



Formulation and evaluation of rivastigmine loaded polymeric nanoparticles

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ABSTRACT

The purpose of the present study was to formulate polyethylene oxide and polyvinyl alcohol nanoparticulate system of Rivastigmine for drug delivery to brain. Nanoparticles were prepared with poly ethylene oxide (PEO) by solvent displacement technique stabilized by polyvinyl alcohol (PVA). The Prepared nanoparticles were characterized for pH, particle size, surface morphology, entrapment efficiency, In vitro release, zeta potential. Compatibility studies indicated replication of spectral peaks in the study indicated drug and excipients were compatible with each other. The particle size was determined by Malvern zeta sizer. The average particle size was 100.7 nm with polydispersity index of 0.232. The zeta potential of the optimized formulation D1 was determined and found to be 34.8 mV. Surface properties of the nanoparticles were studied by Transmission electron microscopy (TEM) and nanoparticles found to have smooth surface. The Drug entrapment efficiency was found to be in between 67.5 to 86.53% indicated fairly good drug loading in the formulations indicated increased bioavailability of the drug. Percentage drug release data of optimized formulation D1 fitted in Higuchi's plot indicating diffusion and erosion mechanism of drug release from the developed formulations.

Key words: Rivastigmine, Nanoparticles, Solvent displacement technique, Release kinetics.

INTRODUCTION

Among several causes of dementia, Alzheimer's Disease is the most common. It involves progressive degeneration of neurons that are responsible for learning and memory processes. According to WHO it is estimated that there are currently about 18 million people worldwide suffering from Alzheimer's Disease. Symptoms include gradual development of forgetfulness, progressing to disturbances in language, disability to calculate, difficulty to judge location of objects in space and problem in moving.

Neuropathologically, the disease is characterized by a progressive loss of neurons and synapses with the presence of largenumbers of extracellular amyloid plaques and intracellular neurofibrillary tangles. The earliest pathological event that occurs in the process of Alzheimer's disease is the deposition of the amyloid- β peptide in insoluble forms within the brain. Other pathological features include extracellular senile plaques (mainly composed of amyloid-b peptide), intracellular neurofibrillary tangles, synaptic loss, and brain atrophy.

Two classes of drugs are approved for the treatment of Alzheimer's disease (AD). The first were the cholinesterase inhibitors (ChEI). The first drug of this class was tacrine in 1994, followed by donepezil, rivastigmine, and galantamine. All these cholinesterase inhibitors are approved for the treatment of mild to moderate AD. Tacrine has shown severe hepatic side effects. Donepezil has a long half-life and is effective as a once daily drug administration with very less side effects. It is therefore a poor candidate for sustained drug delivery dosage form. Galantamine also exhibits very long half-life of 7 hours. Rivastigmine has demonstrated favorable efficacy and safety in patients with dementia of the Alzheimer type and is widely approved for the treatment of mild to moderate AD. It also inhibits Butyrylcholinesterase. In patients with dementia related to Alzheimer's disease, rivastigmine has symptomatic effects

that enable patients to do their work individually. Administered orally, it has short half-life of 1.5 hours due to hepatic first pass metabolism.[1]

Rivastigmine tartrate was approved by the US Food and Drug Administration for the treatment of AD, but its current therapy has many drawbacks that include restricted entry into brain due to its hydrophilicity, necessitating frequent dosing and cholinergic side effects. Targeting of drugs to the brain is one of the most challenging issues for pharmaceutical research, as many hydrophilic drugs and neuropeptides are unable to cross the blood brain barrier.[2]

Drug delivery to the brain requires advances in both drug delivery technologies and drug discovery. Drugs that are effective against diseases in the CNS and reach the brain via the blood compartment must pass the BBB. The management of brain-related diseases with presently available therapeutic system is very difficult, as insufficient amount of drug reaches the brain, due to highly lipophilic nature of the BBB. Many strategies have been developed to overcome this problem which includes chemical delivery systems, magnetic drug targeting or drug carrier systems such as antibodies, liposomes or nanoparticles.[3-6]

Among these, polymeric nanoparticles have recently attracted great attention as potential drug delivery systems. Due to their small size, nanoparticles penetrate into even small capillaries and are taken up within cells, allowing an efficient drug accumulation at the targeted sites in the body. The use of biodegradable materials for nanoparticles preparation allows sustained drug release at the targeted site over a period of days or even weeks after injection.[7]

Rivastigmine tartrate is a reversible cholinesterase inhibitor used for the treatment of Alzheimer's disease. Rivastigmine has been shown to improve or maintain patients' performance in three major domains: cognitive function, global function and behavior. However, limitations with its oral therapy include restricted entry into brain due to its hydrophilicity, necessitating frequent dosing and cholinergic side effects like severe bradycardia, nausea, dyspepsia, vomiting and anorexia.[8] Hence, the present study was aimed at formulating nanoparticulate systems of rivastigmine tartrate that can improve brain targeting, provide sustained release, reduce dosing frequency and minimize side effects.

EXPERIMENTAL SECTION

Rivastigmine tartrate was received as a gift sample from Micro labs, Bangalore. Polyethylene oxide (PEO) was received as a gift sample from Medreich pharma, Bangalore, and Polyvinyl alcohol (PVA) was received as a gift sample from Medreich pharma, Bangalore. All the other chemicals and reagents used in the study were of analytical grade.

Preformulation studies[9]

FTIR spectral studies lie more in the qualitative identification of substances either in pure form or in combination with polymers and excipients and acts as a tool in establishment of chemical interaction. Since FT-IR is related to covalent bonds, the spectra can provide detailed information about the structure of molecular compounds. In order to establish this point, comparisons were made between the spectrum of the substances and the pure compound.

FTIR spectra were recorded with a Thermo Nicolet. Japan in the range 450–4000 cm^{-1} using a resolution of 4 cm^{-1} and 16 scans. Samples were diluted with KBr mixing Powder, and pressed to obtain self-supporting disks. Liquid samples formulations were analyzed to form a thin liquid film between two KBr disks.

Formulation of Rivastigmine Nanoparticles[10]

Rivastigmine polymeric nanoparticles were prepared with poly ethylene oxide (PEO) by solvent displacement technique. Briefly, different concentrations of PEO (0.1, 0.2, 0.3, 0.4 gm) and 10 mg Rivastigmine were dissolved by heating and sonication in specified volume of acetone and methanol. This organic phase was injected drop wise into water (aqueous phase) containing 1% PVA as hydrophilic surfactant, added under mechanical stirring. Formulation chart of Rivastigmine Nanoparticles was given in Table No:1

Optimization of formulation [11]

The amount of drug (ie, 10 mg) was kept constant, and the concentration of polyethylene oxide (PEO) was varied accordingly (ie, 100, 200, 300, 400 mg). The phase ratios (organic: aqueous) was also varied accordingly for the formulations as 1:2, 1:3, and 1:4. Based upon the entrapment values, the formulation with highest entrapment was selected to be the optimized formulation. Formulation D1 with drug: polymer ratio of 1:40 and organic: aqueous phase ratio of 1 : 2 was found to be appropriate with encapsulation efficiency of 86.53%.

Table 1: Formulation of Rivastigmine Nanoparticles

S.no	Formulation code	Drug : polymer ratio	Organic : aqueous phase ratio	Drug (gm)	PEO (gm)	PVA (gm)	Acetone (ml)	Methanol (ml)	Water (ml)
1.	A1	1 : 10	1 : 2	0.01	0.1	0.4	15.0	5.0	40.0
2.	A2	1 : 10	1 : 3	0.01	0.1	0.6	15.0	5.0	60.0
3.	A3	1 : 10	1 : 4	0.01	0.1	0.8	15.0	5.0	80.0
4.	B1	1 : 20	1 : 2	0.01	0.2	0.4	15.0	5.0	40.0
5.	B2	1 : 20	1 : 3	0.01	0.2	0.6	15.0	5.0	60.0
6.	B3	1 : 20	1 : 4	0.01	0.2	0.8	15.0	5.0	80.0
7.	C1	1 : 30	1 : 2	0.01	0.3	0.4	15.0	5.0	40.0
8.	C2	1 : 30	1 : 3	0.01	0.3	0.6	15.0	5.0	60.0
9.	C3	1 : 30	1 : 4	0.01	0.3	0.8	15.0	5.0	80.0
10.	D1	1 : 40	1 : 2	0.01	0.4	0.4	15.0	5.0	40.0
11.	D2	1 : 40	1 : 3	0.01	0.4	0.6	15.0	5.0	60.0
12.	D3	1 : 40	1 : 4	0.01	0.4	0.8	15.0	5.0	80.0

Characterization of Polymeric Nanoparticles

Determination of Particle Size Analysis and zeta-potential analysis [10,11]

Nanoparticle size distribution and zeta potential (ζ) were determined using photon correlation spectroscopy (Zetasizer, HAS 3000; Malvern Instruments, Malvern, UK). The size distribution analysis was performed at a scattering angle of 90° and at a temperature of 25°C using samples appropriately diluted with filtered water whereas zeta potential was measured using a disposable zeta cuvette.

Particle morphology [10,11]

Morphologic evaluation of the nanoparticles was performed using transmission electron microscopy (TEM; Philips CM-10, Eindhoven, The Netherlands). Samples of the nanoparticle suspension (5 to 10 μ L) were dropped onto Formvar-coated copper grid (Plano GmbH, Wetzlar, Germany). After complete drying, the samples were stained using 2% w/v phospho-tungstic acid. Digital Micrograph and Soft Imaging Viewer software (Olympus, Singapore) were used to perform the image capture and analysis.

Entrapment Efficiency [10,11,12]

Entrapment efficiency of drug loaded PNPs was determined by centrifugation of samples at 13,000 rpm for 20 min. The amount of free drug was determined in the clear supernatant by UV spectrophotometer at 263 nm using supernatant of non loaded nanoparticles on basic correction.

The entrapment efficiency (EE %) could be achieved by the following equation.

$$EE (\%) = \frac{\text{Total amount of Rivastigmine} - \text{Free Rivastigmine}}{\text{Total amount of Rivastigmine}} \times 100$$

In vitro release profile [9,10,12]

In vitro release studies were performed using modified Franz diffusion cell. Dialysis membrane having pore size 2.4 nm; molecular weight cut off 12,000–14,000, was used (Membrane was soaked in double-distilled water for 12 hr before mounting in a Franz diffusion cell). A volume equivalent to 6 mg of Rivastigmine (Practically calculated) loaded PNPs formulation was placed in the donor compartment and the receptor compartment was filled with 10 ml of PBS. The content of the cell was stirred with the help of magnetic stirrer at 37°C. Aliquots were withdrawn from receiver compartment through side tube at every hour time interval up to 12 hours. Fresh medium of PBS was replaced each time to maintain constant volume. Samples were analyzed by UV visible spectroscopy at 263 nm.

Drug-release kinetics [9,10]

The drug release data was subjected various analyses like Zero-order, First-order, Higuchi, Hixson Crowell model and Korsmeyer-Peppas.

RESULTS AND DISCUSSION

Preformulation Studies FTIR Spectroscopy:

Compatibility study of drug with the excipients was determined by FTIR spectroscopy. The spectra of the drug and other ingredients used in the formulation were compared with the spectra of binary mixture of drug and excipients mixed in the ratio of 1:1. The standard FTIR spectra of the drug matches with the FTIR of the drug sample taken for the study confirms the authenticity of the drug. There was no significant appearance of new peaks or disappearance of characteristic peaks implies that there was no incompatibility between drug and the excipients taken for the study.

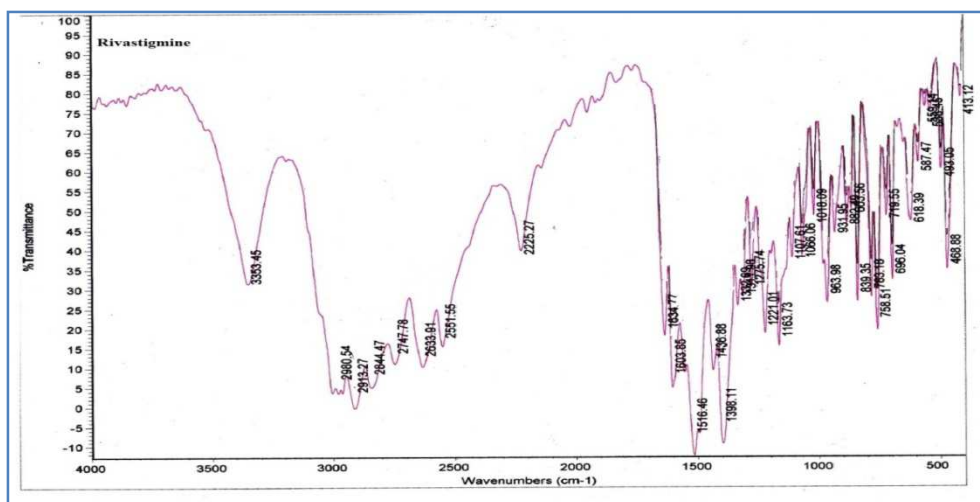


Fig 1: FTIR Spectra of Rivastigmine

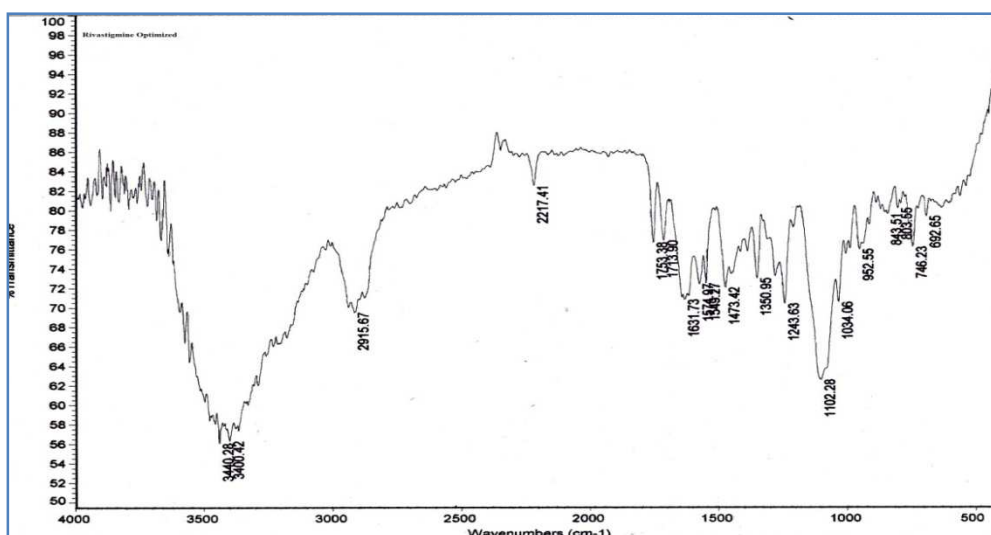


Fig 2: FTIR Graph of optimized formulation

Particle size and Particle size distribution

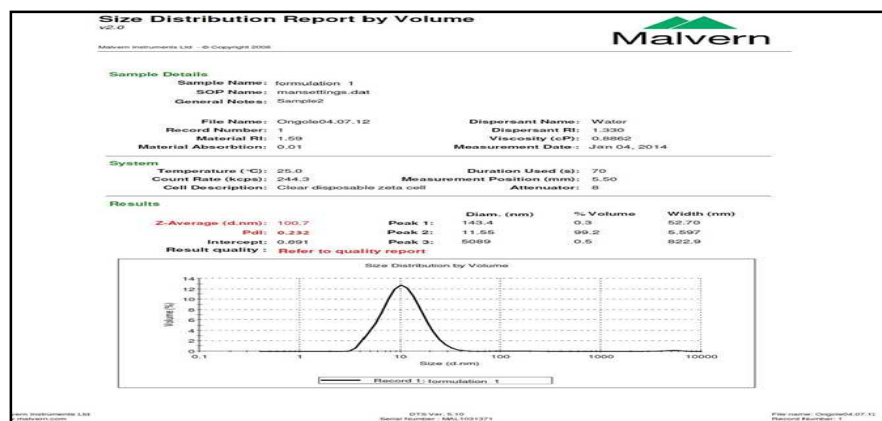
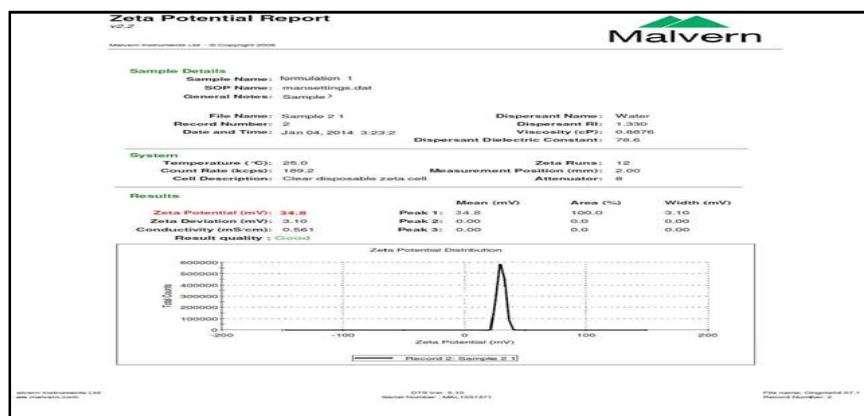


Fig 3: Particle size distribution of Optimized Formulation

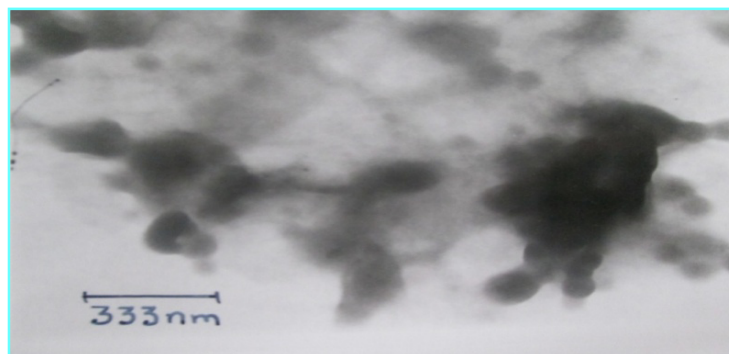
The particle size and particle size distribution of the best formulation[D1] was determined by Malvern zeta sizer. The results confirmed that the average particle size was 100.7 nm and polydispersity index of 0.232. The particle size distribution is found to be normal and uniform.

Zeta Potential (ζ):**Fig 4: Zeta Potential of Optimized Formulation**

The zeta potential of the best formulation [D1] was determined and found to be 34.8 mV. Zeta potential is an important physico-chemical parameter that influences stability of the nanosuspension. Extremely positive or negative zeta potential values cause larger repulsive forces, whereas repulsion between particles with similar electric charge prevents aggregation of the particles and thus ensures easy dispersion. In case of a combined electrostatic and steric stabilization, a minimum zeta potential of ± 20 mV is desirable.

Particle Morphology:

The morphology of the Rivastigmine loaded nanoparticles produced with polyethylene oxide (PEO) was assessed by Transmission electron microscopy (TEM) shown in Figure No: 5 confirming the spherical shaped particles.

**Fig 5: Transmission Electron Microscopy of Rivastigmine loaded Nanoparticles****Table 2: % Entrapment efficiency of different formulations**

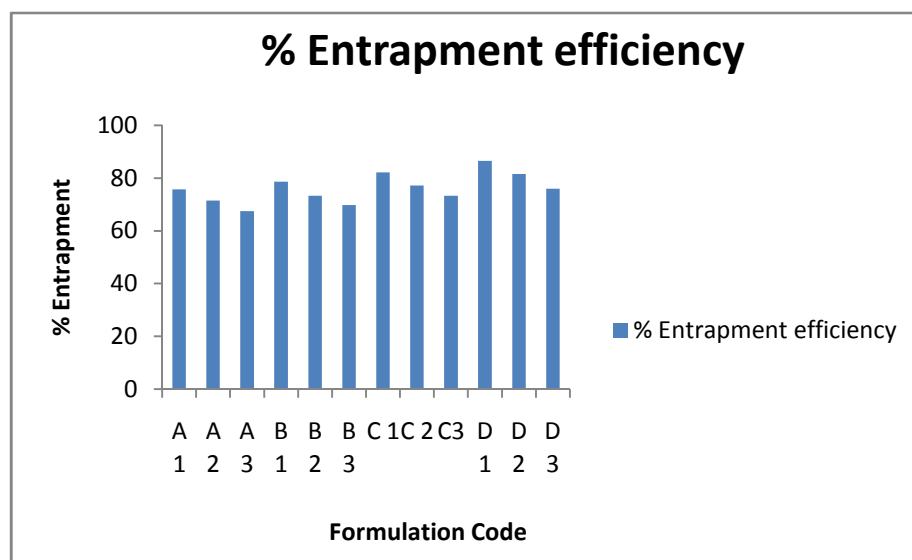
S.No	Formulation code	%Entrapment Efficiency
1	A 1	75.69
2	A 2	71.49
3	A 3	67.5
4	B 1	78.69
5	B 2	73.32
6	B 3	69.75
7	C 1	82.21
8	C 2	77.24
9	C 3	73.35
10	D 1	86.53
11	D 2	81.52
12	D 3	76.00

Drug Entrapment Efficiency:

The Drug entrapment efficiency of the Rivastigmine polymeric nanoparticles was determined by using centrifugation method. The %Entrapment of drug was tabulated in the Table No:7. The % Entrapment efficiency

varied from 67.5% to 86.53% for the formulations prepared. The entrapment efficiency was affected by drug:polymer ratio. The entrapment efficiency was changed when drug and polymer ratio has been changed. It has been showed that increase in polymer concentration in organic phase increases drug entrapment due to increase in organic phase viscosity, which increases the diffusional resistance to drug molecules from organic phase to aqueous phase, there by entrapping more drugs in the polymer nanoparticles. Percentage entrapment depends on organic phase and aqueous phase volume ratio. It suggests that change in phase volume ratio changed the entrapment efficiency. This may be considered due to solvent-drug interaction.

Fig 6: Percentage Drug Entrapment of Various Nanoparticle Formulations



In vitro release profile:

The *In vitro* release profiles of 12 formulations are shown in the figureNo:7. The formulations shows a release to maintain sustained and controlled release of the drug. The apparatus Franz diffusion cell with constant temperature bath used for the study of diffusion studies

Table No:3 Cumulative % Drug Release for Rivastigmine Nanoparticle formulations

Time (hrs)	Cumulative % Drug Release											
	Formulation Code											
	A1	A2	A3	B1	B2	B3	C1	C2	C3	D1	D2	D3
0.5	18.77	20.27	20.08	24.48	20.81	18.18	22.13	19.02	17.85	18.77	16.69	13.60
1	22.47	26.14	23.77	27.94	25.30	24.81	25.89	22.89	21.31	22.47	19.08	19.77
2	25.33	31.06	27.62	33.783	28.43	29.46	29.40	26.69	25.72	25.33	23.50	23.28
3	29.28	35.06	32.99	38.94	32.71	33.44	37.46	30.39	29.71	29.28	27.75	27.42
4	33.69	40.28	38.20	44.73	37.54	37.58	42.31	33.96	33.65	33.69	31.14	31.65
5	37.23	45.76	40.77	46.80	40.42	42.16	45.53	39.42	37.76	37.23	35.04	35.28
6	41.28	48.35	45.25	49.55	45.06	45.50	49.09	43.73	42.36	41.28	38.75	38.02
7	44.30	51.83	48.57	52.09	50.19	48.975	52.31	48.21	44.18	44.30	42.10	40.90
8	54.93	66.58	61.32	64.76	63.34	59.64	64.55	60.59	55.90	54.93	52.77	51.64
9	59.88	72.45	66.68	70.24	69.75	64.93	69.64	65.14	60.24	59.88	57.86	56.74
10	64.57	77.95	74.76	75.88	75.77	70.15	74.78	70.21	64.67	64.57	63.22	61.81
11	70.44	84.09	82.69	80.80	81.42	76.50	79.34	74.67	70.18	70.44	70.00	66.35
12	76.73	92.18	88.27	88.27	87.09	82.65	83.74	78.99	77.88	76.73	76.02	71.66

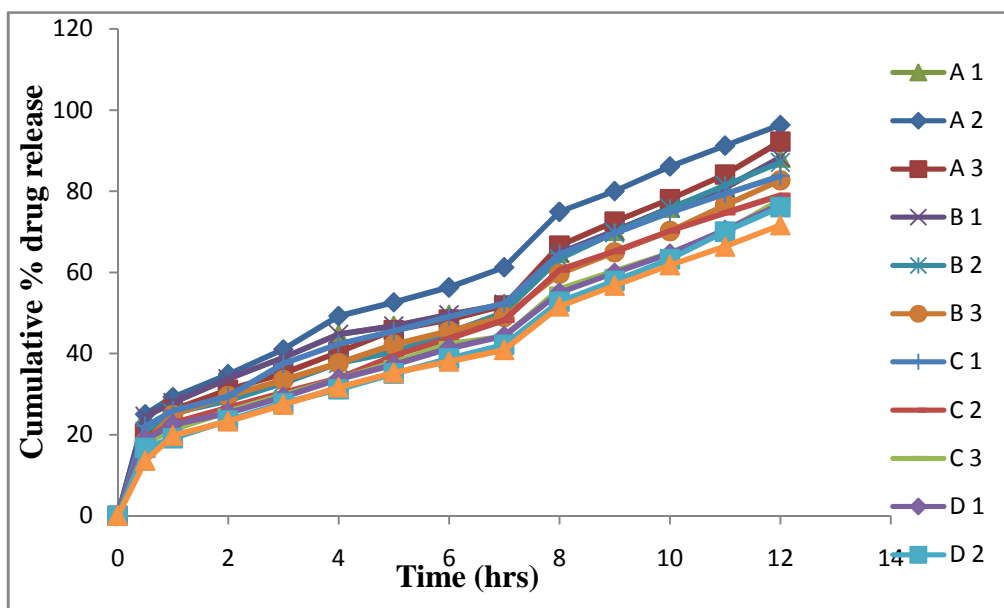


Fig 7: *In Vitro* Drug Release Profile of different Nanoparticle Formulations

Drug-release kinetics:

The release constant was calculated from the slope of the appropriate plots, and the regression coefficient (r^2) was determined. Percentage drug release data of best formulation D1 fitted in Higuchi's plot which was indicative of an diffusion and/or erosion mechanisms followed by zero order

Table 4: Drug-release kinetics data of optimized formulation [D1]

Formulation code	Zero order Regression (R^2)	First order Regression (R^2)	Higuchi's model Regression (R^2)	Korsmeyer-Peppas model		Best fit model
				Regression (R^2)	Slope (n)	
D 1 (Optimized formulation)	0.9841	-0.9813	0.9819	0.9609	0.41	Zero order and Higuchi's model

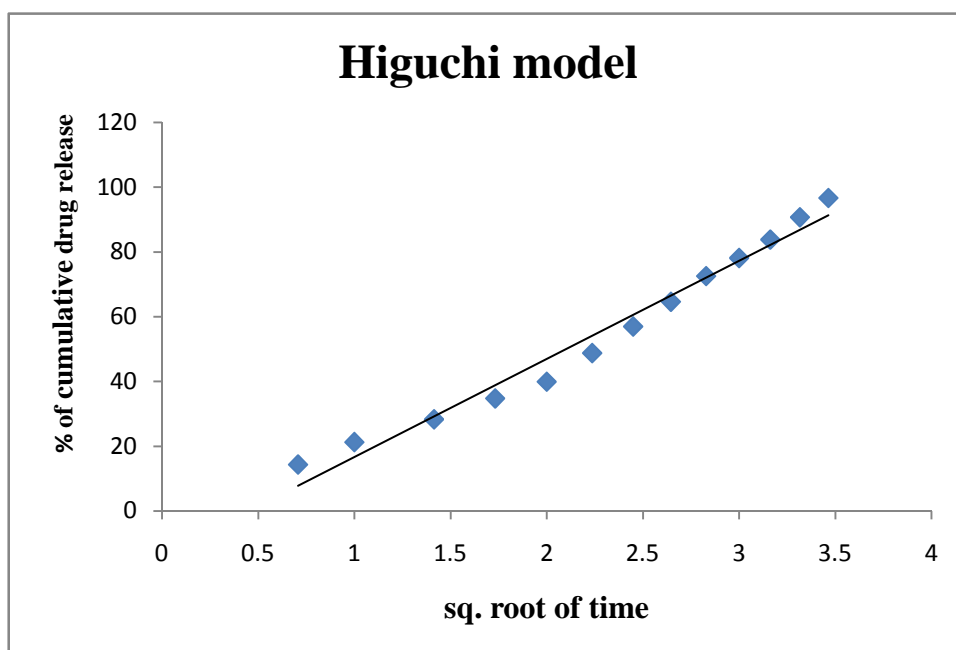


Fig 8 : Higuchi model Drug-release kinetics profile of optimized formulation

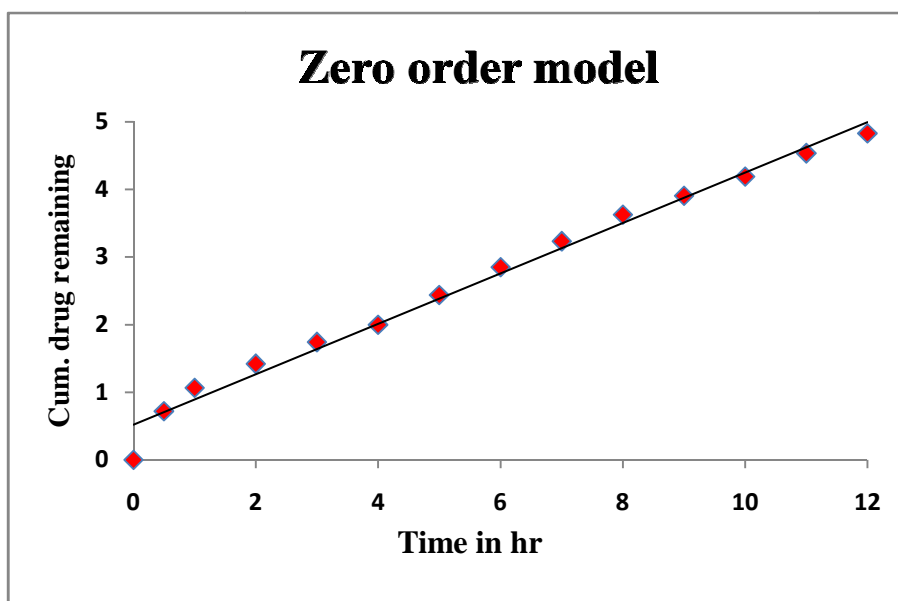


Fig 9 : Zero order model Drug-release kinetics profile of optimized formulation

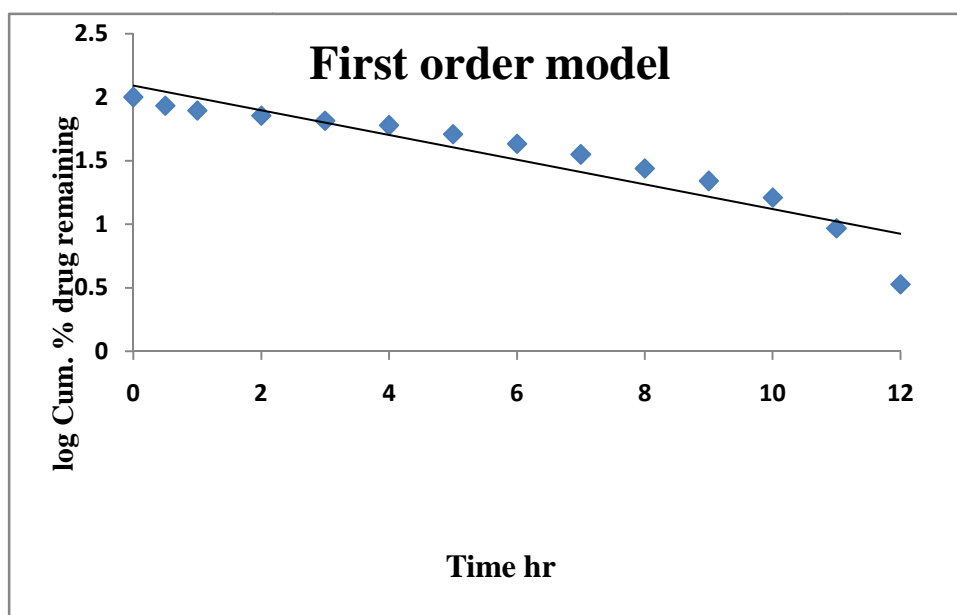


Fig 10: First order model Drug-release kinetics profile of optimized formulation

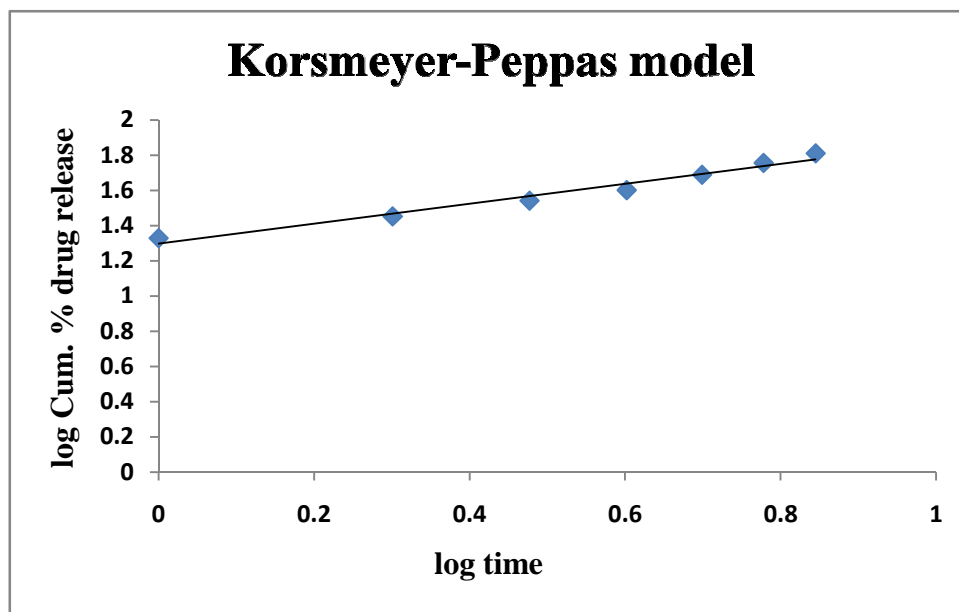


Fig 11:Korsmeyer-peppas Drug-release kinetics profiles of optimized formulation

CONCLUSION

The present work is intended to formulate and evaluate the nanoparticles of Rivastigmine, in view of increasing drug bioavailability, timed release of drug molecule, the sustained effect of rivastigmine was obtained from the present nanoparticles formulations which enables precision drug targeting to the brain. In the current study, the potential of Polyethylene oxide (PEO) for the specific delivery of competitive inhibitor of AchE's drug was investigated.

The polymeric nanoparticles of Rivastigmine were made up of drug and polyethylene oxide. Polyvinyl alcohol (PVA) is used as stabilizer and surfactant. The various formulations were designed by changing the drug: polymer and organic : aqueous phase ratio. The prepared polymeric nanoparticles so formed were evaluated for % Entrapment efficiency, and *in vitro* release profiles.

The % Entrapment efficiency of the formulation was affected by the drug: polymer ratio in the formulation. It has been showed that increase in polymer concentration in organic phase increases drug entrapment due to increase in organic phase viscosity, which increases the diffusional resistance to drug molecules from organic phase to aqueous phase, there by entrapping more drugs in the polymer nanoparticles. Percentage entrapment depends on organic phase and aqueous phase volume ratio. It suggests that change in phase volume ratio changed the entrapment efficiency. This may be considered due to solvent-drug interaction.

The *in vitro* drug release studies were performed with Franz diffusion cell. The formulation D1 having drug: polymer ratio 1 :40 and organic : aqueous phase ratio 1:2 showed a drug release of 76.73% for 12 hours indicates the increased bioavailability of the drug.

The formulation [D1] was selected as best formulation based on % Entrapment efficiency and drug release and was subjected to determination of particle size and zeta potential, particle morphology, drug-release kinetics, *in-vitro* release studies.

The formulation [D1] had particle size of 100.7nm and poly dispersity index of 0.232. The zeta potential (ζ) is 34.8 mV. A particle size below 250 nm with a polydispersity index near 0.25 was considered optimum. The particle morphology of Rivastigmine loaded NPs was confirmed by Transmission electron micrographs.

Percentage drug release data of best formulation [D1] fitted in Higuchi's plot which was indicative of an diffusion and/or erosion mechanisms.so the present work was fulfilled by formulating Rivastigmine polymeric nanoparticles for passive targeting of brain controlled drug delivery system there by increasing timed release of drug and bioavailability of the drug. Furtherstudies are needed toinvestigate these formulations forits performance*in vivo*.

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