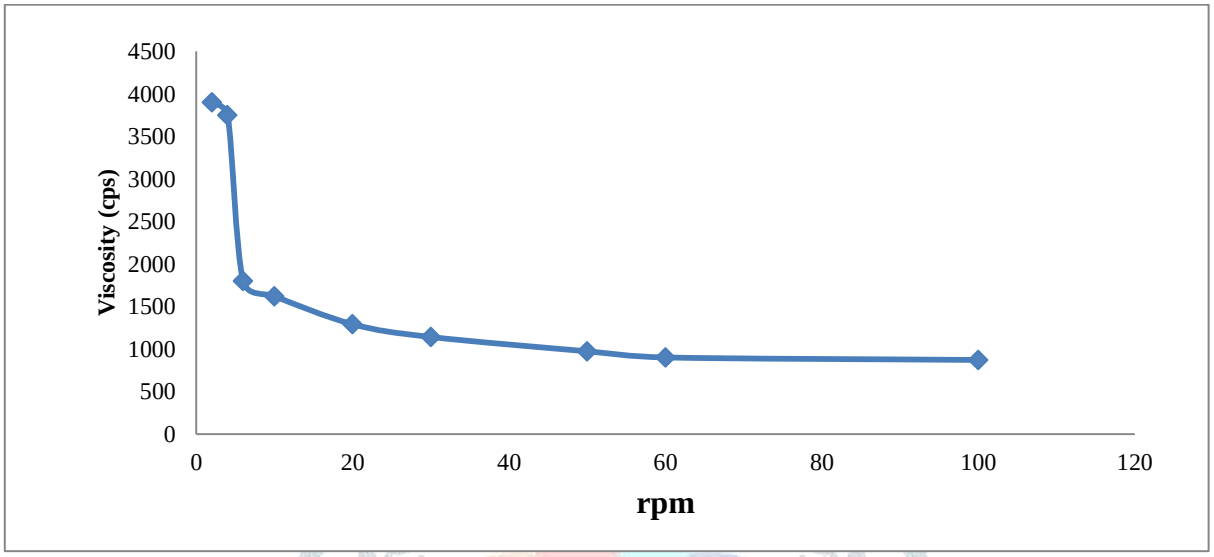

Fig (vi) Graph of viscosity against rpm of the prepared gel

Table (viii) Result of Spreadability and pH of the optimized gel formulation

Formulation code	pH	Spreadability
FF1	7.0±0.98	13.08±5.14

Table (ix) Drug content of the donepezil loaded nanoparticles

S.no	Formulation code	Percentage drug content (%) n=3 (Mean±S.D.)
1	FS1	94.8±1.45
2	FS2	86.4±2.3
3	FS3	92.7±2.76
4	FR1	90.1±2.02
5	FR2	92.02±1.88
6	FR3	88.5±1.54

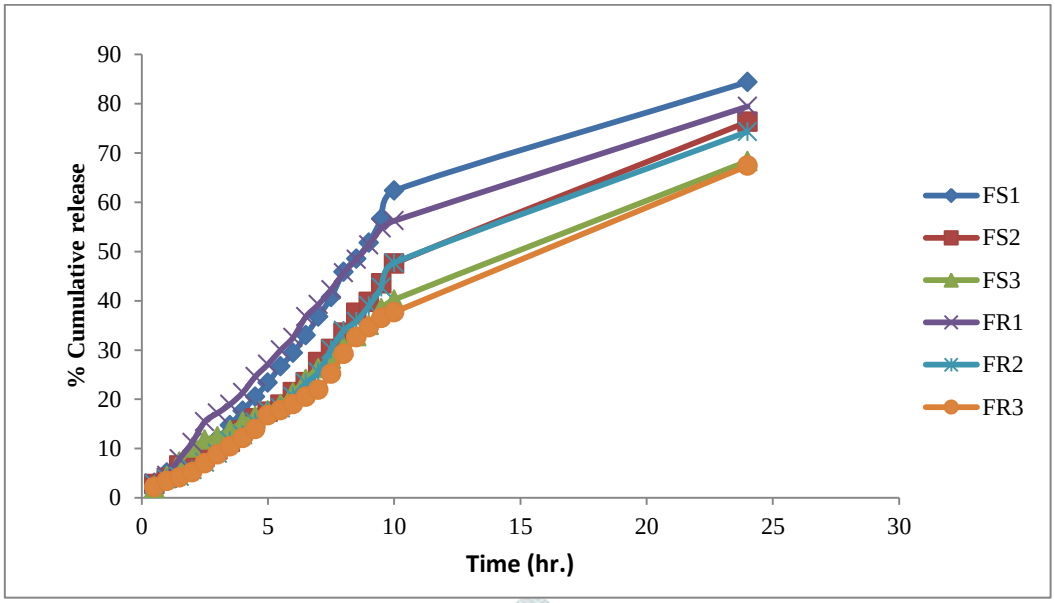

Fig (vii) *In-vitro* zero order release profile of formulation FS1-FR3

Table (x) Comparison of correlation coefficient of release data of formulation FS1-FR3 by using curve fitting models.

Formulation code	r^2				n^*	Best fit model	Mechanism of release
	Zero order	First order	Higuchi kinetics	Hixon crowell			
FS1	0.9792	0.9316	0.9345	0.9764	1.0549	Zero order	Supercase II Transport
FS2	0.9702	0.9369	0.9297	0.9494	0.9798	Zero order	Anomalous Transport
FS3	0.9807	0.9625	0.9228	0.9695	0.8943	Zero order	Anomalous Transport
FR1	0.9982	0.9817	0.9325	0.9900	1.01887	Zero order	Supercase II Transport
FR2	0.9679	0.9332	0.9330	0.9463	1.0299	Zero order	Supercase II Transport
FR3	0.9809	0.9615	0.9333	0.9688	1.0242	Zero order	Supercase II transport