FORMULATION, CHARACTERIZATION AND IN-VITRO EVALUATION OF FLOATING MICROSPHERES OF RASAGILINE MESYLATE

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Abstract: Systematic studies were conducted using two different polymers in different concentrations to prepare Rasagiline Mesylate Floating Microspheres. All the prepared systems were evaluated for the different properties. From the Preformulation studies for drug excipients compatibility, it was observed that no physical incompatibility existed between the drug and excipients. All the four different formulations prepared contain drug about 97%-102%. In vitro drug release profile indicated that drug release was retarded due to the presence of higher concentration of polymer. Formulation F2 has only 68%drug release in 9 hrs due to higher ratio of the polymer. Formulated Microspheres gave satisfactory results for various evaluation parameters like Angle of Repose, Drug Entrapment Efficiency, Scanning Electronic Microscopy and in vitro drug release. Comparing the two different Polymers such as HPMC and Chitosan provided better-sustained release characteristics with excellent in-vitro drug release. From the above results also indicated that at higher viscosity grades of polymer concentrations drug release was retarded greatly.

Keywords: formulation, characterization and in-vitro evaluation of floating microspheres of rasagiline mesylate.

INTRODUCTION

The oral route is the most feasible and oftenused mode of drug delivery. However, this approach has several physiological drawbacks: the drug-specific absorption window in the upper small intestine, a quick gastrointestinal transit time, and an unpredictable gastric emptying rate.[1] There are several difficulties in designing controlled-release systems for improved absorption and bioavailability. One of these difficulties is limiting the dosage form in the target part of the gastrointestinal tract. Drug absorption from the gastrointestinal tract is a complex process that depends on a number of variables. It is widely acknowledged that how much of the drug is absorbed in the digestive tract depends on how long it is in contact with the small intestinal mucosa.[2] To achieve optimum therapeutic efficiency with the least amount of toxicity and side effects, the medicine must be delivered to the target tissue at the ideal time and in the ideal quantity.[3] A medicinal drug can be delivered to the target area using a variety of methods that allow for a continuous, regulated release. Some of the drawbacks of traditional therapy can be avoided with the use of a well-designed controlled drug delivery system, and the therapeutic potency of a certain medicine can be improved. The use of floating microspheres as medication carriers are one such strategy. In controlled conditions, a microsphere can be utilized to deliver hormones, antibiotics, and other pharmaceuticals. In addition to their fundamental advantages, microspheres can be used in ways that increase their surface area and make it simpler to estimate diffusion and mass transfer properties. Microspheres are tiny granules, typically between 1 and 1000 micrometers in size.[4] Microspheres have advanced technologically to a number of processes, including combinations of phase separations or precipitations, emulsion or solvent evaporation, and spraying techniques.[5] Floating microspheres are one of the most promising methods for buoyant gastro retentive drug delivery.[6] As a result, a delayed transit through the stomach occurs because the preparations for controlled gastrointestinal transit are made to float on gastric fluids with a specific density of less than one.[7] Less variation in plasma drug concentration and improved GI retention result from the drug being released at the desired rate.[8]

Rasagiline is an irreversible inhibitor of monoamine oxidase and is used as a monotherapy in early Parkinson's disease or as an adjunct therapy in more advanced cases.[9-11]

To design, formulate & carryout the in-vitro evaluation studies on floating microspheres of Rasagiline Mesylate.The main objective of the study was to formulate and evaluate Floating microspheres of Rasagiline Mesylate which is expected to deliver the drug in controlled manner with reduced frequency of drug administration, improve patient compliance & bioavailability of Rasagiline Mesylate. This study mainly deals in the design of a formulation which produces time controlled prolonged drug release and to enhance the bioavailability of the drug to about 90% and also

to reduce the dosing interval of the drug.

MATERIALS AND METHODS

The following materials were used as supplied by the manufacturers.

Table 1: Materials Used

S. No.	Chemicals	Supplied by
1	Rasagiline Mesylate	Microlabs, Bangalore
2	HPMC	Oxford Laboratories, Mumbai
3	Chitosan	Central Institute of Fisheries, Kerala
4	Sodium Alginate	S.D. Fine Chem. Ltd, Mumbai
5	Sodium Bicarbonate	S.D. Fine Chem. Ltd, Mumbai

Pre-formulation study

Prior to the development of the dosage forms the preformulation study was carried out. Hence Infrared spectra of the physical mixture of the drug and the polymers chosen were taken. The infra-red spectra of the drug and polymers were also taken.

The application of infra-red spectroscopy lies more in the qualitative identification of substances either in pure form or in the mixture and as a tool in establishment of the structure. Since I.R. is related to covalent bonds, the spectra can provide detailed information about the structure of molecular compounds. In order to establish this point, comparisons can be made between the spectrum of the substance and the drug.

STANDARD PLOT FOR ESOMEPRAZOLE MAGNESIUM TRIHYDRATE

Standard Graph by using Phosphate Buffer (pH 7.4)

Accurately weighed 10 mg of Rasagiline Mesylatewas dissolved in 100 ml of 7.4 pH buffer solution to form 100 µg/ml stock solutions.

From this stock solution aliquots of 2.5 ml, 5 ml, 7.5 ml, 10 ml, 12.5 ml, 15 ml, 17.5ml, 20 ml, 22.5 ml, 25 ml were pipette out into a series of 50 ml in order to get a concentration ranging from 5-50µg/ml.

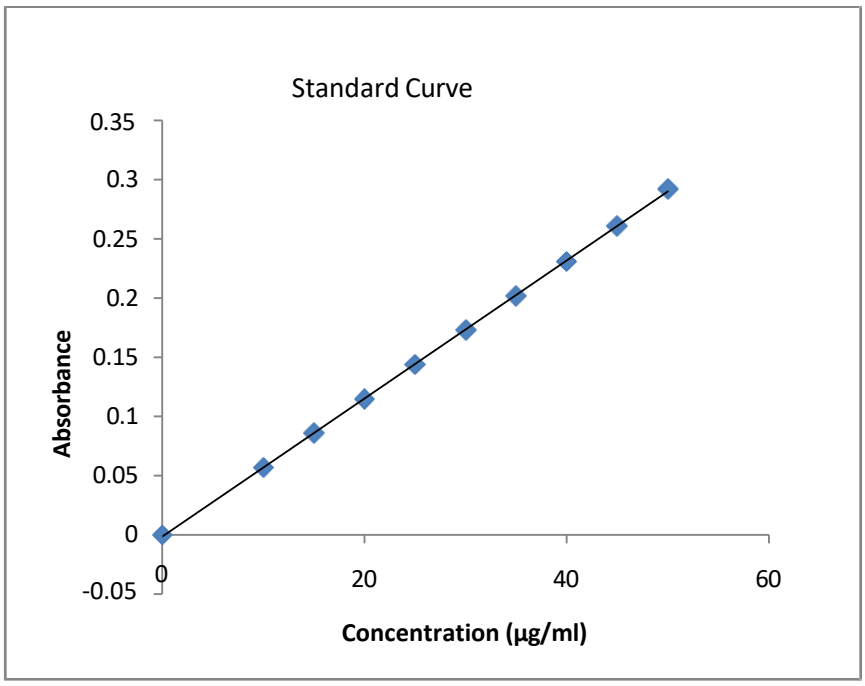
The absorbance of the resulting solution was then measured at 301 nm using UV spectrophotometer against respective parent solvent as a blank. The standard curve was obtained by plotting absorbance Vs. concentration µg/ml.

Table No. 2: Standard Calibration Curve of Rasagiline Mesylate

SL. No	CONCENTRATION(µg/ml)	ABSORBANCE
1	**10**	**0.057**
2	**15**	**0.086**
3	**20**	**0.115**
4	**25**	**0.144**
5	**30**	**0.173**
6	**35**	**0.202**

7	**40**	**0.231**
8	**45**	**0.261**
9	**50**	**0.292**

Graph No.1
Standard Calibaration Curve of Rasagiline Mesylate



b) SOLUBILITY STUDIES

Table No 3: Solubility studies of Rasagiline Mesylate

S. No.	Solvents	Observed
1	PBS-7.4(pH)	Freely soluble
2	Ethanol	Freely soluble

c) FT-IR SPECTRA OF RASAGILINE MESYLATE

The FT-IR analysis of the Rasagiline Mesylatewas carried out for qualitative compound identification. The FT-IR spectra for pure drug and with other excipients was obtained by placing the drug directly into the cavity and was determined by FT-IR spectrophotometer in the wave number region of 4000-400 cm^{-1}.

Table No.4: Comparison of I.R. Spectra of Rasagiline Mesylate and in Combination with Polymers

S. No	Sample	C=O (cm^{-1})	-CC (cm^{-1})	-CH (cm^{-1})
1	Rasagiline Mesylate	3182	1462	919
2	RM + HPMC	3183	1458	915

3	RM + Chitosan	3182	1462	919
4	RM + Na_2CO_3	3182	1495	919
5	RM + HPMC + Chitosan	3182	1462	919

Preparation of Microspheres

In the present study, microspheres are prepared using **Emulsion-Polymerization** method. In this method, polymeric drug solution i.e., drug + polymer and solvent system (DicloroMethane + Ethanol) of 10 ml is added to 10 % solution of egg albumin. This polymeric phase is stirred continuously to form a uniform dispersion. In another beaker 86 ml of coconut oil containing 1 ml of 0.5% Sodium Lauryl Sulphate is taken which forms the organic phase. The polymeric phase is added drop wise using needle into the organic phase. It is continuously stirred for 2 hrs with a speed of 700 rpm using stirrer. After stirring 1 ml of formaldehyde is added and obtained microspheres are washed thrice with 20 ml of □–hexane and the obtained final microspheres are stored in a dessicator.

Table No. 5: Formulation Design For Floating Microspheres of Rasagiline Mesylate

Formulation No.	**Drug in mg**	**HPMC (mg)**	**Chitosan (mg)**	**Sodium Bicarbonate (% W/V)**	**Sodium Alginate (% W/V)**
F_1 (1:1)	50	50	-	1 %	2 %
F_2 (1:1.5)	50	75	-	1 %	2 %
F_3 (1;1)	50	-	50	1 %	2 %
F_4 (1;1.5)	50	-	75	1 %	2 %

PARTICLE SIZE DETERMINATION

The particle size of a pharmaceutical preparation is strictly maintained in order to get optimal biological activity.

Methods to estimate particle size are

a. Optical Microscopy

b. Sieving Method

c. Sedimentation Method

d. Elutriation Method

e. Centrifugal refractometry

f. Permeability Method

g. Light scattering Method

Table No. 6: Common techniques for measuring fine particles of various sizes

S. No.	Technique	Particle sizes in (µm)
1	Optical Microscopy	1-100 µm
2	Sieving	> 50 µm
3	Sedimentation	> 1 µm
4	Elutriation	1-50 µm
5	Centrifugation	< 50 µm
6	Permeability	> 1 µm
7	Light Scattering	0.5-50 µm

RESULTS

SCANNING ELECTRON MICROSCOPY

Procedure

Morphology details of the specimens were determined by using a Scanning Electron Microscope (SEM), Model JSM 35CF, JEOL, Japan.

The samples were dried thoroughly in Vaccum dessicator before mounting on brass specimen studies. The samples were mounted on specimen studies using Double sided adhesive tape. The sputtering was done for nearly 3 minutes to obtain uniform coating on the sample to enable good quality SEM images. The SEM was operated at low accelerating voltage.

The condenser lens position was maintained between 4.4-5.1. The objective lens aperture has a diameter of 240 microns and the Working Distance WD is 39 mm.

Fig No.2
SEM of Prepared Microspheres Under Low Magnification

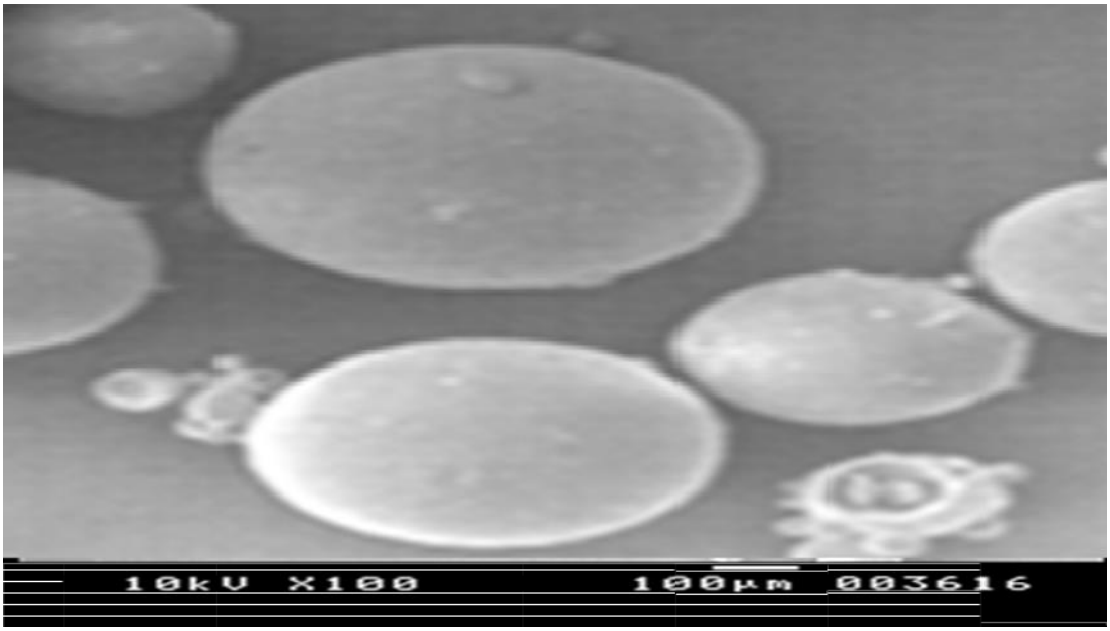
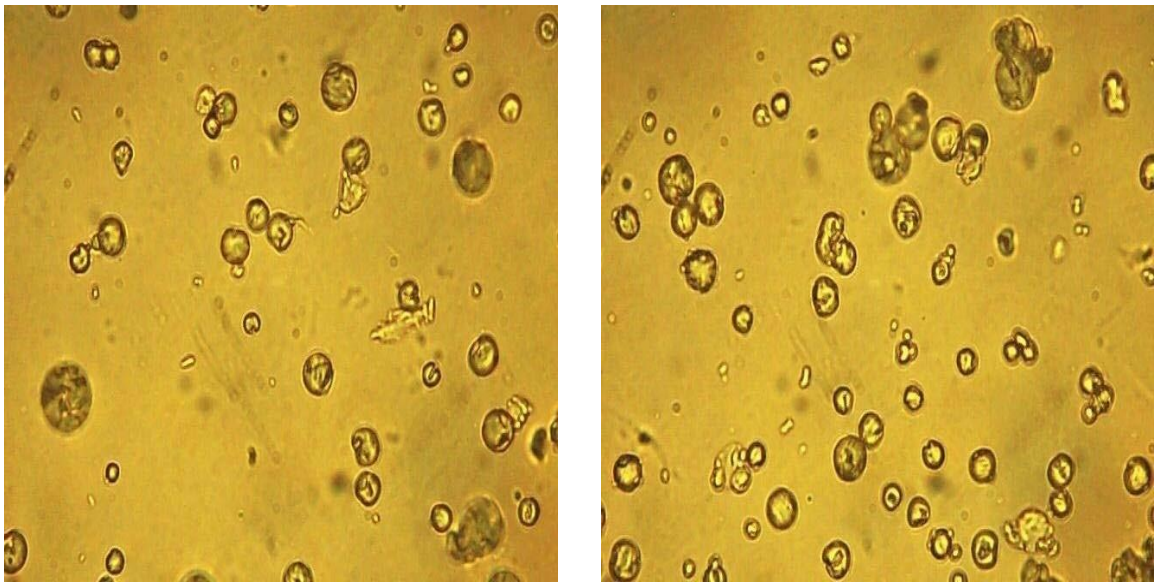


Fig No. 3
Microscopic Pictures of Rasagiline Mesylate Floating Microspheres



Flow Properties
Flow property depends on particle size, shape, porosity and density of microspheres.
Angle of Repose
The flow characteristics are measured by angle of repose. Improper flow is due to frictional forces between the particles. These forces are quantified by angle surface of the pile of the powder and the horizontal plane. The flow of powder and the angle of repose is depicted in following table.

Definition
The lower the angle of repose, the better the flow property. Rough and irregular surface of particles gives higher angle of repose. Decreased in the particle size leads to a higher angle of repose.
tan □ = h / r

□ = tan $^{-1}$ (h / r)

Where, h= height of pile

r = radius of the base of the pile

□ = angle of repose

Method
A glass funnel is held in place with a clamp on a ring support over a glass plate. The glass plate is placed on a stand. Approximately 100 g of particles is transferred into funnel keeping the office of the funnel blocked by the lower thumb. As the thumb is removed, the particles are emptied from funnel, and the angle of repose s determined by above mentioned formula.

Table No.7
Angle of Repose of Microparticles

S. No.	Formulation	Angle of repose
1	F1	25º70’
2	F2	28º29’

3	F3	29º74’
4	F4	30º96’

DRUG ENTRAPMENT EFFICIENCY

Drug entrapment efficiency of Rasagiline Mesylate was performed by accurately weighing 100 mg of microparticles and suspend in 100 ml of simulated intestinal fluid of pH 7.4±0.1 and it was kept for 12 hrs. Next day it was stirred for 15 min, and subjected for filtration. After suitable dilution, Rasagiline Mesylate content in the filtrate was analyzed Spectrophotometrically at 301 nm using Shimadzu 1201 UV-visible spectrophotometer.

The absorbance found from the UV-Spectrophotometer was plotted on the standard curve to get the concentration of the entrapped drug. Calculating this concentration with the dilution factor we get the percentage drug encapsulated in microparticles.

Table No.8`

Drug Entrapment Efficiency of Microparticles

Formulation	Absorbance at 301 nm	Theoretical yield (mg)	Practical yield (mg)	Drug Entrapment Efficiency
F1	0.059	50	20.34	79.66
F2	0.054	50	18.62	81.38
F3	0.032	50	11.03	88.97
F4	0.0481	50	16.58	83.42

In-vitro Dissolution Studies

A drug is expected to release from the solid dosage forms (granules, tablets, capsules etc) and immediately go into molecular solution. This process is called as Dissolution.

Drug release studies

The method specified in USP for the drug release study was followed.

Apparatus

USP XXIII dissolution test apparatus employing the round bottom dissolution vessel and rotating basket assembly.

Buffer stage

900 ml of pH 7.4 intestinal fluid (phosphate buffer) is used as dissolution media.

Time

At every 1 hr interval upto 12 hours.

Procedure

In-vitro release profile of the microparticles was evaluated using rotating basket dissolution apparatus. 900 ml of phosphate buffer (pH 7.4) maintained at 37±0.5ºC is used as dissolution Media, and the basket was rotated at a constant speed of 75 rpm. Accurately weighed amount of microparticles were placed in the baskets.

Aliquots of samples were withdrawn at the interval of 1 hour for 9 hours in phosphate buffer pH 7.4. The samples withdrawn were filtered, diluted suitably and analyzed at 301 nm spectrophotometrically for drug release.

Table No.9
In-vitro Dissolution Profile for Formulation F_3

Time (hrs)	Absorbance	Concentration	Cumulative % Drug Released
1	0.09	0.31	11.17
2	0.13	0.044	16.13
3	0.23	0.724	26.06
4	0.25	0.852	31.03
5	0.29	1.068	38.48
6	0.34	1.172	42.20
7	0.41	1.413	50.88
8	0.53	1.827	65.79
9	0.58	2.006	72.28
10	0.62	2.137	76.96
11	0.68	2.379	85.65
12	0.78	2.689	96.82

Table No.10
Cumulative % Drug Release Vs Time

	Cumulative % Drug Release			
Time (hrs)	**F_1**	**F_2**	**F_3**	**F_4**
1	9.931	8.68	11.17	8.6
2	14.89	11.17	16.13	14.9
3	22.34	16.13	26.06	23.5
4	27.31	18.62	31.03	28.55
5	31.03	26.03	38.48	38.48
6	36.01	34.75	42.20	47.17
7	43.44	42.20	50.88	57.10
8	53.37	45.93	65.79	65.79

9	70.75	54.62	72.28	73.24
10	80.68	59.58	76.96	78.20
11	88.13	67.03	85.65	80.68
12	93.10	91.86	96.82	94.34

Graph No.11

Accelarated Stability Studies

The formulations were stored in an oven at 37+1ºc and 60+1ºc for a period of six weeks. The samples were analyzed for drug content every week by Spectrophotometer at 301nm.

Method

Microspheres were individually wrapped in aluminium foil and packed in amber colured screw capped bottle and put under specified condition in incubator for 3 months. After 3 months the microspheres were evaluated for In-vitro drug release.

Table No.11

Results of Assay of Formulation After Accelarated Stability Studies

Days	**37ºC**	**60ºC**
1	82.13	81.08
7	82.08	80.35
14	81.05	79.01
21	80.92	78.03
28	79.87	78.26
35	77.86	77.22
42	76.26	76.28

DISCUSSION

Floating Microspheres of Rasagiline Mesylate were prepared by emulsion polymerization technique and various evaluation parameters were assessed, with a view to obtain oral controlled released of Rasagiline Mesylate.

The prepared microparticles were then subjected to granulometric study, angle of repose, scanning electron microscopy, drug entrapment efficiency, in-vitro dissolution and stability studies.

A standard calibration curve for the drug was obtained by measuring absorbance at 301 nm, and by plotting the graph of absorbance Vs concentration.

To check the compatibility of the drug with various polymers, IR spectra of drugs, polymers and combinations. The characteristics absorption peaks of Rasagiline Mesylatewere obtained.

Angle of Repose
Acceptable range of angle of repose is 22º61' to 31º60'.

Formulations F_1 to F_4 showed an angle of repose in the acceptable range, which indicates a good flow property.

The drug entrapment efficiency of all the formulations were in the range between

78.62 % to 91.25%.

The dissolution studies were conducted by using dissolution medias, a pH 7.4.

The data obtained in the in-vitro dissolution studies were grouped according to modes of data treatment as follows:

- Cumulative percent drug release Vs. Time (Zero-order).
- Cumulative percent drug retained Vs. Square root of Time (Higuchi Model).
- Log Cumulative percent drug retained Vs. Time (First-order).
- Cumulative percent drug release in (mg) Vs. Time (Korsmeyer-Peppas Model).

Morphology of the microparticles were investigated by Scanning Electron Microscopy.

Stability study was carried out for the formulation F_3 at 40ºC ± 1ºC for a period of 45 days.

SUMMARY

Systematic studies were conducted using two different polymers in different concentrations to prepare Rasagiline Mesylate Floating Microspheres. All the prepared systems were evaluated for the different properties.

From the Preformulation studies for drug excipients compatibility, it was observed that no physical incompatibility existed between the drug and excipients.

All the four different formulations prepared contain drug about 97%-102%.

In vitro drug release profile indicated that drug release was retarded due to the presence of higher concentration of polymer.

Formulation F2 has only 68%drug release in 9 hrs due to higher ratio of the polymer.

Formulated Microspheres gave satisfactory results for various evaluation parameters like Angle of Repose, Drug Entrapment Efficiency, Scanning Electronic Microscopy and in vitro drug release.

Comparing the two different Polymers such as HPMC and Chitosan provided better-sustained release characteristics with excellent in-vitro drug release. From the above results also indicated that at higher viscosity grades of polymer concentrations drug release was retarded greatly.

CONCLUSION

Floating microspheres of Rasagiline Mesylate can be formulated as an approach to increase residence time and thereby improve its bioavailability. Formulation F3 gave better-controlled drug release in comparison to the other formulations. Among the polymers used to improve the gastric residence, Chitosan showed better control over drug release.
The drug release pattern from the optimized formulations was best fitted to Korsmeyer-Peppas model and zero order kinetics. Drug – excipients interaction of optimized formulations was carried out by using FTIR studies. In this analysis drug – excipients compatibility interactions were not observed.
In conclusion, very promising in vitro drug release results were observed with Floating microspheres of Rasagiline Mesylate, further there is a scope to conduct the bioavailability studies in human volunteers to know the exact pharmacokinetics of the developed floating microspheres of Rasagiline Mesylate

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