https://doi.org/10.48047/AFJBS.6.Si3.2024.2850-2876

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Formulation and Evaluation of Microparticulate Drug Delivery of Rivastigmine for Effective Treatment of Alzheimer's Disease

Tannu Singh[1]*,Mr. Manish Kumar Patel[2],Mr. Seraj Alam Siddique[3], Dr.Prashant Kumar Katiyar[4]

[1*]Research Scholar, Kanpur Institute of Technology and Pharmacy, Kanpur, (U.P.)
[2]Assistant Professor, Kanpur Institute of Technology and Pharmacy, Kanpur, (U.P.)
[3]Assistant Professor, Kanpur Institute of Technology and Pharmacy, Kanpur, (U.P.)
[4]Professor&Director,KanpurInstituteofTechnologyandPharmacy,Kanpur, (U.P.)

CorrespondingAuthor:Tannu Singh
[1]*Research Scholar, Kanpur Institute of Technology and Pharmacy, Kanpur, (U.P.)

Article Info

Volume 6, Issue Si3, July 2024
Received: 11 May 2024
Accepted: 19 June 2024
Published: 08 July2024
doi:
10.48047/AFJBS.6.Si3.2024.2850-2876

ABSTRACT:

The purpose of this research was to formulate and evaluate of microparticulate drug delivery of Rivastigmine for effective treatment of Alzheimer's disease**.** Rivastigmine microspheres were prepared by non-aqueous solvent evaporation technique using Ethyl cellulose, Eudragit S 100 and Eudragit RS 100 as polymers. Results of preliminary trials indicate that volume of different solvents used, time taken for preparation of microspheres, polymer-to-drug ratio, and speed of rotation affected characteristics of microspheres. Prepared microspheres were discrete, spherical, and free flowing. The microspheres exhibited high percentage drug entrapment efficiency and yield. The drug release was also sustained for more than 10 hours. So from the above study, it can be concluded that Rivastigmine loaded microspheres can serve as an effective tool for the treatment of Alzheimer's disease.

Keywords: Rivastigmine, Microsphere, Alzheimer's disease, Non aqueous solvent, evaporation technique, Eudragit, entrapment efficiency

1. Introduction:

Alzheimer's disease (AD) is a neurological condition that progressively deteriorates thinking, memory, learning, and intellectual abilities in addition to the ability to do even the simplest of tasks. AD accounts for 50 to 80 percent of dementia cases [1]. Other types of dementia include vascular dementia, mixed dementia, dementia with Lewy bodies and front temporal dementia. It has been well established that the brain of patients with AD is characterized by the build-up of a signature plaque containing an abundance of the protein amyloid-β (Aβ) [2]. As a consequence, hundreds of millions of research dollars are currently being invested by the pharmaceutical industry towards finding and testing drugs that interfere with Aβ synthesis. A chemical in the brain that facilitates communication among nerve cells and is important for memory [3]. Alzheimer's disease is associated with inadequate levels of this Presently five drugs have been approved by the U.S. Food and Drug Administration (FDA) for treating the cognitive symptoms of AD [4]. Galantamine, Rivastigmine, Donepezil and Tacrine belong to a class of drugs known as cholinesterase inhibitors. Each acts in a different way to delay the breakdown important neurotransmitter [5]. Rivastigmine is a non-competitive acetylcholinesterase inhibitor of the carbamate type. It has been shown, in animal and man, to inhibit central and peripheral acetylcholinesterase and Butyrylcholinesterases, proportionally with the dose. It is an irreversible and progressive brain disease that slowly destroys memory and thinking skill and eventually even the ability to carry out the simplest task. In most people with AD, symptoms first appear at the age 60 [6, 7].

Formulation scientists need to pay more attention to the problem of directing medications to the brain, even with the technological improvements and developments in the field of brain research [8]. This is a highly tough subject. A review of the worldwide market trends for CNS medications reveals the underdevelopment of these pharmacological categories. The main reason for this is that the majority of the novel pharmacological compounds have not been able to effectively cross the blood-brain barrier (BBB) [9, 10]. Microspheres can be defined as solid, approximately spherical particles ranging from1to1000μm, containing dispersed drug in either solution (or) microcrystalline form. Microspheres are sometimes referred to as micro particles. Microspheres can be manufactured from various natural and synthetic materials [11]. Microspheres carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems [12]. Microspheres form an important part of such novel drug delivery systems[7]. This can be achieved by coupling bioadhesion characteristics to microspheres and developing bioadhesive microspheres. Microspheres carrier systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems[13, 14].

The different methods used for various microspheres preparation depends on particle size, route of administration, duration of drug release and these above characters related to rpm, method of cross linking, drug of cross linking, evaporation time, co-precipitation. Microspheres containing anti-biotic drug as a core material were prepared by a Non-aqueous Solvent Evaporation method. Briefly, drug and ethyl cellulose were mixed in acetone at various ratios. The processes are carried out in a liquid manufacturing vehicle. The microcapsule coating is dispersed in a volatile solvent which is immiscible with the liquid manufacturing vehicle phase [15, 16]. A core material to be microencapsulated is dissolved

or dispersed in the coating polymer solution. Solvent evaporation involves the formation of an emulsion between polymer solution and an immiscible continuous phase whether aqueous (o/w) or non-aqueous. The comparison of mucoadhesive microspheres of hyaluronic acid, Chitosan glutamate and a combination of the two prepared by solvent evaporation with microcapsules of hyaluronic acid and gelating prepared by complex coacervation were made[17, 18].Thispresent research is designed to prepare and characterize the rivastigmine loaded microspheres which was confirmed by preformulation study, drug polymer compatibility testing, in-vitro and rheological evaluations.

2. Materials and Methods:

2.1. Materials

The Drug sample Rivastigmine was obtained as gift sample from Ind-Swift Pvt. Ltd., Chandigarh and Eudragit S100, Eudragit RS100 along with Ethyl cellulose were obtained from S.D. Fine, Mumbai and other chemicals employed for the study were of analytical grade.

2.2. Formulation of Rivastigmine loaded Microspheres

Microspheres containing anti-biotic drug as a core material were prepared by a Non-aqueous Solvent Evaporation method. Briefly, drug and ethyl cellulose were mixed in acetone at various ratios. The slurry was slowly introduced into 30ml of liquid paraffin while being stirred at 1200 rpm by a mechanical stirrer equipped with a three bladed propeller at room temperature. The solution was stirred for 2 h to allow the solvent to evaporate completely and the microspheres were collected by filtration. The microspheres were washed repeatedly with petroleum ether (40o-60oC) until free from oil. The collected microspheres were dried for 1 h at room temperature and subsequently stored in desiccators over fused Calcium chloride**.** The composition and preparation of Rivastigmine loaded microsphere are shown in Table 1-3 [19, 20].

Table 1: Composition of formulations of ethyl cellulose

S.No.	Ingredients	Formulation Code				
		FE1	FE2	FE3	FE4	FE5
1	Rivastigmine (mg)	100	100	100	100	100
2	Ethyl cellulose(mg)	200	300	400	500	600
3	Acetone(ml)	15	15	15	15	15
4	Liquid paraffin	30	30	30	30	30

Table 2: Composition of formulations of Eudragit S100

S.No.	Ingredients	Formulation Code				
		FS1	FS2	FS3	FS4	FS5
1	Rivastigmine (mg)	100	100	100	100	100
2	Eudragit S100(mg)	200	300	400	500	600
3	Acetone(ml)	15	15	15	15	15
4	Liquid paraffin	30	30	30	30	30

Table 3: Composition of formulations of Eudragit RS 100

S. No.	Ingredients	Formulation Code				
		FR1	FR2	FR3	FR4	FR5
1	Rivastigmine (mg)	100	100	100	100	100
2	Eudragit RS 100 (mg)	200	300	400	500	600

3	**Acetone(ml)**	15	15	15	15	15
4	**Liquid paraffin**	30	30	30	30	30

2.3. Determination of Solubility of Rivastigmine

Solubility is the method that use for determination of purity of drug. Solubility was performed in accordance with "Indian Pharmacopoeia-1996", during preformulation of drug in different solvents (Acetone, Methanol, Dichloromethane, Chloroform and Water etc.). A saturated Solution of Rivastigmine was prepared in different Solvent systems and then drug concentration was measured in these solvent systems by using UV Spectrophotometer at 210 nm wavelength[21].

2.4.Drug Excipient Compatibility study

IR studies of Rivastigmine, Sodium CMC, Sodium alginate, Ethyl cellulose and physical mixture of drug with polymers was carried out to find out the interaction. Fourier Transform Infrared (FT-IR) analysis measurements of pure drug, carrier and drug-loaded microcapsules formulations were obtained using a Perkin- Elmer system 200 FT-IR spectrophotometer) to check the drug-polymer interaction and chemical integrity of the drug in the microspheres. The pellets were prepared on KBr - press under a hydraulic pressure of 150 kg/cm2; the spectra were scanned over the wave number range of 4000 - 400 cm-1 at the ambient temperature [22].

2.5.Estimation of Absorbance of Rivastigmine by U.V. Spectrophotometer

In order to determine λmax for Rivastigmine, standard stock solution of Rivastigmine, (10 mg /ml) in Dichloromethane was prepared and scanned for absorbance in between 300-200 nm by using UV Spectrophotometer (Shimadzu UV-1700, Double beam UV spectrophotometer). The peak was found at 210 nm[23].

2.6 Determination of pH on Partition Co-efficient of Rivastigmine

The Partition coefficient of Rivastigmine was determined in solvent system: n-Octanol/distilled water and n-Octanol/saline phosphate buffer. Accurately weighed quantity of drug (20 mg) taken in one stoppered glass vial containing 5.0 ml of n-Octanol. After dissolving the drug in n-Octanol, 5ml distilled water was added to vial and in another vial Octanol/saline phosphate buffer was taken. Then the glass vial was kept to equilibrate by shaking in vertex mechanical shaker for 6 hrs and after shaking, the vial were transferred into separating funnel, kept overnight at 37±2OC for equilibrium. The contents and both phases were separated. After appropriate dilutions, the aqueous phase was analyzed for Rivastigmine against reagent blank solution using Shimadzu-1700 E U.V. spectrophotometer. The drug concentration in n-Octanol phase was determined by subtracting the amount in aqueous phase from the total quantity of drug added to the vials[24].

The partition coefficient value "P" was calculated by given equation:

P o/w = Conc. (organic)/ Conc. (aqueous); P w/o = Conc. (aqueous)/ Conc. (organic).

2.7. Determination of Yield of Microspheres

Thoroughly dried microspheres were collected and weighed accurately. Determination of % yield of microspheres was calculated by weighing the mass of obtained microspheres divided by total weight of drug and polymer combined which was multiplied by 100. This shows the percent yield of the microsphere [25].

% Yield= Mass of obtained microsphere/ Total weight of drug and polymer *100

2.8.Determination of Particle Size of Microspheres

The microspheres size distribution was determined by the optical microscopy method using a calibrated stage micrometer with the help of equation. $Xg = 10x [(n_i x \log X_i)/N]$ where Xg is geometrical mean diameter, n_i number of particles in range,X_i is the midpoint of range and N is a total number of particles [25].

2.9. Measurement of Percent Moisture Loss in Microsphere

This was done to check the hydrophobicity of the formulations and resulted were found promising as depicted in the Figure [26]. Percent moisture loss was calculated by given formula.

Percentage of moisture loss of microspheres = (Initial weight- Final weight) × 100
Initial weight

2.10. Determination of Drug Content and Entrapment Efficiency

Drug loaded microspheres (100mg) were powdered and suspended in 100 ml 0.1 N HCl solutions and kept for 24hours. It was stirred for 5minutes and filtered. Ofloxacin content in the filtrate was determined spectrophotometrically at 278nm using a regression derived from the standard curve. The entire test was performed in triplicate.The drug entrapment efficiency was calculated by the given equation [23, 25].

EE = (Pc/Tc) x 100

Pc = Practical drug content, Tc = Theoritical drug content.

2.11. Determination of Rheological Properties

The rheological properties such as angle of repose, Carr's index, Bulk density, True density, Porosity and Hausner's ratio were determined to assess the flow ability of the prepared microspheres. The procedures provided in the literature was followed [26-28].

2.12. Determination of Swelling Index

The dynamic swelling property of microspheres in the dissolution medium was determined. Microspheres of known weight were placed in dissolution solution for 3hr and the swollen microspheres were collected by a centrifuge and the wet weight of the swollen microspheres was determined by first blotting the particles with filter paper to remove absorbed water on surface and then weighing immediately on an electronic balance. The percentage of swelling of microspheres in the dissolution media was then calculated by using the mentioned equation [29].

$S_w = (W_t - W_o) / W_o \times 100$

2.13. *In Vitro* Dissolution Study

In vitro drug release studies were carried out using USP II dissolution test apparatus Type II, paddle apparatus (100 rpm, 37±0.5 ^{0}C). *In vitro* release study for drug loaded multilayered tablets was carried out by keeping the tablets for 2 h in 0.1 N HCl (900 ml), simulated gastric fluid (SGF). The dissolution medium was then replaced with pH 7.4 phosphate buffer solution (900 ml), simulated intestinal fluid (SIF), and tested for 3 h. The time dependent coated tablets were evaluated by exposing them to 900 ml SIF for 3 h which was later replaced by pH 6.8 phosphate buffer solution (900 ml), simulated colonic fluid (SCF), and tested for release [30].

2.14. Determination of morphological characteristics of Rivastigmine loaded microsphere through Scanning Electron Microscopy (SEM)

Scanning electron microscopy was carried out to study the morphological characteristics of Rivastigmine loadedmicrospheres. The dried microspheres were coated with gold (100 A$^\circ$) under an argon atmosphere in a gold coating unit and Scanning electron micrographs were observed [31].

3. Results:

3.1. Solubility Study

The solubility study was investigated by measuring solubility of rivastigmine in both hydrophilic and lipophilic solvents as shown in Table 4. Weigh specific amount of drug which was added to 250ml of solvent medium and stirred mechanically in a water bath at 37^{0}C.One hour later 5ml were withdrawn and assayed spectrophotometrically.

Table 4: Solubility study of drug in different solvents

Solvent	Solubility
Acetone	Soluble
Methylene chloride	Soluble
Isopropyl alcohol	Soluble
Ethanol	Soluble
Chloroform	Soluble

3.2. Drug Excipient Compatibility Study

FTIR spectra for Rivastigmine (Figure 1) given in National formulary were completely matched with FTIR spectra of obtained drug sample which gives a preliminary identification about drug. Drug polymer compatibility studies have been performed by using FTIR instrument as shown in Table 5. To fulfill the study process first IR peaks of functional groups of pure drug has been scan out followed by mixture of drug & polymer. The peaks obtained were compared to conclude the result as shown in Figure 2-4.

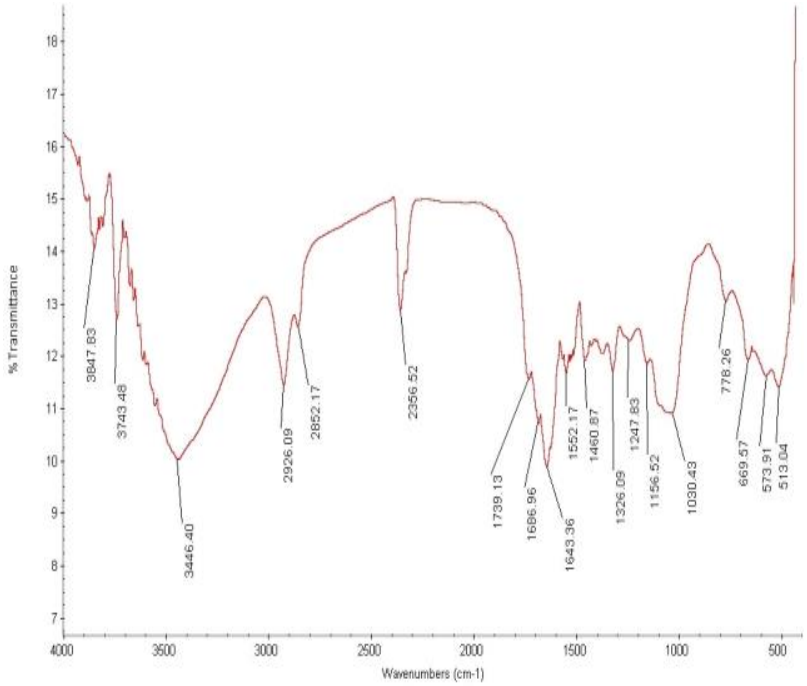

Figure 1: FTIR Spectrum of Rivastigmine

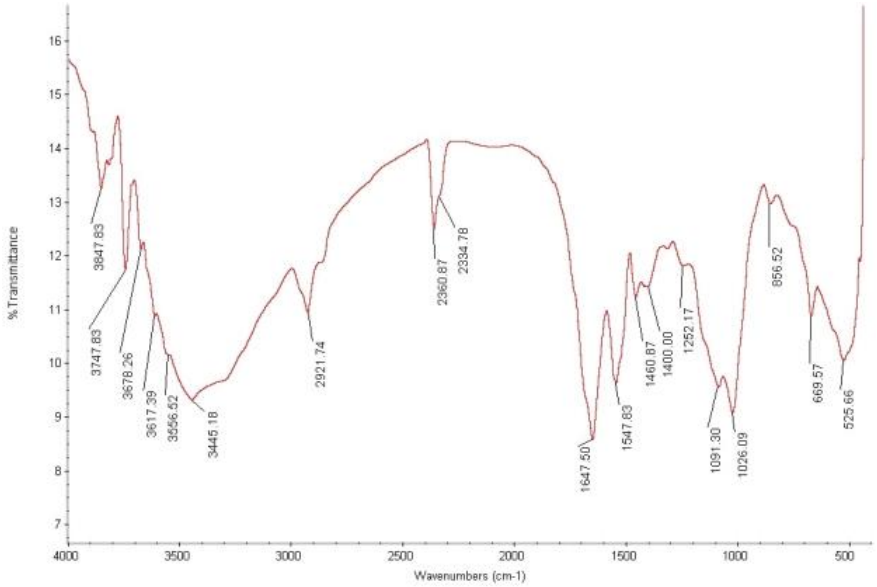

Figure 2: FTIR Spectrum of Rivastigmine and Ethyl Cellulose.

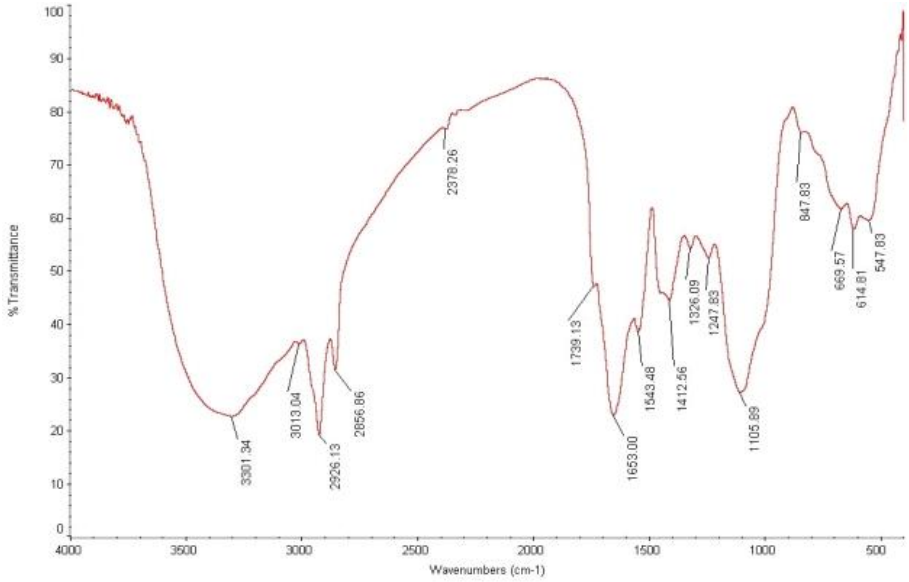

Figure 3: FTIR Spectrum of Rivastigmine and Eudragit-RS 100

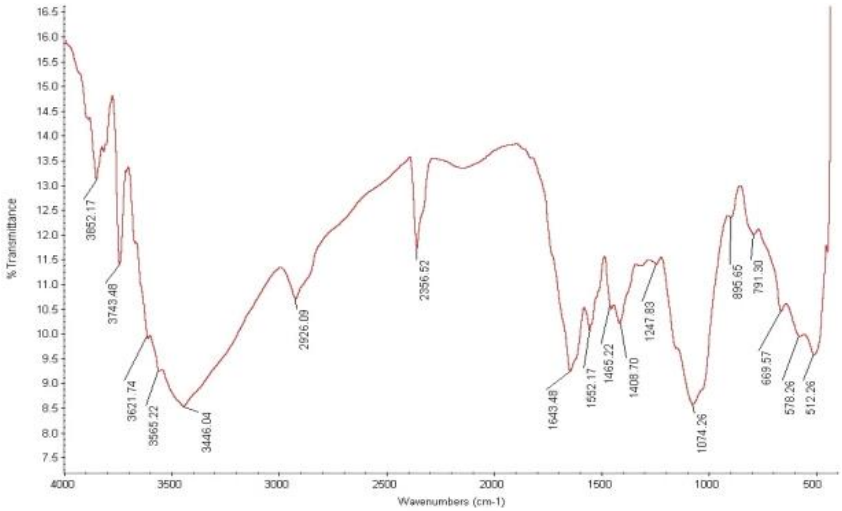

Figure 4:FTIR Spectrum of Rivastigmine and Eudragit-S 100

Table 5: Drug- Excipients Compatibility

S. No.	Drug	Drug+Ethyl Cellulose	Drug+Eudragit-RS 100	Drug+Eudragit-S 100
1	3847.83	3847.83	3301.34	3852.17
2	3743.48	3747.83	3013.04	3743.48
3	3446.40	3678.26	2926.13	3621.74
4	2926.09	3617.39	2856.86	3565.22
5	2852.17	3556.52	2378.26	3446.04
6	2356.52	3445.18	1739.13	2926.09
7	1739.13	2921.76	1653.00	2356.52
8	1686.96	2360.87	1543.48	1643.48
9	1643.36	2334.78	1412.56	1552.17
10	1552.17	1647.50	1326.09	1465.22
11	1460.87	1547.83	1247.83	1408.70
12	1326.09	1460.87	1105.89	1247.83
13	1247.83	1400.00	847.83	1074.26
14	1156.52	1252.17	669.57	895.65
15	1030.43	1091.30	614.81	791.30
16	778.26	1026.09	547.83	669.57
17	669.57	856.52	542.45	578.26
18	573.91	669.57	511.12	512.26
19	513.04	525.66	502.16	504.21

3.3. Estimation of Absorbance of Rivastigmine by U.V. Spectrophotometer

A. In Water: Model drug Rivastigmine was dissolved in water at a concentration of 10µg/ml. The prepared solution was scanned in the spectrum mode from 400 nm to 200 nm. Obtained λmax (263.5 nm) was used for preparation of calibration curve. Six concentrations 1, 2, 4, 6, 8 and 10 µg/ml of Rivastigmine was prepared and calibration curve of drug was plotted.

B. InPhosphate Buffer pH 7.4: Model drug Rivastigmine was dissolved in phosphate buffer pH 7.4 at a concentration of 10µg/ml. The prepared solution was scanned in the spectrum mode from 400 nm to 200 nm. Obtained λmax (263.5 nm) was used for preparation of calibration curve. Six concentrations 1, 2, 4, 6, 8 and 10 µg/ml of Rivastigmine was prepared and calibration curve of drug was plotted. As per the experimental result (Figure 5 and 6) all three prepared standard curve having regression value above 0.99 which signify the reproducibility and linearity.

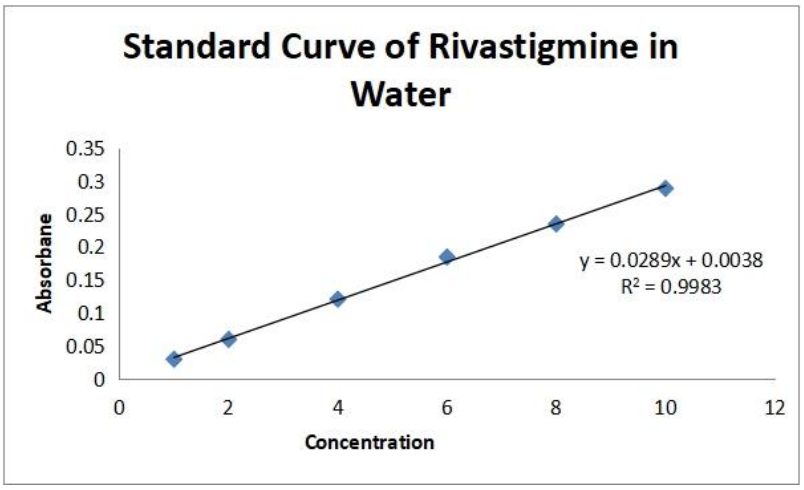

Figure 5: Standard Curve of Rivastigmine in Water

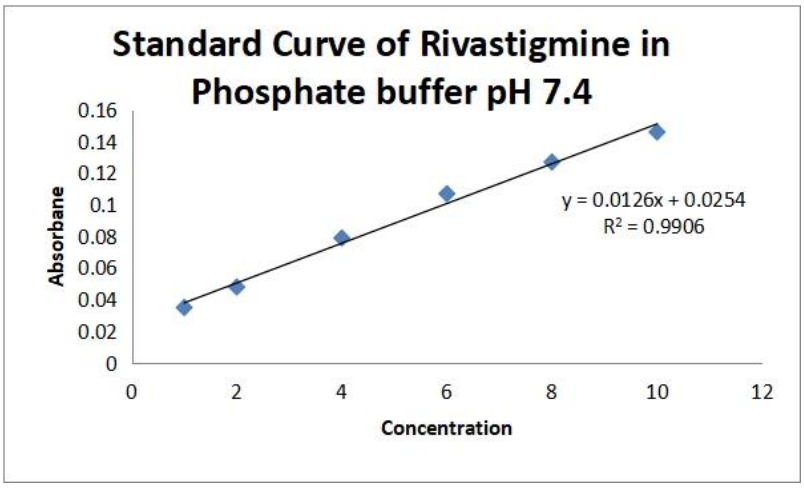

Figure 6: Standard Curve of Rivastigmine in Phosphate buffer pH 7.4

3.4. Determination of pH on Partition Co-efficient of Rivastigmine

The calculated partition coefficientof various sample S1 to S5 are depicted in Table 6. The maximum value of partition coefficient 0.375 is at pH 7.4 signifies that, in that particular pH maximum permeation of drug could be obtained.

Table 6: Calculation of Partition Co-efficient.

S. No	Buffer pH	Abs.	Abs.	(X)	(Y)	Partition coefficient (Y/X)
S1	2.2	3.135	39.22	98.05	1.95	0.0198
S2	4.0	3.135	39.22	98.05	1.95	0.0198
S3	6.8	2.960	37.01	92.52	7.48	0.0808
S4	7.4	2.334	29.08	72.7	27.3	0.375
S5	8.0	2.834	35.41	88.52	11.47	0.129

X: Amount of Rivastigmine in 25 ml of buffer
Y: Amount of Rivastigmine 25 ml of n-octanol

3.5. Determination of Yield of Microspheres

From experimental result it was observed that all the prepared formulation having more than 66% yield and as the polymer concentration was increased, the %yield was also increased.

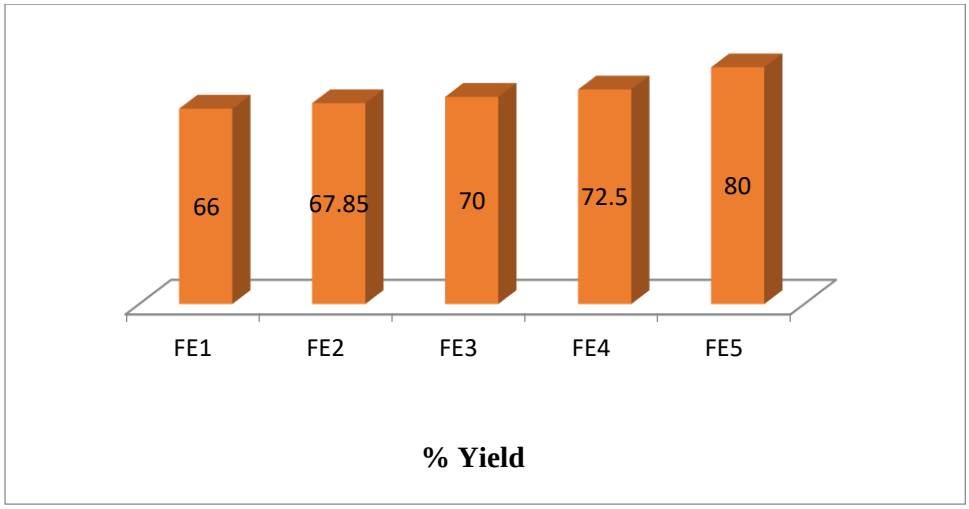

Figure 7: Percent Yield of Formulations (FE1-FE5)

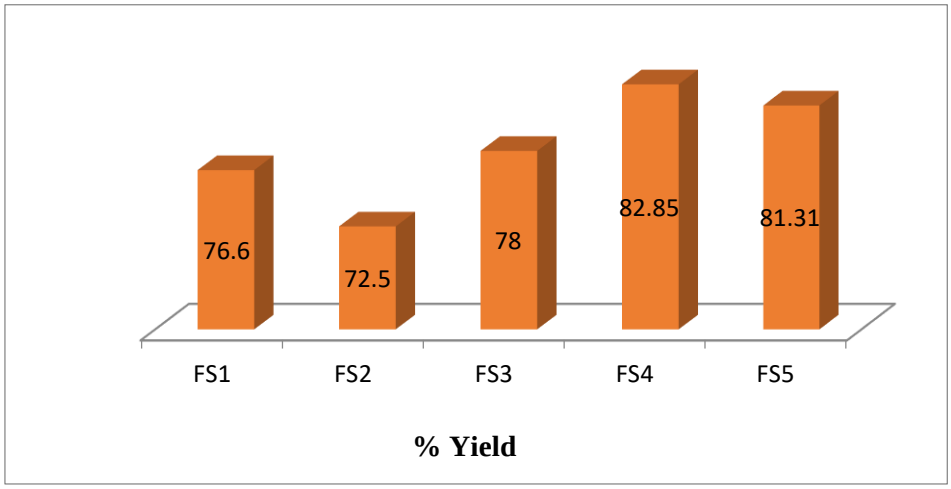

Figure 8: Percent Yield of Formulations (FS1-FS5)

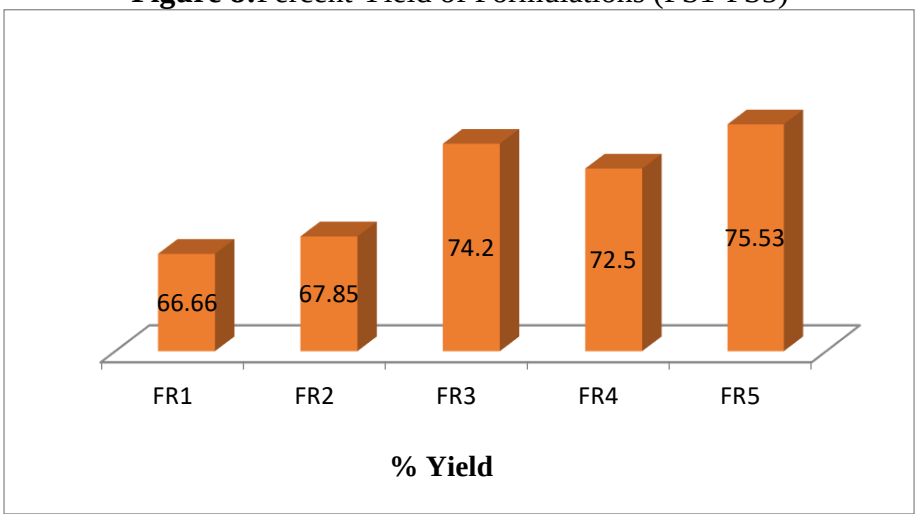

Figure 9: Percent Yield of Formulations (FR1-FR5)

3.6.Determination of Particle Size of Microspheres

The microspheres size distribution in (µm) was determined by the optical microscopy method using a calibrated stage micrometer with the help of equation:

$Xg = 10x\ [(n_i x\ \log X_i)/N]$

Xg is geometrical mean diameter, n_i number of particles in range,X_i is the midpoint of range and N is a total number of particles.

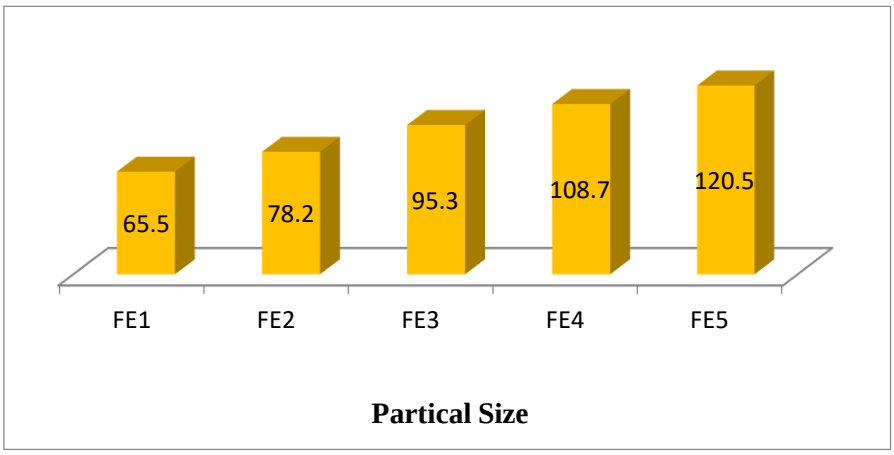

Figure 10: Particle Size Analysis of Microspheres (FE1-FE5)

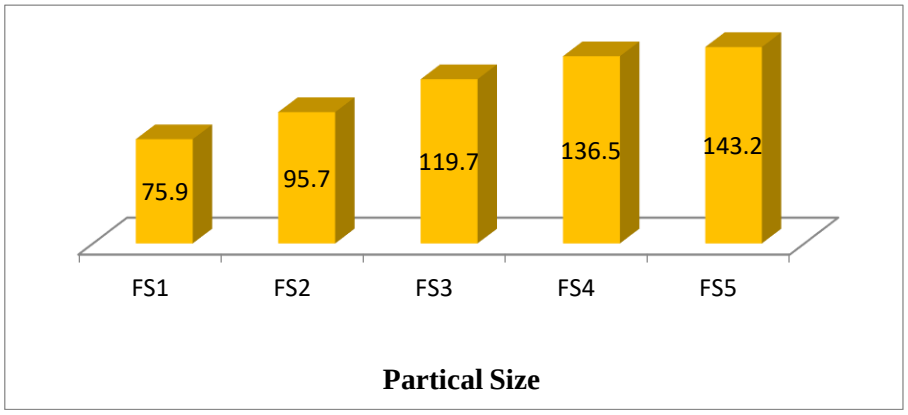

Figure 11: Particle Size Analysis of Microspheres (FS1-FS5)

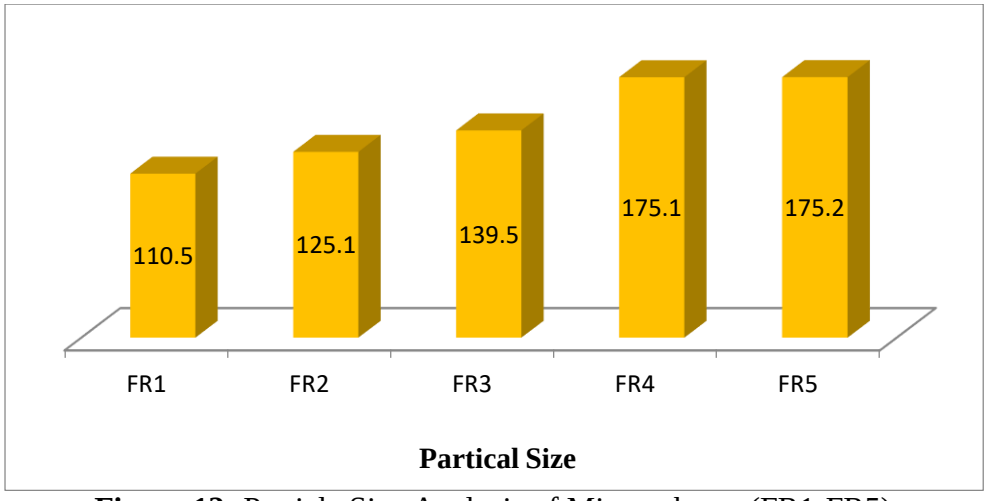

Figure 12: Particle Size Analysis of Microspheres (FR1-FR5)

3.7. Measurement of Moisture Loss

It can be concluded that the prepared microspheres were dried properly as well as the strong hydrophobic nature of selected polymer prevent the preparation of water as shown in Figure 13-15.

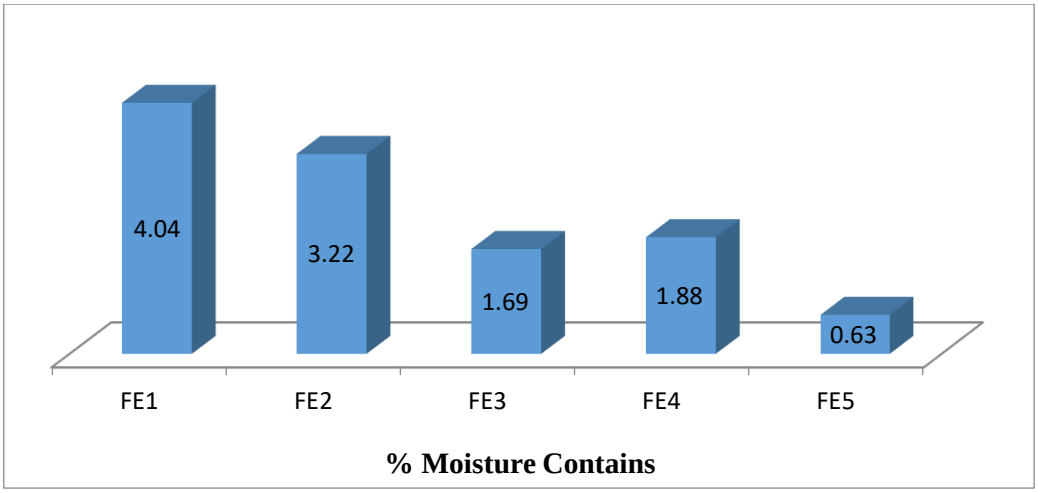

Figure 13: % Moisture ContentAnalysis of Microspheres (FE1-FE5)

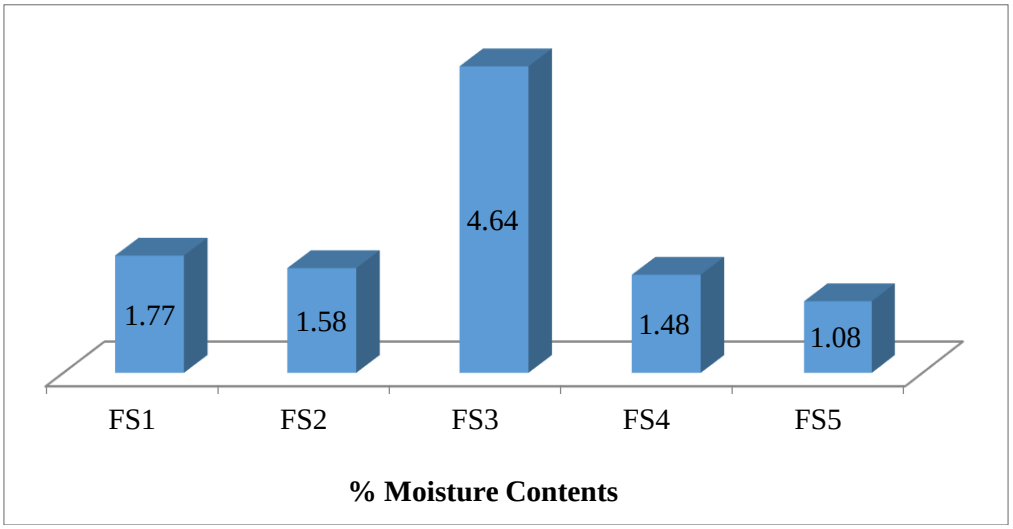

Figure 14: % Moisture ContentAnalysis of Microspheres (FS1-FS5)

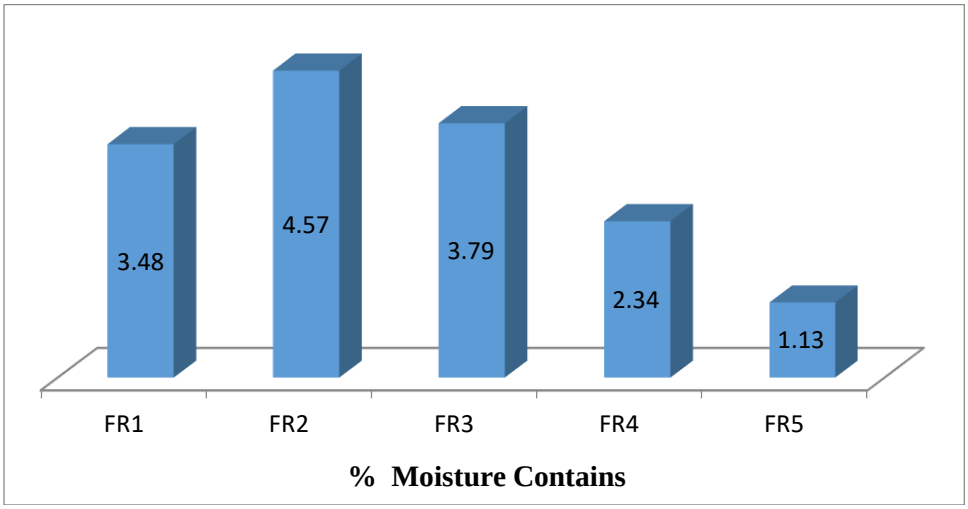

Figure 15: % Moisture ContentAnalysis of microspheres (FR1-FR5)

3.8. Determination of Drug content and Drug entrapment efficiency

The Drug content and Drug entrapment efficiency was determined and it showed prominent results for the various formulations as shown in Figure 16-21.

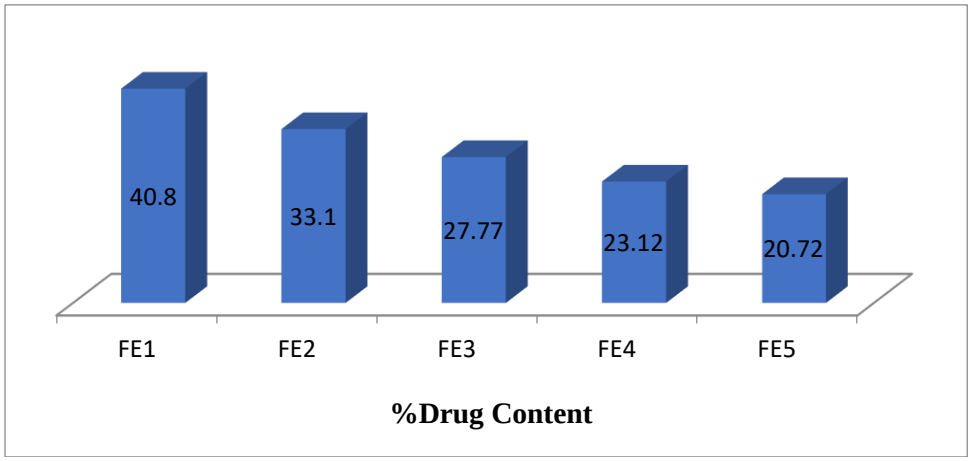

Figure 16: % Drug ContentAnalysis of Microspheres (FE1-FE5)

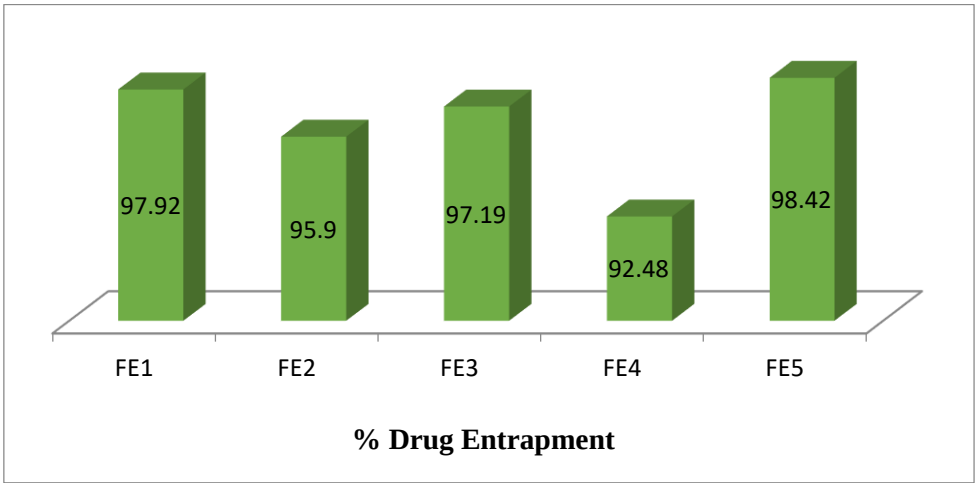

Figure 17: % Drug entrapmentAnalysis of Microspheres (FE1-FE5)

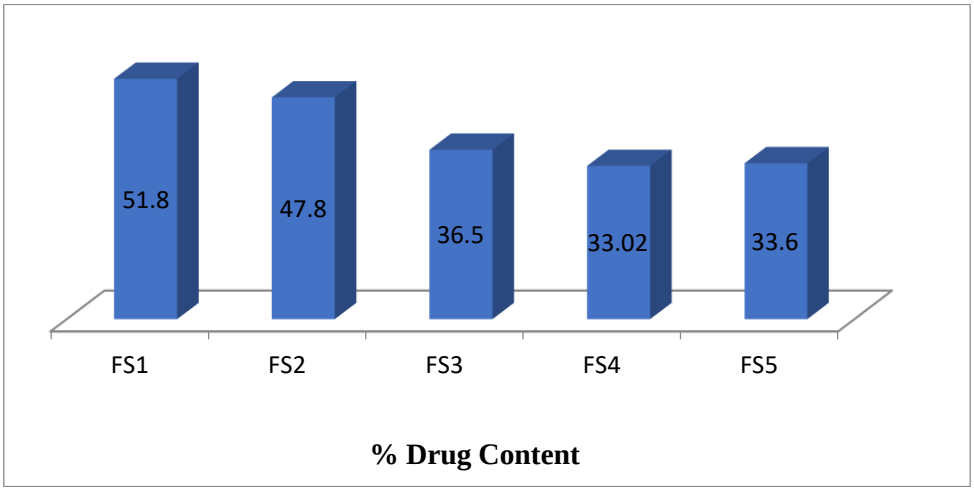

Figure 18: % Drug ContentAnalysis of Microspheres (FS1-FS5)

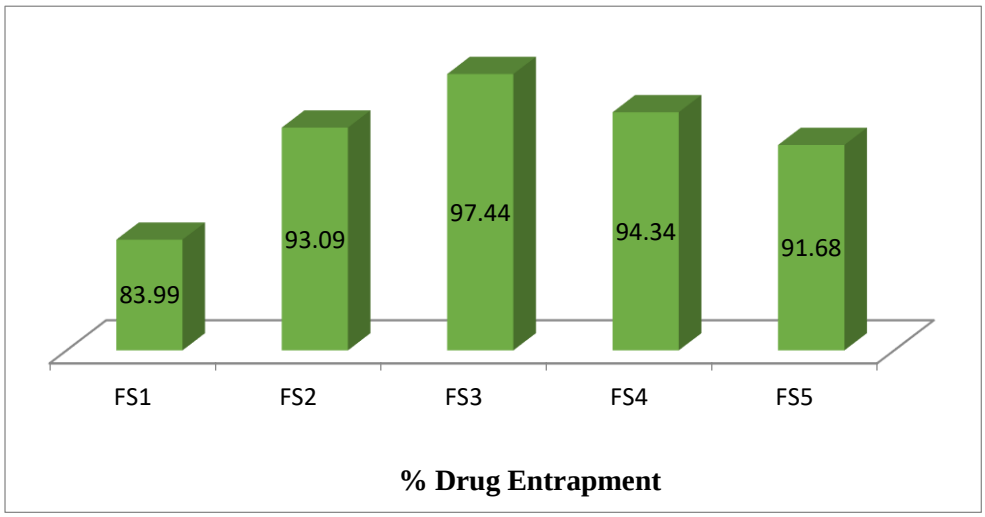

Figure 19: % Drug entrapmentAnalysis of Microspheres (FS1-FS5)

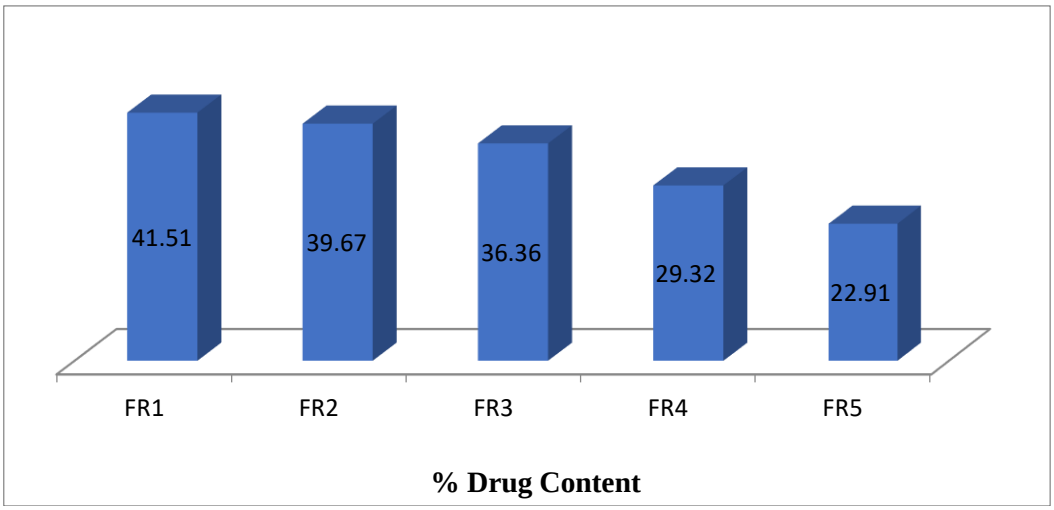

Figure 20: % Drug ContentAnalysis of Microspheres (FR1-FR5)

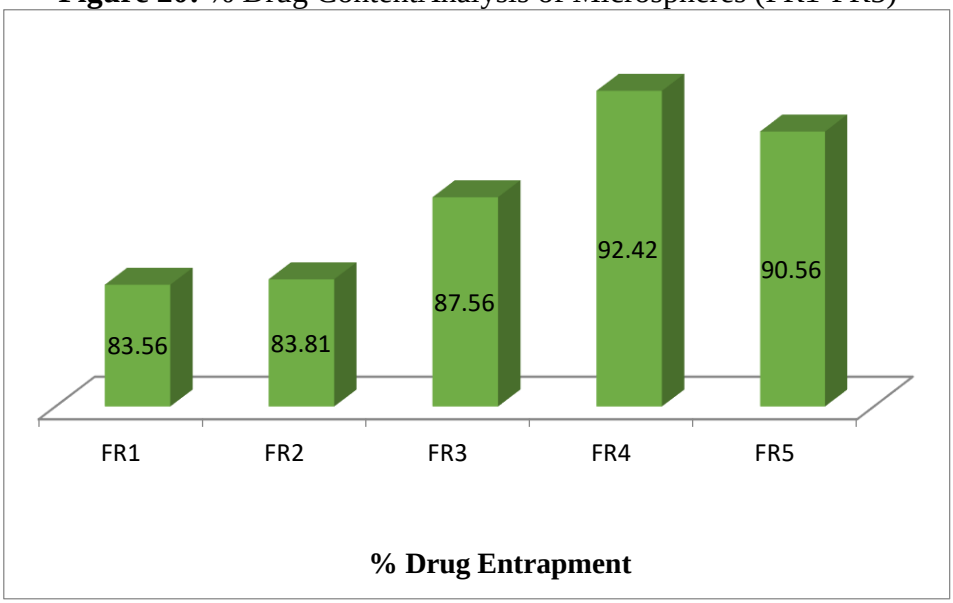

Figure 21: % Drug entrapmentAnalysis of microspheres (FR1-FR5)

3.9. Determination of Rheological Properties

The various rheological parameters were evaluated like angle of repose, bulk density, tapped density, carr's index and hausner's ratio were calculated and are listed in Table 7 for different formulation codes. The bulk density of prepared microspheres were found to be in the range of 0.53 to 0.65 g/cc, tapped density was found to be in the range of 0.61 to 0.74 g/cc, the Carr`s Index values were in the range of 11.12 to 13.43%, the angle of repose was in the range of 24.97to 28.36 and Hausner`s ratio was in the range of 1.122 to 1.155.

Table 7: Evaluation of Rheological Characteristics.

Batch Code	Evaluation of Rheological Characteristics				
	Angle of Repose (º)	**Bulk Density (g/cm^3)**	**Tapped Density (g/cm^3)**	**%Carr's Index**	**Hausner's ratio**
FE1	24.33	0.444	0.487	8.829	1.096
FE2	21.32	0.408	0.465	12.25	1.139
FE3	27.54	0.416	0.487	14.57	1.170
FE4	24.32	0.350	0.390	10.25	1.114
FE5	25.12	0.344	0.434	10.71	1.107
FS1	25.29	0.400	0.44	9.90	1.110
FS2	27.89	0.4	0.425	5.882	1.062
FS3	27.86	0.571	0.666	14.2	1.166
FS4	29.72	0.500	0.571	12.43	1.142
FS5	23.52	0.444	0.500	11.2	1.126
FR1	21.60	0.500	0.571	12.43	1.142
FR2	23.02	0.400	0.425	5.882	1.062
FR3	29.54	0.571	0.666	14.26	1.166
FR4	23.21	0.406	0.476	14.28	1.166
FR5	24.91	0.465	0.540	13.88	1.161

3.9. Determination of swelling index

% swelling index of different formulationsas listed in Table 1-3 was reported in Figure 22-24.

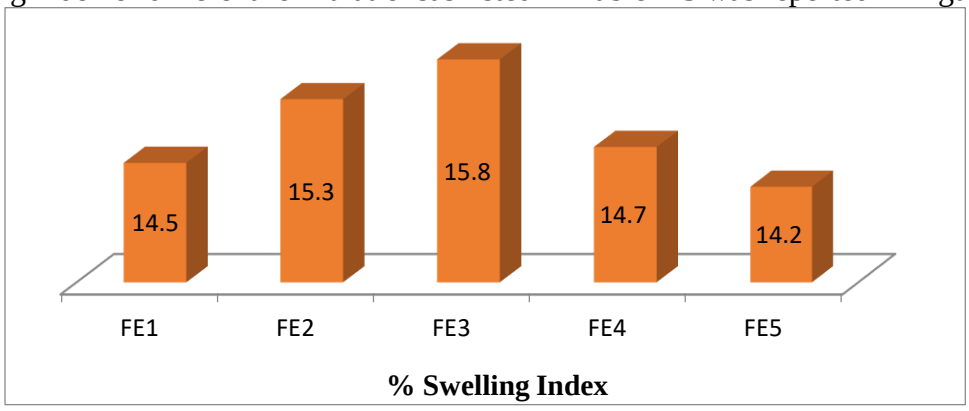

Figure 22: % Swelling IndexAnalysis of Microspheres (FE1-FE5)

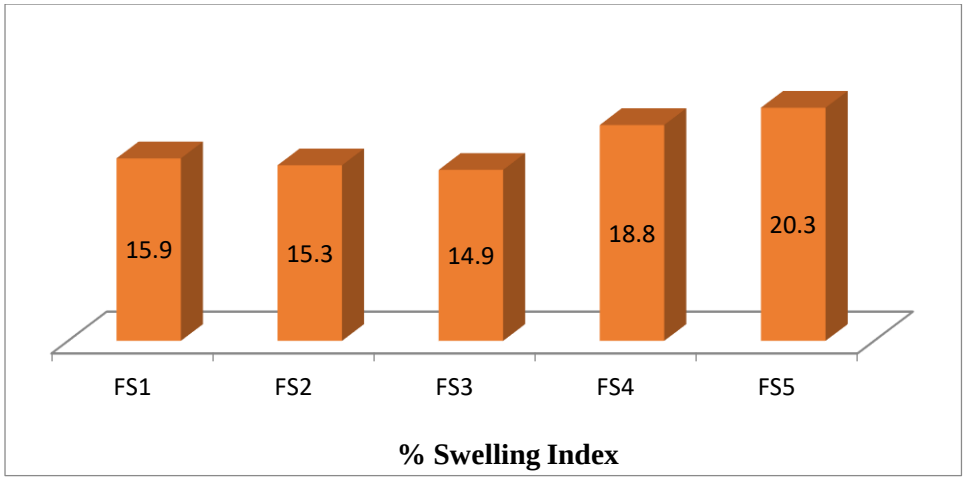

Figure 23: % Swelling IndexAnalysis of microspheres (FS1-FS5)

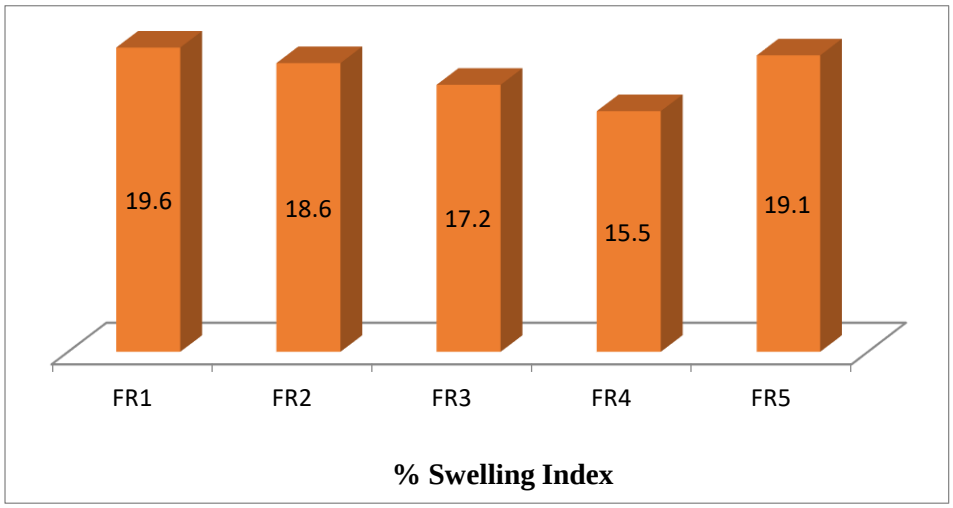

Figure 24:% Swelling IndexAnalysis of microspheres (FR1-FR5)

3.10. *In Vitro* Dissolution Study

All the prepared microspheres containing Ethyl Cellulose in the ratio of 1:2 to 1:6 (FE1-FE5) along with FS1-FS5 were subjected to *invitro*dissolution studies using phosphate buffer pH 7.4. The percent cumulative amount of drug release (%CR) and time taken to release 50 % from the formulations along with Korsmeyer – Peppas Release Profile of the formulations has been reported in Figure 25-39.

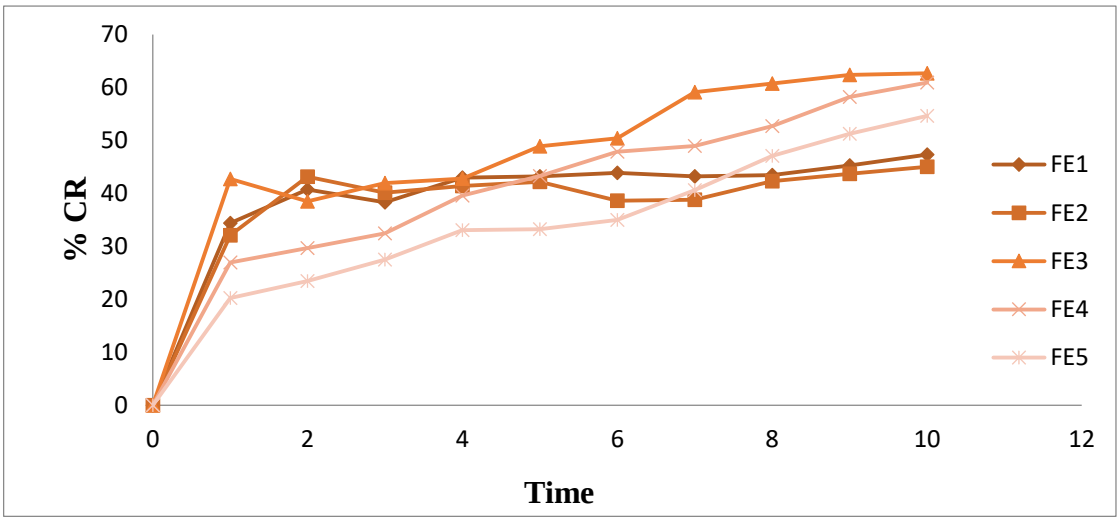

Figure 25: Zero Order Release Profile (FE1-FE5)

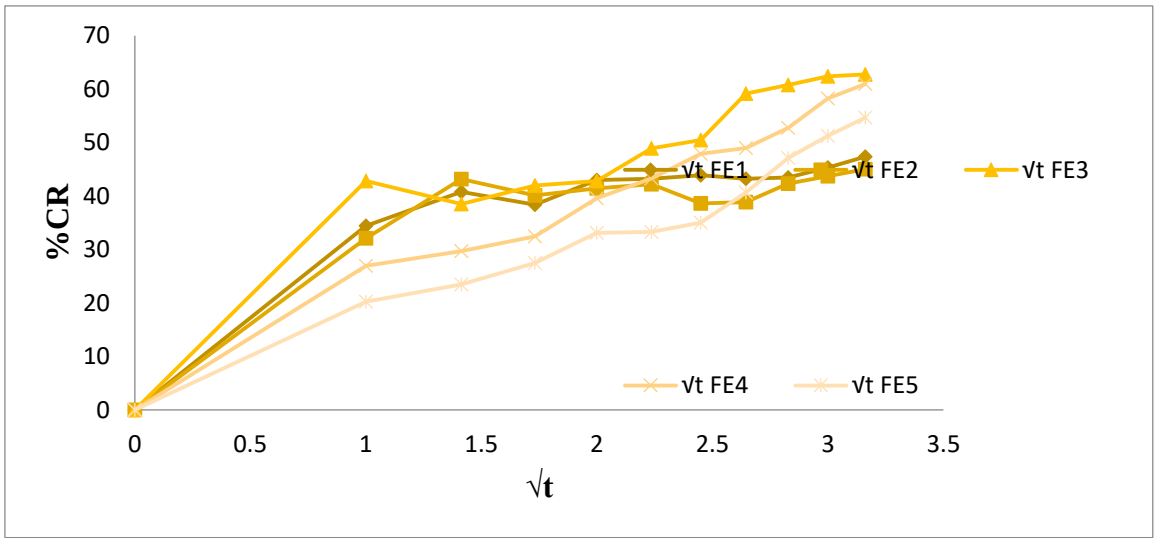

Figure 26: Higuchi Model release profile (FE1-FE5)

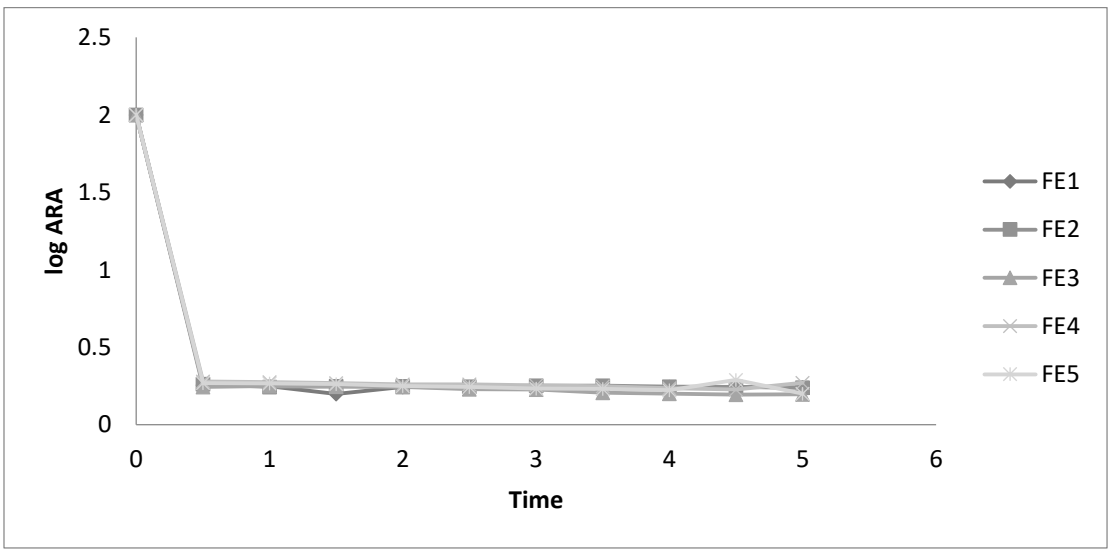

Figure 27: First Order Release Profile (FE1-FE5)

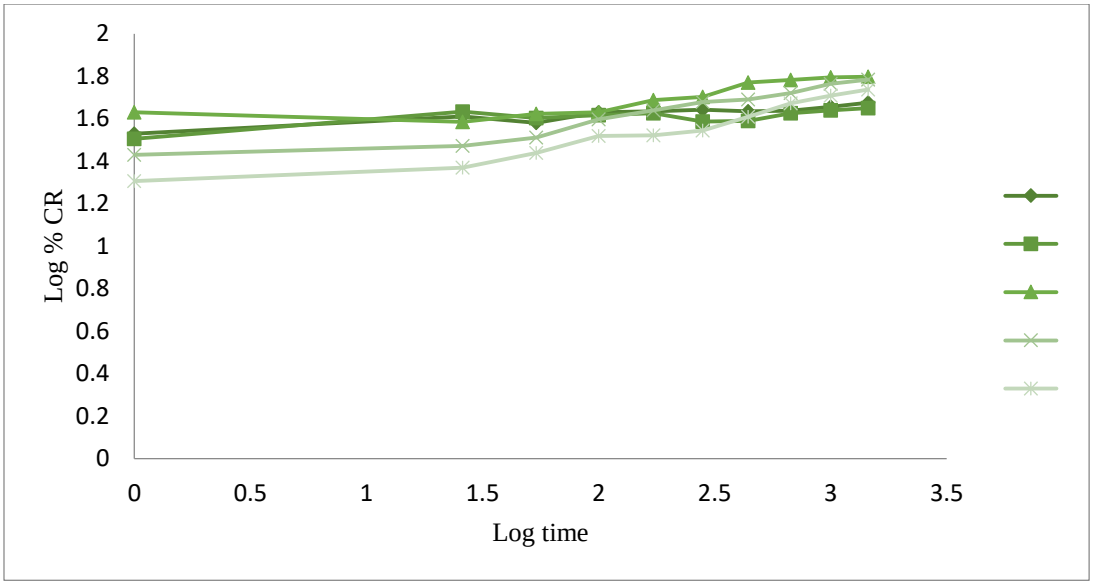

Figure 28: Korsmeyer–Peppas Release Profile (FE1-FE5)

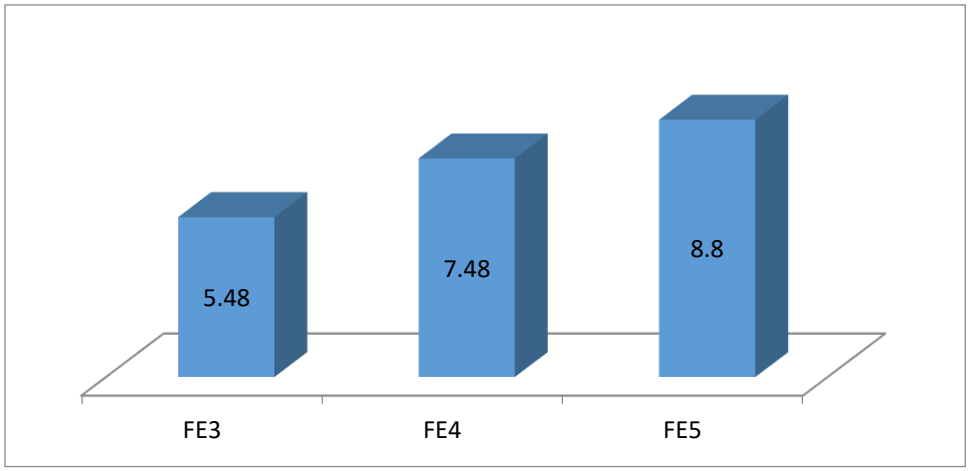

Figure 29: Time to release 50 percent of drug from formulation (FE1-FE5)

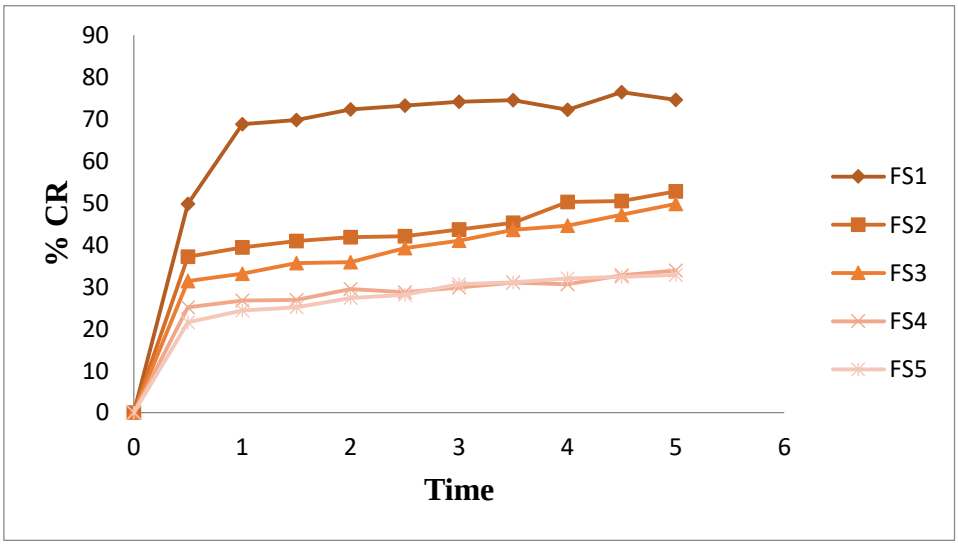

Figure 30: Zero Order Release Profile (FS1-FS5)

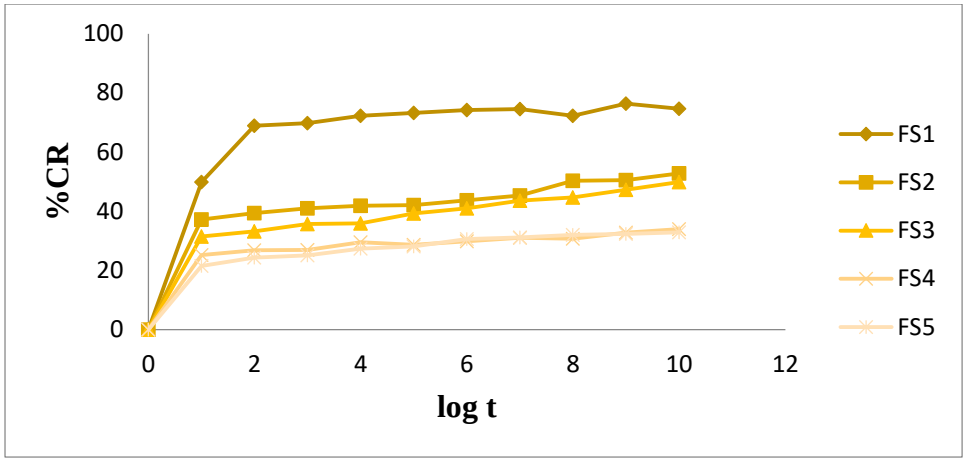

Figure 31: Higuchi Model Release Profile (FS1-FS5)

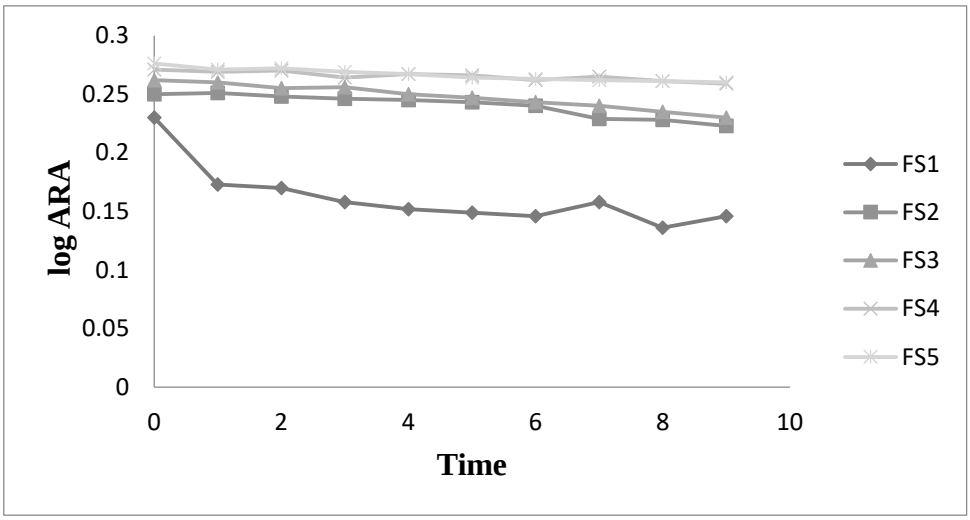

Figure 32: First Order Release Profile (FS1-FS5)

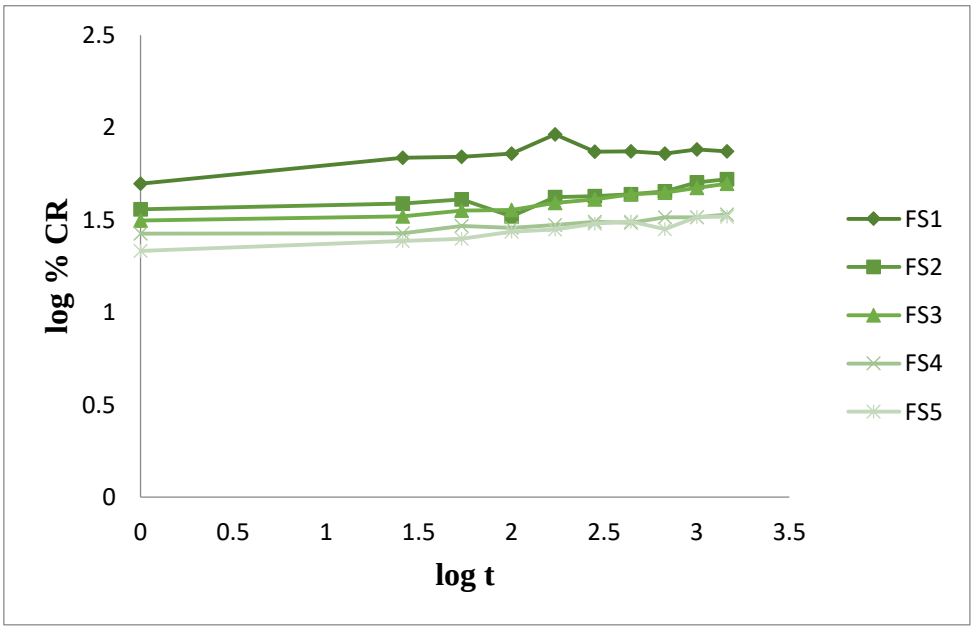

Figure 33: Korsmeyer – Peppas Release Profile (FS1-FS5)

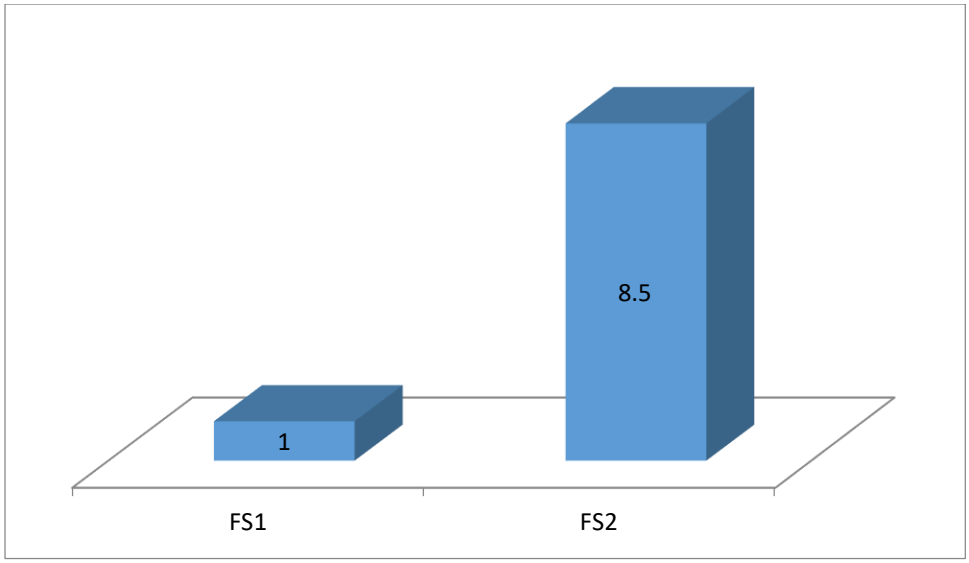

Figure 34: Time (in hours) to release 50 percent of drug from formulation (FS1-FS5)

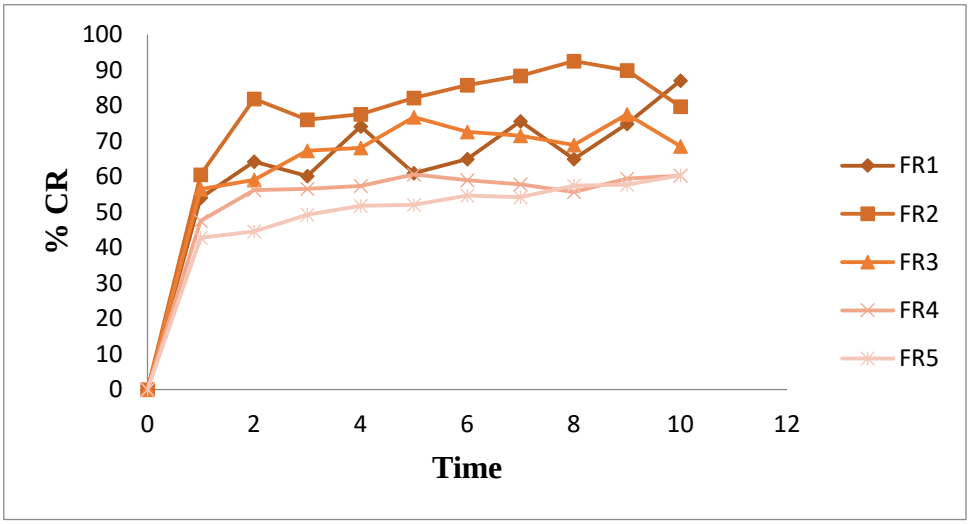

Figure 35: Zero Order Release Profile (FR1-FR5)

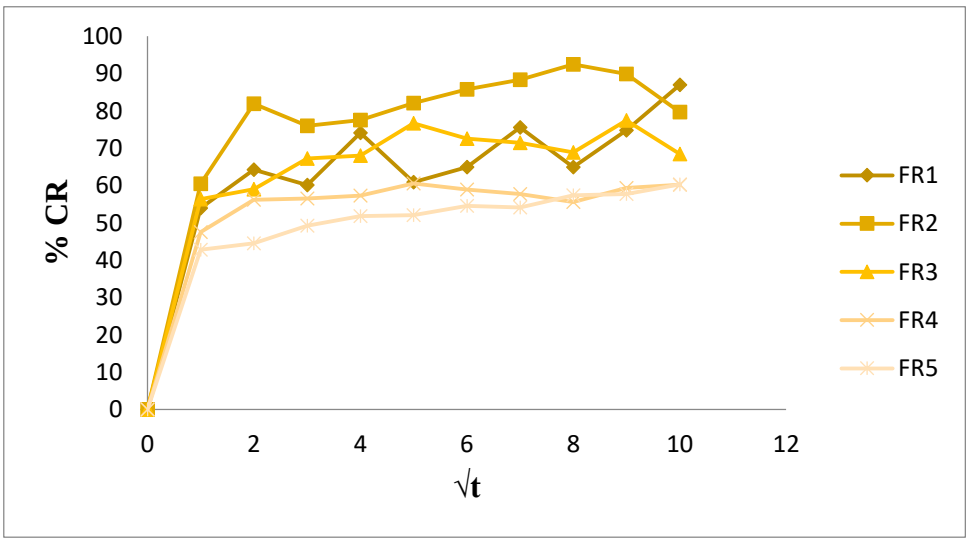

Figure 36: Higuchi Model Release profile (FR1-FR5)

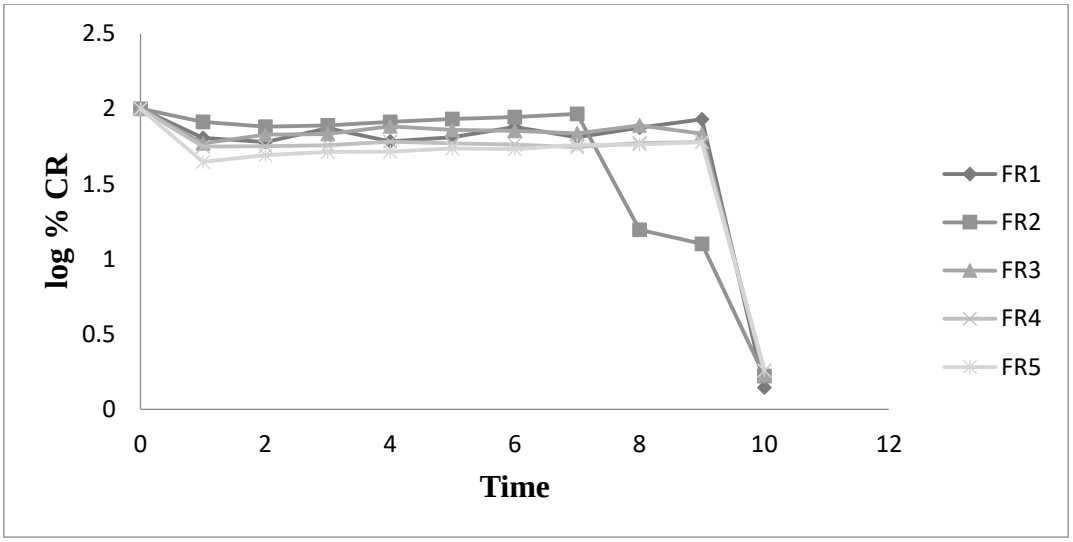

Figure 37: First order Release profile (FR1-FR5)

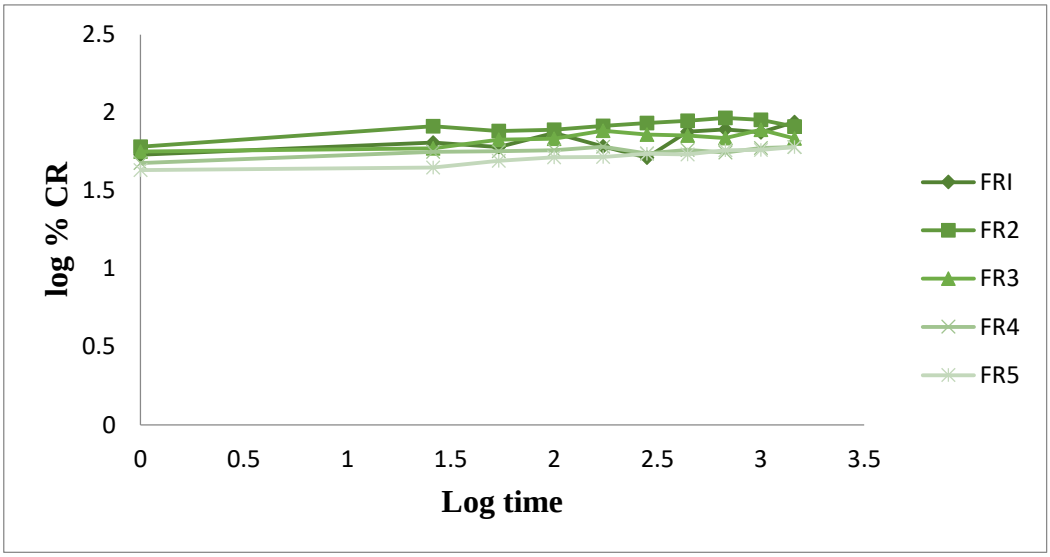

Figure 38: Korsmeyer – Peppas Release Profile **(**FR1-FR5)

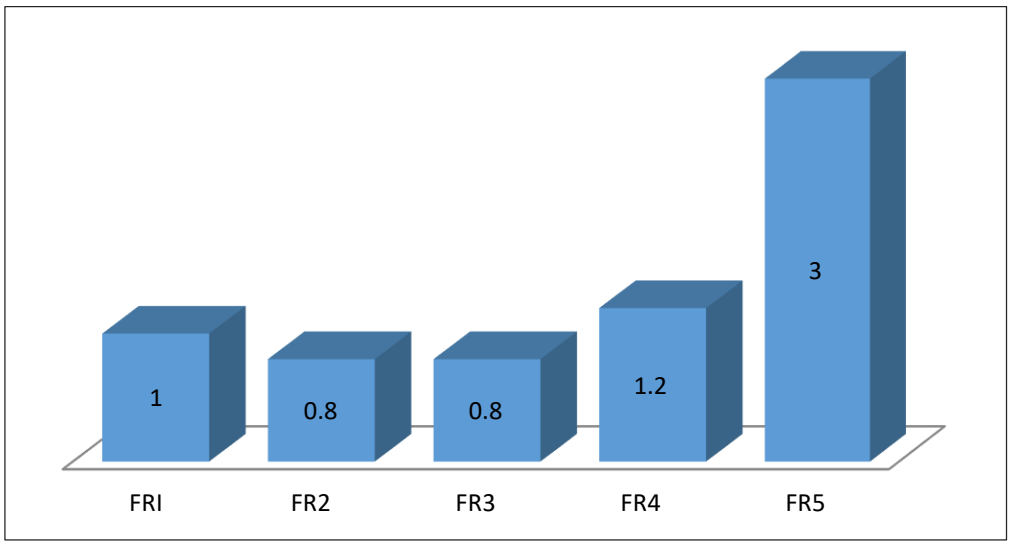

Figure 39: Time to release 50 percent of drug from formulation (FR1-FR5) (in hours).

3.11. Determination of morphological characteristics of Rivastigmine loaded microsphere through Scanning Electron Microscopy (SEM)

The surface morphology study of selected microspheres was carried out and as per the photographic evidence, it was clearly concluded that the selected formulation had smooth surface with no sign of aggregation.The correlation coefficient r^2 values were used as criteria to choose the best model for the drug release from the drug loaded multilayered tablet formulation. From the respective table (Table 8) it was observed that the individual formulation having different r^2 value for different model. On the basis of higher value of r^2we select the best fit model. Now Korsmeyer-Peppas model possess great importance to know the release mecahnism of the drug from the formulation. To predict the mechanism of diffusion release , the following equation $M_t/M_\infty = kt^n$ was used to analyze data of sustained release of drug loaded formulation. Now n= 0.5 means fickian diffusion, 0.5< n<1.0 non-fickian diffusion, and n=1.0 case II diffusion. Considering the n values calculated for the studied loaded multilayered tablet formulation (Table 8).

3.11.1. SEM imaging Of Rivastigmine and Ethyl Cellulose

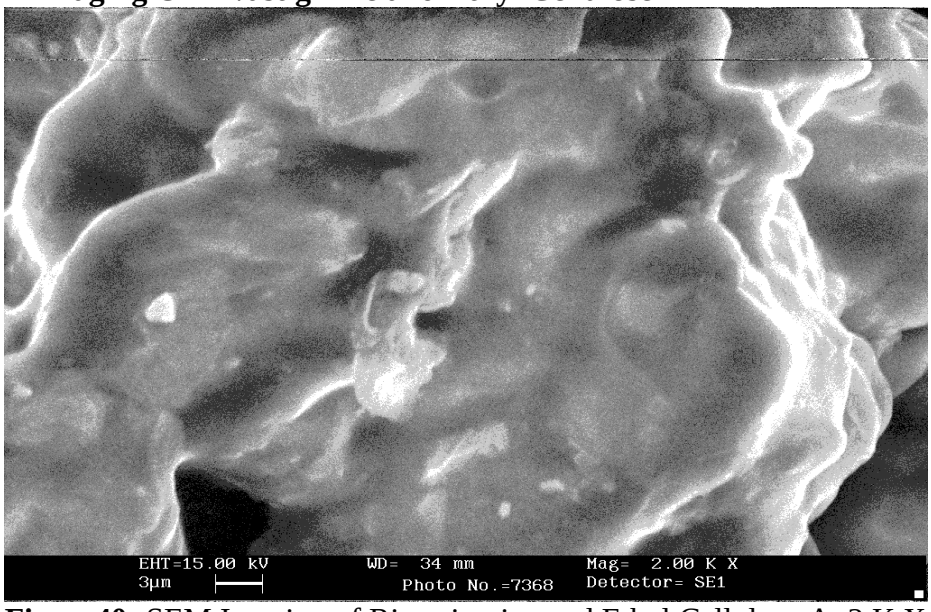

Figure40: SEM Imaging of Rivastigmine and Ethyl Cellulose At 2 K X

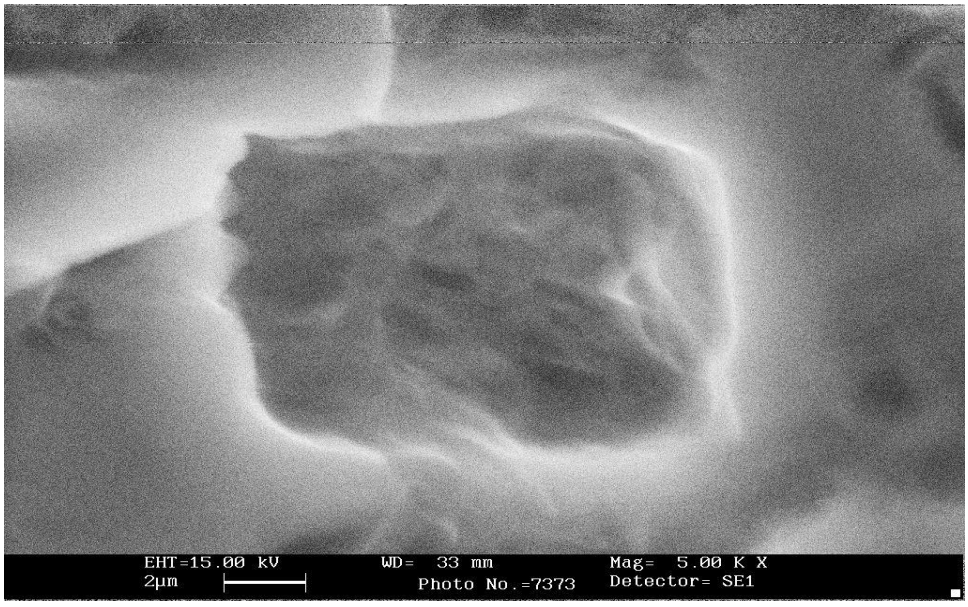

Figure 41: SEM Imaging of Rivastigmine and Ethyl Cellulose at 5K X.

3.11.2. SEM Reports of Rivastigmine and Eudragit- S 100

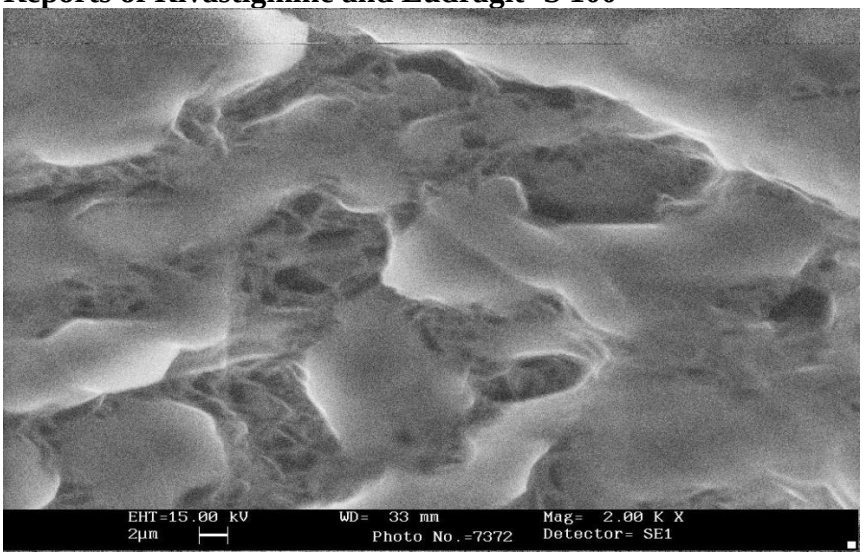

Figure 42: SEM imaging of Rivastigmine and Eudragit S 100 at 2K X.

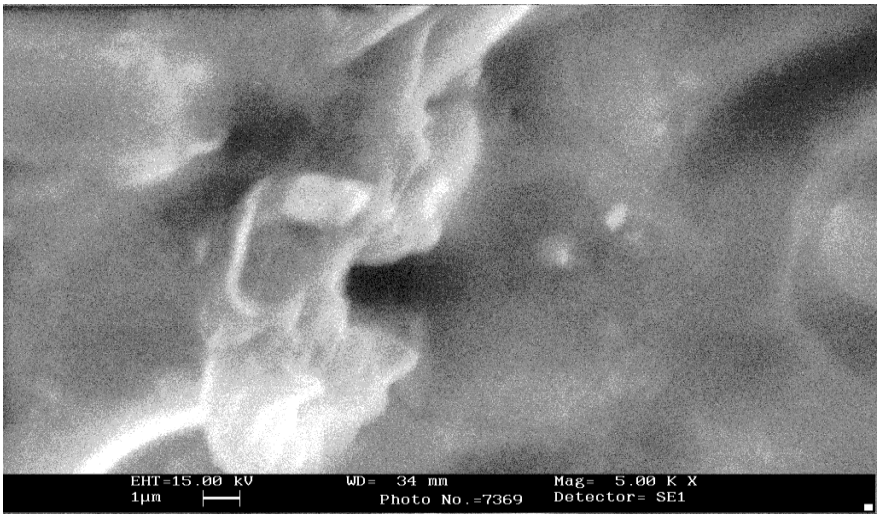

Figure 43: SEM imaging of Rivastigmine and Eudragit S 100 at 5KX

3.11.3. SEM Reports of Rivastigmine and Eudragit RS 100

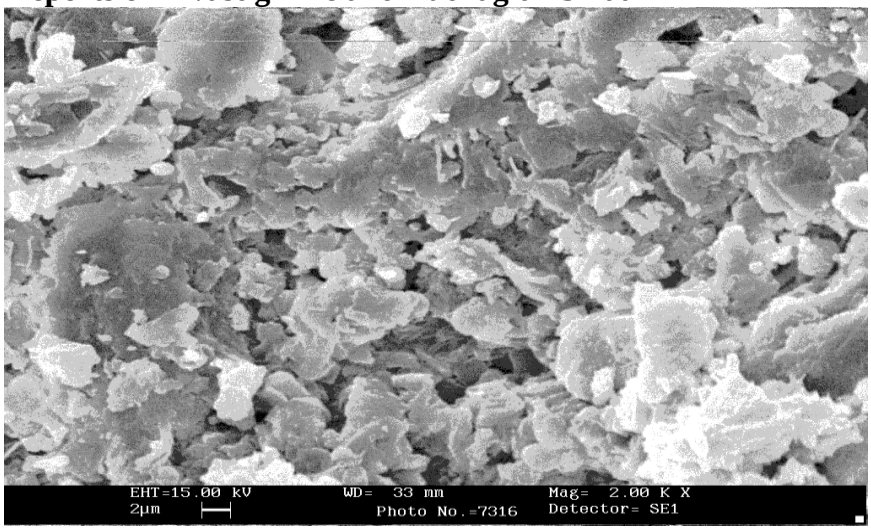

Figure 44: SEM imaging of Rivastigmine and Eudragit RS 100 at 2K X

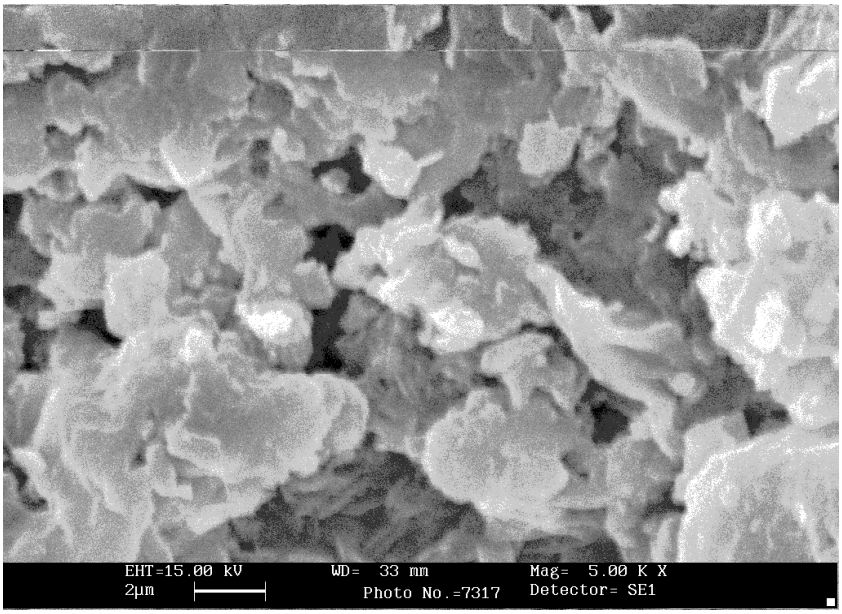

Figure 45: SEM imaging of Rivastigmine and Eudragit S 100 at 5K X.

Table 8: Release Kinetics of Optimized Microspheric Formulations

Formulation	r^2			Korsmeyr r^2	Peppas plotN
	Zero order plot	First order plot	Higuchi plot		
FE1	0.705	First-order	0.909	.248	0.159
FE2	0.621	0.448	0.913	0.251	0.123
FE3	0.797	0.620	0.915	0.282	0.171
FE4	0.934	0.862	0.963	0.267	0.163
FE5	0.930	0.953	0.973	.270	0.165
FS1	0.652	0.951	0.938	0.597	0.006
FS2	0.719	0.698	0.724	.898	0.008
FS3	0.350	0.636	0.563	0.980	0.003
FS4	0.762	0.319	0.952	0.974	0.002
FS5	0.711	0.857	0.929	0.952	0.001
FR1	0.793	0.667	0.950	0.219	0.083
FR2	0.698	0.842	0.936	0.574	0.145
FR3	0.761	0.723	0.966	0.249	0.083
FR4	0.673	0.832	0.959	0.213	0.079
FR5	0.484	0.719	0.419	0.206	0.069

4. Discussions:

The most well-established class of medications for treating Alzheimer's-type dementia is cholinesterase inhibitors. Drugs like Rivstigmine acts by blocking acetylcholine esterase enzyme inhibition, cholinesterase inhibitors increase the availability of acetylcholine (ACh) at the synapse. The penetration of such drugs inside the brain through BBB becomes the limitation for which more lipophilic properties need to be added which was done by the prepared formulations. Numerous investigations have been carried out on the drug delivery system after the announcement of biodegradable polymers more than thirty years ago. This study focused on the evaluation of formulated microsphere by the help of various studies.The solubility study BCS class I drug dissolves in both hydrophilic and lipophilic solvent so it can

penetrate the blood brain barrier very easily and its absorption is also very high [22]. FTIR spectra for Rivastigmine given in National formulary was compared with FTIR spectra of obtained drug sample which gives a preliminary identification about pure drug [23]. Under spectroscopy analysis all three prepared standard curve had regression value above 0.99 which signified the reproducibility and linearity as reported in the literature [24].All the prepared formulation had atleast 66% yield and as the polymer concentration was increased, the %yield was also increased. From experimental result, it was reported that all the prepared microspheres having particle size ranging between 65.5 µm to 175. 2 µm and just like %yield as the polymer concentration increased, particle size increased which was also reported in a literature [25, 26]. It was reported that all the prepared microspheres having particle size ranging between 65.5 µm to 175. 2 µm and just like %yield as the polymer concentration increased, particle size increased which fall within the range of reported studies [27].The result % moisture loss clearly revealed all the prepared formulation contained less amount of moisture. So from the experimental results, it can be concluded that the prepared microspheres were dried properly as well as the strong hydrophobic nature of selected polymer prevent the preparation of water. As the experimental results were obtained, all the prepared formulations possessed low to moderate drug content value though the value of drug entrapment is quite high. The maximum drug content was observed in formulation FS1 (51.8), while formulation FE5(20.72) contained less amount of drug among the lots that resembles the reported study [28, 32].All these parameters values as shown in Table 7 were within the pharmacopoeia limit and hence it can be concluded that the prepared microspheres possess good flow ability [29, 33].The value of obtained swelling index is largely dependent of water uptake tendency of selected polymer as reported in Figure 22-24. The entire selected polymerhaving hydrophobic property that is why the value of swelling index is quite low that makes our formulation most suitable for BBB penetration due to its hydrophobic nature [30].

All the prepared microspheres containing Ethyl Cellulose in the ratio of 1:2 to 1:6(FE1-FE5) were subjected to *invitro* dissolution studies using phosphate buffer pH 7.4.From the obtained release profile data, it was clearly observed that except formulation FE5 all the prepared microspheres released more than 25% drug during first hour study. This may be due to surface entrapment drug and less drug entrapment. Although after initial burst release, all the prepared microspheres showed a controlled release profile and it was confirmed by the analyzed $t_{50\%}$ data. FE5 provide better controlled release and possess a higher value of $t_{50\%}$ i.e. 8.8 hours among the other formulations.All the prepared microspheres containing Ethyl Cellulose in the ratio of 1:2 to 1:6(FE1-FE5) were subjected to *invitro* dissolution studies using phosphate buffer pH 7.4.From the obtained release profile data, it was clearly observed that except formulation FE5 all the prepared microspheres released more than 25% drug during first hour study. This may be due to surface entrapment drug and less drug entrapment. Although after initial burst release, all the prepared microspheres showed a controlled release profile and it was confirmed by the analyzed $t_{50\%}$ data. FE5 provide better controlled release and possess a higher value of $t_{50\%}$ i.e. 8.8 hours among the other formulations. Formulations prepared by using Eudragit S 100 were also subjected for *invitro* dissolution study. The initial drug release patterns were quite same as in case of formulations prepared from ethyl cellulose. Formulations prepared by using Eudragit S 100 were also subjected for *invitro* dissolution study. The initial drug release patterns were quite same as in case of formulations prepared from ethyl cellulose. From *invitro* release profile, it was quite clear that as we were increasing the polymeric concentration, more sustained release profile was obtained.From *In-vitro* release profile, it was quite clear that as we were increasing the polymeric concentration, more sustained release profile was obtained. Formulations prepared from Eudragit RS 100 when subjected to *invitro* release profile study,the release pattern sujested that the perticular lot provide excess burst release and less controlled release. This

may be due to the following reasons such as less amount of drug entrapment and more amount of surface entrapment and improper formation of polymeric matrix formation as shown in figure 25-39 [30, 31].

After studying the surface morphology of a few chosen microspheres and analyzing photographic data, it was evident that the chosen formulation had a smooth surface free of any signs of aggregation.After studying the surface morphology of a few chosen microspheres and analyzing photographic data, it was evident that the chosen formulation had a smooth surface free of any signs of aggregation. Kinetic models describe drug release from immediate and modified release dosage forms. Thus the model fitting analysis (zero order, First oreder, Higuchi and Korsmeyer-peppas model) were done by compairing the coefficient of regression (r^2) values and corresponding n values of all the kinetic equation. In case of formulations prepared by Ethyl Cellulose,the drug release pattern governed by fickian diffusion.that means the release of drug from polymeric matrix depends on squre root of time. In case of formulations of Eudragit S 100, non fickian diffusion is dominant.This type of mechanism is generally obtained due to release from initially dry hydrophobic glassy material that swells when in contact with water and become rubbry and shows anamolus diffusion as a result of re-arangement of macromolecule chain. In case of formulation prepared by Eudragit RS 100,execpt formulation FR2 all the formulation released via non fickian diffusion mechanism. On the basis of all above evaluation parameters i.e. Rheological property, *In Vitro*release profile, Comparision of $T_{50\%}$ values and Release kinetic study, FS3(1:3) r^2=0.980, FE4(1:4) r^2=0.267 and FR5(1:5) r^2=0.206 were selected as optimized formulationas depicted in figure 40-45 which was similar to that reported in previous established literatures [31, 34].

5. Conclusions:

This study clearly demonstrated that the Rivastigmine can be successfully prepared as microspheres. The microspheres showed ideal properties with an added advantage of circumventing sustained release. Well defined evaluation parameters were provided to produce potential therapeutic benefit. It is possible to control the disease at faster rate after administration of Rivastigmine from microparticulate. However further work is essential to stabilize Rivastigmine in microspheres for promising controlled drug delivery along with this route of drug delivery and Further more studies need to be done for establishing*in vivo* effects of theRivastigmine loaded microsphere must be executed in order to prove the pharmacological action of the formulated microspheres.

6. References:

1. Scheltens P, De Strooper B, Kivipelto M, Holstege H, Chételat G, Teunissen CE, Cummings J, van der Flier WM. Alzheimer's disease. The Lancet. 2021 Apr 24;397(10284):1577-90.
2. Masters CL, Bateman R, Blennow K, Rowe CC, Sperling RA, Cummings JL. Alzheimer's disease. Nature reviews disease primers. 2015 Oct 15;1(1):1-8.
3. Scheltens P, Blennow K, Breteler MM, De Strooper B, Frisoni GB, Salloway S, Van der Flier WM. Alzheimer's disease. The Lancet. 2016 Jul 30;388(10043):505-17.
4. Wenk GL. Neuropathologic changes in Alzheimer's disease. Journal of Clinical Psychiatry. 2003 Jul 1;64:7-10.
5. Citron M. Alzheimer's disease: strategies for disease modification. Nature reviews Drug discovery. 2010 May;9(5):387-98.

6. Melnikova I. Therapies for Alzheimer's disease. Nature Reviews Drug Discovery. 2007 May 1;6(5).
7. Zhang Y, Chen H, Li R, Sterling K, Song W. Amyloid β-based therapy for Alzheimer's disease: Challenges, successes and future. Signal transduction and targeted therapy. 2023 Jun 30;8(1):248.
8. DeTure MA, Dickson DW. The neuropathological diagnosis of Alzheimer's disease. Molecular neurodegeneration. 2019 Aug 2;14(1):32.
9. Gribkoff VK, Kaczmarek LK. The Difficult Path to the Discovery of Novel Treatments in Psychiatric Disorders. InDrug Development in Psychiatry 2023 Mar 17 (pp. 255-285). Cham: Springer International Publishing.
10. Guo J, Cheng J, North BJ, Wei W. Functional analyses of major cancer-related signaling pathways in Alzheimer's disease etiology. BiochimicaBiophysicaActa (BBA)-Reviews on Cancer. 2017 Dec 1;1868(2):341-58.
11. Gurung BD, Kakar S. An overview on microspheres. Int J Health Clin Res. 2020;3(1):11-24.
12. Karan S, Debnath S, Kuotsu K, Chatterjee TK. In-vitro and in-vivo evaluation of polymeric microsphere formulation for colon targeted delivery of 5-fluorouracil using biocompatible natural gum katira. International journal of biological macromolecules. 2020 Sep 1;158:922-36.
13. Benoit JP, Faisant N, Venier-Julienne MC, Menei P. Development of microspheres for neurological disorders: from basics to clinical applications. Journal of controlled release. 2000 Mar 1;65(1-2):285-96.
14. Yawalkar AN, Pawar MA, Vavia PR. Microspheres for targeted drug delivery-A review on recent applications. Journal of Drug Delivery Science and Technology. 2022 Sep 1;75:103659.
15. Singh K, Ahmad Z, Shakya P, Ansari VA, Kumar A, Zishan M, Arif M. Nano formulation: a novel approach for nose to brain drug delivery. J Chem Pharm Res. 2016;8(2):208-15.
16. Desai SA, Vidyasagar GA, Shah VI, Desai DH. Preparation and in vitro characterization of mucoadhesive microspheres of midazolam: Nose to brain administration. Asian J Pharm Clin Res. 2011;4(1):100-2.
17. Dhadde GS, Mali HS, Raut ID, Nitalikar MM, Bhutkar MA. A review on microspheres: types, method of preparation, characterization and application. Asian Journal of Pharmacy and Technology. 2021;11(2):149-55.
18. Belwal SD, Joshi D, Rautela A, Kumar P. Formulation and evaluation of microsphere of Aceclofenac. Journal of Advancement in Pharmacology. 2020;1(1):65-70.
19. Asif HM, Kumar RA, Rao TR, Anjum MA. Preparation and evaluation of ethyl cellulose microspheres prepared by solvent evaporation technique. Int J Pharm Pharm Sci. 2014;6(7):264-6.
20. Chella N, Yada KK, Vempati R. Preparation and evaluation of ethyl cellulose microspheres containing diclofenac sodium by novel w/o/o emulsion method. Journal of pharmaceutical sciences and research. 2010 Dec 1;2(12):884.
21. Shabaraya A.R. and Narayanacharyulu R., Design and Evaluation Of Chitosan Microspheres of Metoprolol Tartrate for Sustained Release, Indian J. Pharm. Sci., 2003, 65(3), 250-252.
22. Asha Patel, Subhabrata Ray, and Ram Sharnagat Thakur.In vitro evaluation and optimization of controlled release floating drug delivery system of metformin hydrochloride, Daru;;14:p57-64 2006.

23. Y.Kawashima, T. Niwa, H. Takeuchi, T. Hino, and Y. Itho. Hollow microspheres for use as a floating controlled drug delivery system in the stomach, J Pharmaceutical sciences;;81:p135-140 1990.
24. Sunil K. Jain, Govind P. Agrawal, and Narendra K. Jain. Evaluation of Porous Carrier-based Floating Orlistat Microspheres for Gastric Delivery, AAPS PharmSciTech;2006;7 (4):pE1-E9.
25. Gadad AP, Naik SS, Dandagi PM, Bolmal UB. Formulation and evaluation of gastroretentive floating microspheres of lafutidine. Indian J Pharm Edu Res. 2016 Apr 1;50:S76-81.
26. Rahman Z, Khar RK. Designand Evaluation of Bilayer Floating Tablets of Captopril. Acta Pharma; 2006:56; p49-57.
27. Levis S.R. and Deasy P., Pharmaceutical Applications of Size Reduced Grades of Surfactant Co-Processed Micro Crystalline Cellulose, Int. J. Pharm., 2001, 230, 25-33.
28. Chowdary K.P.R and Rao Y.S., Mucoadhesive Microcapsules of Glipizide: Characterization, In Vitro and In Vivo Evaluation. Indian J. Pharm. Sci., 2003, 65(3), 279-284.
29. Desai K.G. and Park H.J., Study of Gamma-Irradiation Effects on Chitosan Microparticles, Drug delivery, 2006, 13, 39-50.
30. Levis S.R. and Deasy P., Pharmaceutical Applications of Size Reduced Grades of Surfactant Co-Processed Micro Crystalline Cellulose, Int. J. Pharm., 2001, 230, 25-33.
31. Shovarani K.N. and Goundalkar A.G., Preparation and Evaluation of Microsphere of Diclofenac Sodium, Indian J. Pharm. Sci., 1994, 56(4), 45-50.
32. Ismail MF, ElMeshad AN, Salem NA. Potential therapeutic effect of nanobased formulation of rivastigmine on rat model of Alzheimer's disease. International journal of nanomedicine. 2013 Jan 23:393-406.
33. Lipp L, Sharma D, Banerjee A, Singh J. In vitro and in vivo optimization of phase sensitive smart polymer for controlled delivery of rivastigmine for treatment of Alzheimer's disease. Pharmaceutical research. 2020 Mar;37:1-2.
34. Rompicherla SK, Arumugam K, Bojja SL, Kumar N, Rao CM. Pharmacokinetic and pharmacodynamic evaluation of nasal liposome and nanoparticle based rivastigmine formulations in acute and chronic models of Alzheimer's disease. Naunyn-schmiedeberg's Archives of Pharmacology. 2021 Aug;394(8):1737-55.