FORMULATION AND EVALUATION OF MICROSPHERES OF RABEPRAZOLE SODIUM

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ABSTRACT

Microspheres are spherical particles such as those found in dispersed pharmaceuticals in a specific solution or microcrystalline form, are known as microspheres. The current research was based on the formulation and estimation of microspheres of rabeprazole sodium using diverse excipients. Gift sample of Rabeprazole sodium was obtained from Reddy's Laboratories, India. The Dichloromethane, PVA, PEG, HCl, KH2PO4, HPMC, ethyl cellulose, Sodium hydroxide were purchased from the local Chemical store at Lucknow. The drug and other excipients were determined for pre-formulation profile in terms of solubility, drug-excipient compatibility. Solvent evaporation method was used in the formulation of microspheres. Total 6 formulations were developed and evaluated by diverse parameters e.g., density determination, particles size, flow properties, drug content, entrapment efficiency, in vitro drug release, spreadability, pH range, swelling index, SEM analysis and stability. In results, microspheres were shown to have remarkable formulization properties throughout the study. It has shown for optimum angle of repose, Carr's & Hausner's index. Microspheres M1 and M4 showed better drug release and stability and thus considered as optimized formulation. In conclusion, microspheres of rabeprazole sodium found as optimized formulation in terms of particle size, % drug content, stability etc. It might be effective externally in the cure of fungal infections due to its already proved antibacterial efficacy. It suggests to fellow researchers to determine the pharmacokinetic profile of formulated microspheres of rabeprazole sodium.

Keywords: microspheres, rabeprazole sodium, solvent evaporation method, HPMC.

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INTRODUCTION

Microsphere

Microspheres are spherical particles such as those found in dispersed pharmaceuticals in a specific solution or microcrystalline form, are known as microspheres [1]. Medications with a short $t^{1/2}$ that are absorbed quickly from the gastrointestinal tract are rapidly eliminated from the bloodstream. Controlled release formulations have been created to circumvent this issue by allowing for a gradual release of the substance into the GIT and the maintenance of a constant medicine intensity in the plasma for an extended duration. Appropriate dosage formulations achieve and maintain the specified plasma therapeutic drug concentration throughout the treatment [2].

Polymers used in microsphere

Natural

Proteins, carbs, and even chemically modified forms of carbohydrates are all potential sources. Proteins such as albumin, gelatin, and collagen are also put to use. Agarose, carrageenan, chitosan, starch, and other naturally occurring and synthetic carbohydrates [3-5].

Synthetic

Synthetic polymers are divided into two types-

a) Non-biodegradable e.g., Poly methyl methacrylate, Acrolein Glycidyl methacrylate, etc.

b) Biodegradable e.g., Lactides, Glycolides and their co polymers, etc.

Rabeprazole

Rabeprazole, an inhibitor of proton pumps, is prescribed for ulcer sufferers. The acidic environment of the parietal cells converts this prodrug into an active sulphenamide form. By blocking the H+K+ATPase of the coated gastric cells, rabeprazole reduces stomach acid production under both basal and stimulated conditions [6].

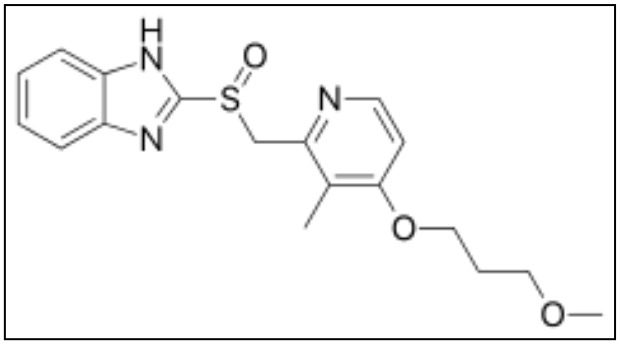


Fig 1. Structure of Rabeprazole

IUPAC name: 2-({[4-(3-methoxypropoxy)-3- methylpyridin-2-yl] methane} sulfinyl)-1H-1, 3- benzodiazole,

Molecular Formula: $C_{18}H_{21}N_3O_3S$

Molecular Weight: 359.45

Category: Anti-Ulcer

Mechanism of Action

By blocking the gastric H+K+ATPase, antisecretory drugs like rabeprazole (substituted benzimidazole proton-pump inhibitors) reduce acid output from the stomach. Because it inhibits PPI, an enzyme hypothesised to serve as the acid (proton) pump in the parietal cell, rabeprazole is commonly referred to as a gastric proton-pump inhibitor. Rabeprazole blocks

the final step in the production of stomach acid. The parietal cells in the stomach store rabeprazole and then protonate it to become an active sulfonamide. In vitro studies have shown that rabeprazole has a half-life of 78 seconds and is chemically activated at a pH of 1.2 [7].
Based on the above ROL, the current research was based on the formulation and estimation of microspheres of rabeprazole sodium.

MATERIALS AND METHODS

Experimental requirements

Gift sample of Rabeprazole sodium was obtained from Reddy's Laboratories, India. The Dichloromethane, PVA, PEG, HCl, KH2PO4, HPMC, ethyl cellulose, Sodium hydroxide were purchased from the local Chemical store at Lucknow. Rotatory evaporator, weighing balance, pH meter, FT-IR and SEM were used in the study.

Pre-formulation study

The drug and other excipients were determined for preformulation profile in terms of solubility, drug-excipient compatibility. It was done for the uniformity of drug content, better dissolution and in turn facilitated bioavailability. It also includes preparation of standard calibration curve of rabeprazole.

Preparation of microspheres

Since rabeprazole is slightly water soluble the microspheres were made using an o/w (oil/water) solvent evaporation process. The 20ml of dichloromethane was used to dissolve the polymers ethyl cellulose and HPMC. When these polymers and the medicine are thoroughly combined, a transparent solution appears. The surfactant polyethylene glycol (0.1%) was then added. The solution was then emulsified by gradually adding 160 ml of an aqueous solution containing 0.46 percent by weight PVA. At a temperature of 35 degrees Celsius, dichloromethane evaporated away. Until the solvent was entirely withdrawn, the emulsifier kept the oil droplets in their spherical shape and prevented them from aggregating, so the microspheres remained intact as single particles [8].
The microspheres were allowed to set, and then they were rinsed five times in distilled water before being dried.

Table 1. Composition of microspheres of rabeprazole sodium

Rabeprazole (g)	HPMC (g)	Ethyl Cellulose (g)	Dichloromethane (ml)	PVA (ml)
0.5	1	2	20	160
0.5	2	1	20	160
0.5	2	1	20	160
0.5	1	2	20	160
0.5	2	1	20	160
0.5	1	2	20	160

0.1N HCl preparation

9 ml of HCl was diluted in distilled water until the volume in a 1000 ml volumetric flask read 0.1N.

Preparation of PBS (pH 7.4)

To prepare a PB with a pH of 7.4, a 100 ml volumetric flask was filled with 25 ml of 0.2M KH2PO4 solution, 19.55 ml of 0.2N NaOH solution, and enough distilled water to make up the volume. The pH was measured as 7.4.

CHARACTERIZATION (IN-VITRO)

Determination of percentage yield

The total number of microspheres produced, along with the amount of product and polymers needed in their creation, can be used to derive a percentage yield [9].

Angle of repose

The fixed funnel technique was used to determine it. A glass funnel is clamped to a ring stand that rests over a glass plate. The thumb is used to cover the funnel's aperture as around 1g of powder is placed into the funnel. The powder is poured out of the funnel at an angle, and that angle is measured with respect to the horizontal plane.

$$\text{Angle of repose } (\theta) = \tan^{-1} \times (h/r)$$

Bulk density

Microspheres were weighed precisely, and their volume in a 10ml measuring cylinder was recorded. Using this formula, we can determine the bulk density in grammes per millimetre.

$$\text{Bulk density } (\rho 0) = M/V0$$

M= mass of powder, V0= Vol. of powder

Carr's index

Powder mix compressibility can be determined by utilising the apparent bulk density and the tapped density-

$$\text{Carr's Index} = \frac{\text{Density at the taps- bulk density}}{\text{Density in bulk}} \times 100$$

Hausner's ratio

It is a non-direct measure of powder flowability. The formula for this is below-

$$\text{Hausner's ratio} = \text{Tapped density/Bulk density}$$

Particles size

A temperature of 25 degrees Celsius was used for the measurements. The microsphere's light-scattering intensity was kept within the instrument's detection range by diluting it with twice-distilled water. The measurements were taken at ambient temperature.

SEM analysis

It was used to the morphology of movies. Samples were attached using double-backed adhesive tape on round brass stubs (12mm in width) before being seen under a scanning electron microscope. The samples were subsequently coated with gold palladium using a sputtering technique for 8 minutes at 1.1 LV in an argon environment.

Spreadability

The ability to spread is an important quality of a good powder. The phrase "spread zone" is used to describe the region over which a gel can be administered without difficulty when treating skin or other affected areas. The medical effectiveness of a formulation is influenced by its spreadability value. After a minute, the diameter (1 gramme) of the gel that had spread

across two 20-by-20-inch plates was measured. When conducting spreading tests, a standard weight of 125 g was placed on the top plate. In order to get an average size for the spread circle in centimetres [10], three independent calculations were done.

Entrapment efficiency

For this dissolution, 10 ml of DMSO solvent was employed. Microcentrifuge centrifugation at 5,000 rpm for 15 minutes was employed on 1 gramme of microspheres. To make 10 ml, DMSO was added to a 1 ml sample of the supernatant. Using a UV spectrophotometer (Shimadzu 1800), it was compared to a blank/control sample of DMSO. Quantities of both EE and drugs were determined [11].

% Drug content

A measured volume of the formulation was dissolved with ethanol in a 10 ml volumetric flask. After three minutes of sonication at room temperature, the absorbance of the resultant solution was measured at a maximum of 240 nm against a blank [12].

In-vitro drug release

Microspheres were embedded in a Franz diffusion cell membrane. At 37 degrees Celsius, 15 millilitres of phosphate-buffered saline (PBS) with a pH of 7.4 is placed in the diffusion cell's receiver section and stirred continuously with a magnetic stirrer. A 3ml sample is taken and replaced at regular intervals of 1, 2, 3, 4, 6, and 12 hours. They are refrigerated until after the analysis has been completed. UV-visible spectrophotometer analysis is performed on the samples to reveal their composition. Measurements of drug concentration are made at a wavelength of 270 nm [13].

Swelling index

Microspheres were measured (W), then placed in an agar gel plate containing 2% w/v agar and incubated for one hour at 37°C. Every hour, for up to three hours, the microsphere was removed from the petri dish and carefully blotted to remove any extra surface water. After reweighing the bloated area (W1), we may apply the formula to get the swelling index:

$$\text{swelling Index} = W1 - W / W\ 100$$

Stability profile

The drug's stability is measured by creating a stability profile under varying conditions of temperature, humidity, and light. Microspheres are tested for stability for up to 2 years, as recommended by the ICH. Ambient (252°/605% RH), refrigerator (53°), and freezer (-205°) conditions were used for storage. Microspheres are stored in airtight, glass vials until it is time to use. At regular intervals, samples are obtained and examined [14][15].

RESULTS AND DISCUSSION

Pre-formulation profile

Solubility

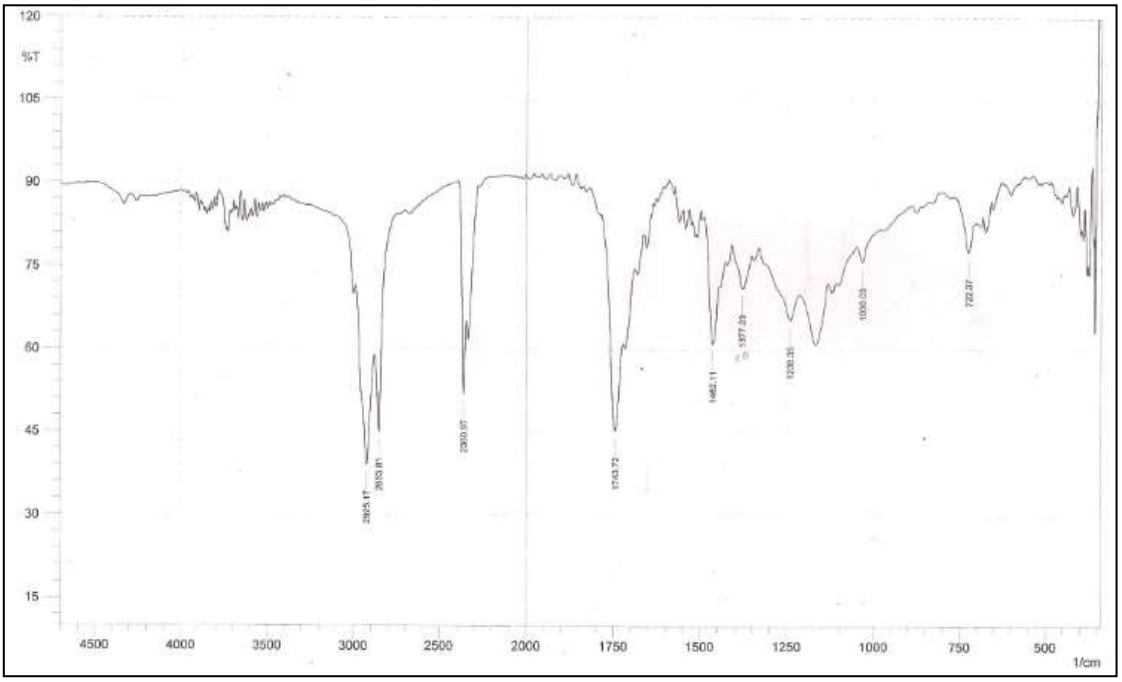
The rabeprazole sodium was confirmed for solubility in various solvents as enumerated below. It was observed soluble in various solvents i.e., ethanol, methanol, NaOH and phosphate buffer; partially soluble in 0.1N HCl and slightly soluble in distilled water. So, it may confirm that rabeprazole is not hydrophilic drug and soluble in ethanol.

Table 2. Solubility of rabeprazole extract

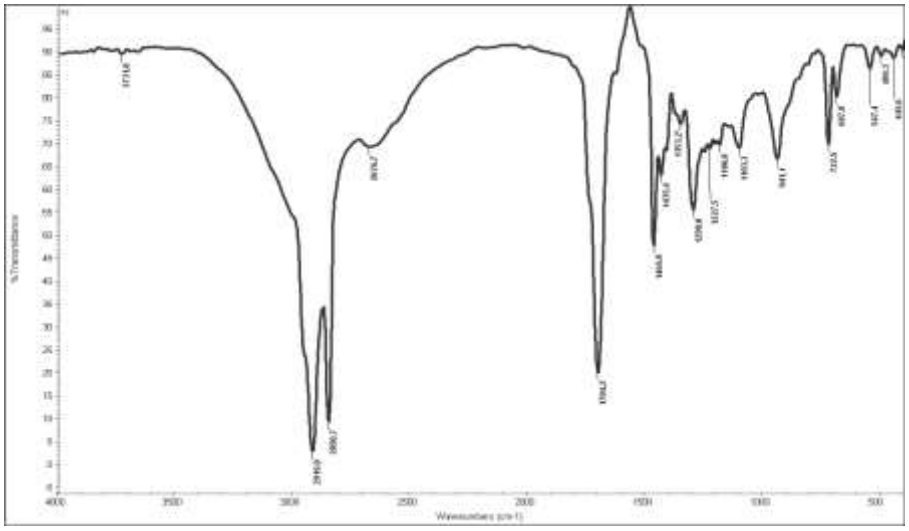
Solvent	Rabeprazole sodium
Ethanol	Soluble
Methanol	Soluble
Dichloromethane	Soluble
0.1N HCl	Partially soluble
Distilled water	Slightly soluble
O.1N NaOH	Soluble
Phosphate Buffer	Soluble

Drug-excipients compatibility

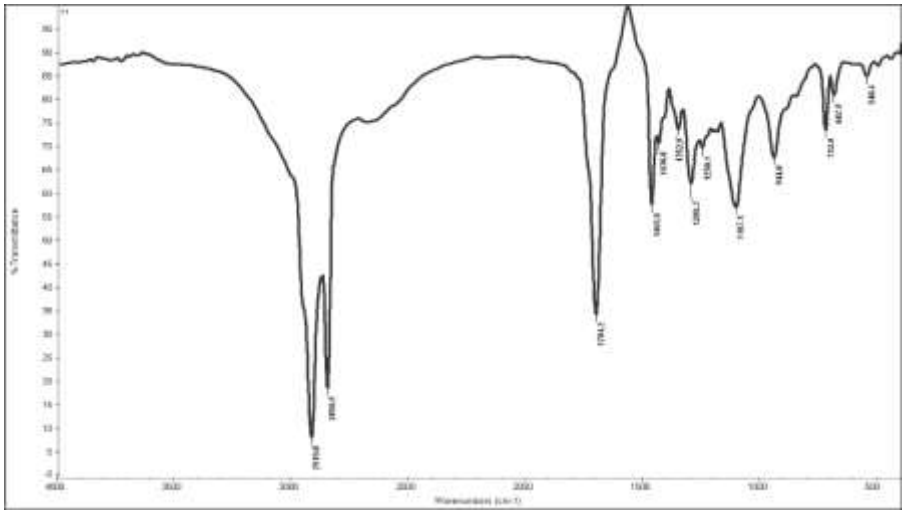
The extract-excipient compatibility investigations including rabeprazole sodium were also conducted, with the FT-IR spectrum being used both singly and in combination.



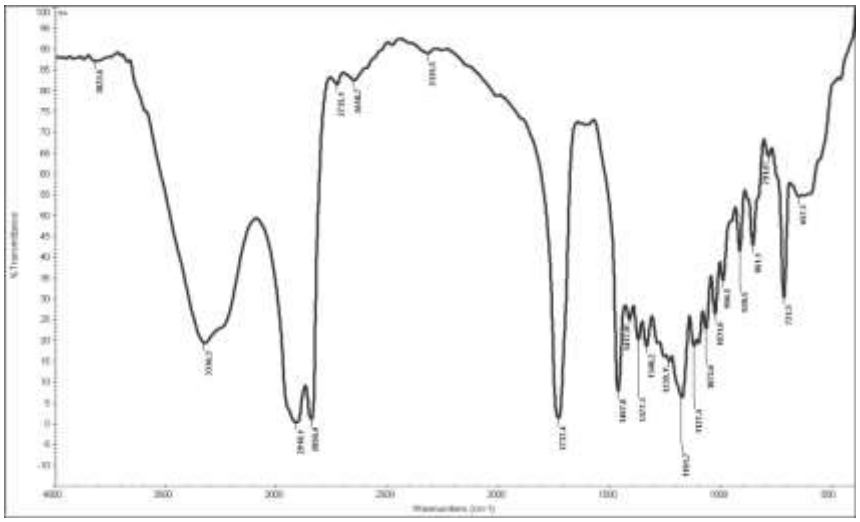
FTIR spectra of Rabeprazole sodium



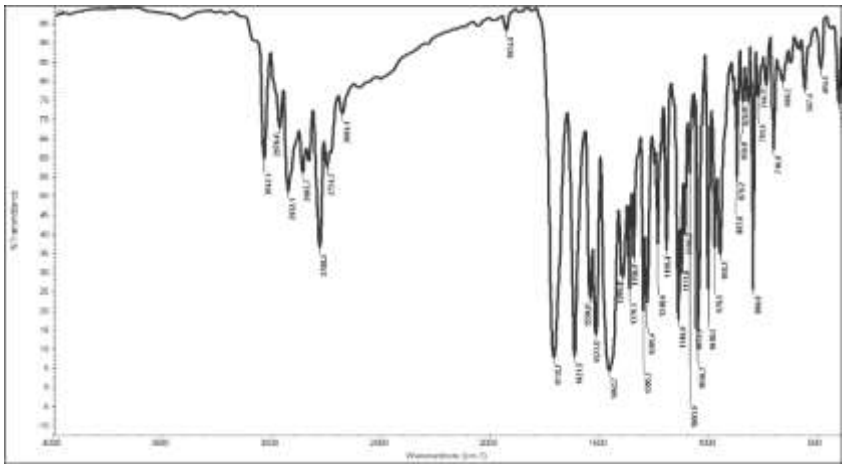
FTIR spectra of Rabeprazole sodium + HPMC



FTIR spectra of Rabeprazole sodium + EC



FTIR spectra of Rabeprazole sodium + Dichloromethane



FTIR spectra of Rabeprazole sodium + PVA

Fig 2. FTIR spectra of rabeprazole-compatibility

Rabeprazole sodium microspheres spectra were compared, and it was found that there were no appreciable changes or functional peak losses.

Standard calibration curve- Rabeprazole sodium

UV-spectrophotometric analysis was used to look at rabeprazole sodium. A PBS (pH 7.4) containing a trace amount of methanol was used to measure the drug's absorbance at 274 nm. From zero to concentrations between 2 and 10 g/ml, the rabeprazole standard curve in PBS at pH 7.4 followed a straight line. The curve conforms to Beer-Lambert's law.

Table 3. Std. calibration curve- Rabeprazole sodium

Conc. (µg/ml)	O. D.
10	0.17
20	0.21
30	0.28
40	0.34
50	0.43
60	0.48

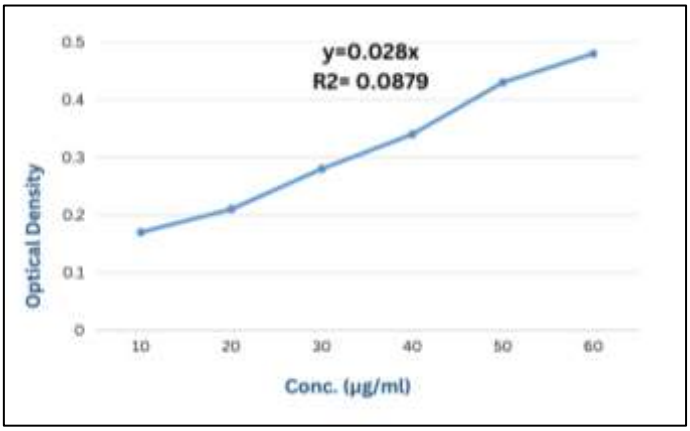


Fig 3. Standard calibration curve at pH 7.4

CHARACTERIZATION (IN-VITRO)

Percentage yield

The % yield demonstrates that how the formulations were efficiently developed using diverse and compatible polymers. % yield was observed maximum in formulation M1 and M4 in contrast to others.
The % yield was showed in following table-

Table 4. % yield of microspheres

Formulation	% yield
M1	92.15
M2	89.42
M3	87.75
M4	93.11
M5	88.53
M6	86.48

Determination of density

Microspheres were estimated for their density in terms of bulk and tapped. Bulk density was estimated as 0.350, 0.372, 0.382 and 0.427 in the M1, M2, M3 and M4 formulations, respectively. However, tapped density was estimated as 0.521, 0.539, 0.513 and 0.546, in the M1, M2, M3 and M4 formulations, respectively.

Table 5. Density of microspheres

Formulation	Density	
	Bulk	**Tapped**
M1	0.350	0.521
M2	0.372	0.539
M3	0.382	0.513
M4	0.459	0.546
M5	0.392	0.529
M6	0.451	0.548

Determination of flow properties

Flow property is an essential property of microspheres or powders. Angle of repose was observed as 29.91±0.82 and 31.29±0.14 in the formulations M4 and M5, respectively. Carr's index was calculated as 12.67±0.62, 12.29±0.53, 13.56±0.38 and 14.56±0.92 in M1, M4, M5 and M6, respectively. Hausner's ratio was found maximum in M5 and M6 as 1.41±0.06 and 1.62±0.03, respectively.

Table 6. Flow properties of microspheres

Formulation	Flow properties		
	Angle of repose	**Carr's index**	**Hausner's ratio**
M1	25.54±0.43	12.67±0.62	1.38±0.07
M2	28.23±0.31	13.72±0.47	1.20±0.04
M3	27.12±0.52	11.34±0.25	1.78±0.02
M4	29.91±0.82	12.29±0.53	1.29±0.01
M5	31.29±0.14	13.56±0.38	1.41±0.06
M6	26.78±0.67	14.56±0.92	1.62±0.03

Drug entrapment

Drug entrapment was observed as 83.48±1.56, 74.19±2.23, 79.37±1.85, 87.12±2.59, 77.48±2.37 and 86.48±2.11, in the M1, M2, M3, M4, M5 and M6, respectively.

Table 7. Drug entrapment

Formulation	Drug entrapment
M1	83.48±1.56
M2	74.19±2.23
M3	79.37±1.85
M4	87.12±2.59
M5	77.48±2.37
M6	86.48±2.11

Determination of particle size

Optimum particles size was noted for all the formulation of microspheres. Where, M1, M4 and M6 showed less particles size as 18.29±0.12µm, 19.59±0.39µm and 19.12±0.40µm, respectively.

Other formulations showed comparatively increased particles size that reduces the surface area.

Table 8. Particle size

Formulation	Particle size (µm)
M1	18.29±0.12
M2	22.26±0.31
M3	20.29±0.10
M4	19.59±0.39

M5	23.92±0.20
M6	19.12±0.40

Swelling index

It has demonstrated a remarkable swelling property when observed. Min. swelling index was seen in M1 (76%) whereas maximum swelling index was calculated in M5 (92%), M6 (90%) and M4 (86%). This power exhibits the concentration of polymers used for the development of microspheres.

Below table represents the swelling power the formulations-

Table 9. Swelling index (%)

Formulation	Swelling index (%) after 8 hours
M1	76
M2	83
M3	78
M4	86
M5	92
M6	90

Determination of % Drug content

Better drug homogeneity and concentration were observed in the produced microsphere, indicating a high drug content of percentage. The maximum % drug content was observed as 92.38±0.43%, 89.29±0.38% and 91.33±0.21% in M1, M5 and M6, respectively. Indicative of consistent medication delivery, all formulations showed a sizable percentage of drug release.

Table 10. % Drug content

Formulation	% Drug content
M1	92.38±0.43
M2	82.22±0.17
M3	78.58±0.20
M4	81.78±0.37
M5	89.29±0.38
M6	91.33±0.21

Measurements of pH

The pH was checked to ensure it was well tolerated and absorbed. The pH was observed as 7.3±0.2, 7.2±0.2, 7.3±0.2, 7.4±0.3, .3±0.2 and 7.6±0.3, in the formulations M1-M6, respectively. Thus, all the preparations showed alkaline pH range.

Table 11. pH range

Formulation	pH± S.D.
M1	7.3±0.2
M2	7.2±0.2
M3	7.3±0.2
M4	7.4±0.3
M5	7.3±0.2
M6	7.6±0.3

5.2.8 Spreadability

The spreadability data showed a remarkable strength when observed. Spreadability was seen as 12.29±0.10 g.cm/s, 12.82±0.23 g.cm/s and 13.50±0.23 g.cm/s in M2, M4 and M6, respectively.

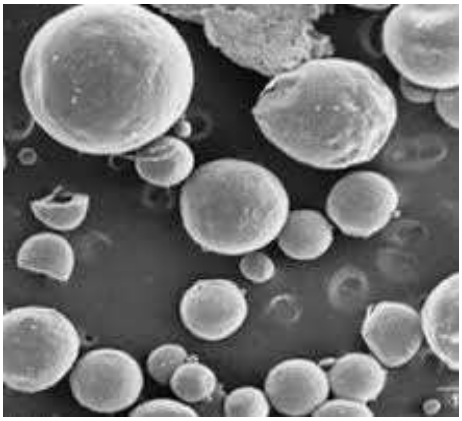
Table 11. shows the spreadability-

Table 12. Spreadability of microspheres

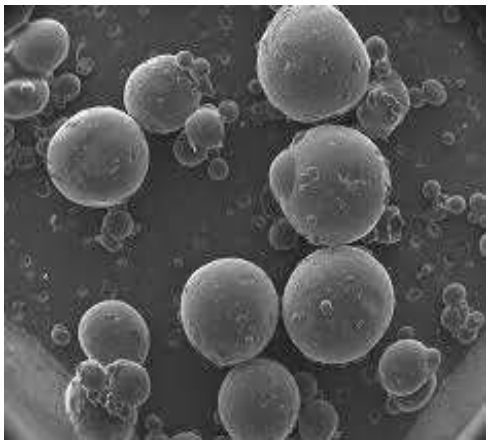
Formulation	Spreadability (g.cm/s)
M1	11.49±0.28
M2	12.29±0.10
M3	13.34±0.22
M4	12.82±0.23
M5	11.62±0.27
M6	13.50±0.23

SEM determination

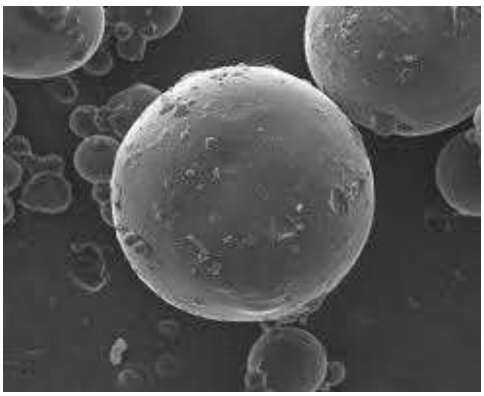
All the formulations of microspheres were determined for SEM. In this parameter, M1- M4 demonstrated an almost near analysis of pictures when observed in SEM but M5 and M6 were not optimized as they shown with some deviation.



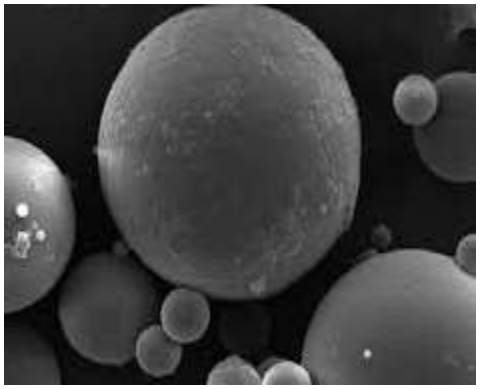
M1



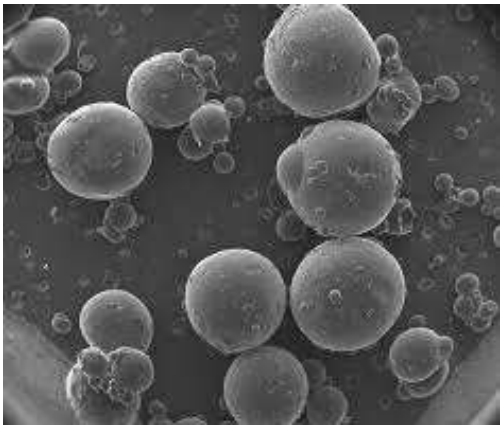
M2



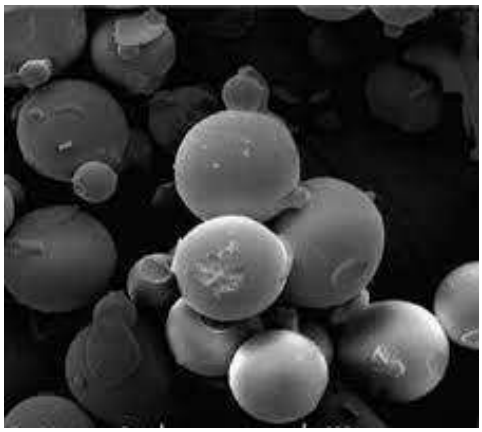
M3



M4



M5



M6

Fig 4. Depiction of SEM determination of microspheres

Estimation of % drug release

The % drug release was calculated for 1 hour to 6 hours when kept in dissolution. At 6 hours, the % drug release was observed maximum as 91.6±0.81%, 93.8±0.34%, 90.4±0.33%, 92.8±0.81%, 91.3±0.27% and 92.5±0.52%, respectively.

Table 13. % Drug release in microspheres

Time (hr)	% drug release ± S D					
	M 1	**M 2**	**M 3**	**M 4**	**M 5**	**M 6**
1	34.3±0.37	33.5±0.60	32.7±0.45	34.3±0.46	32.6±0.37	34.4±0.49
2	38.7±0.55	39.8±0.72	38.5±0.34	39.7±0.65	40.8±0.58	41.9±0.55
3	57.5±0.42	55.2±0.63	58.4±0.23	55.8±0.24	56.9±0.13	57.8±0.74
4	68.4±0.89	68.0±0.86	67.8±0.92	69.7±0.92	66.8±0.67	67.5±0.53
5	83.7±0.32	83.2±0.92	81.6±0.37	84.5±0.73	82.7±0.45	84.8±0.21
6	91.6±0.81	93.8±0.34	90.4±0.33	92.8±0.81	91.3±0.27	92.5±0.52

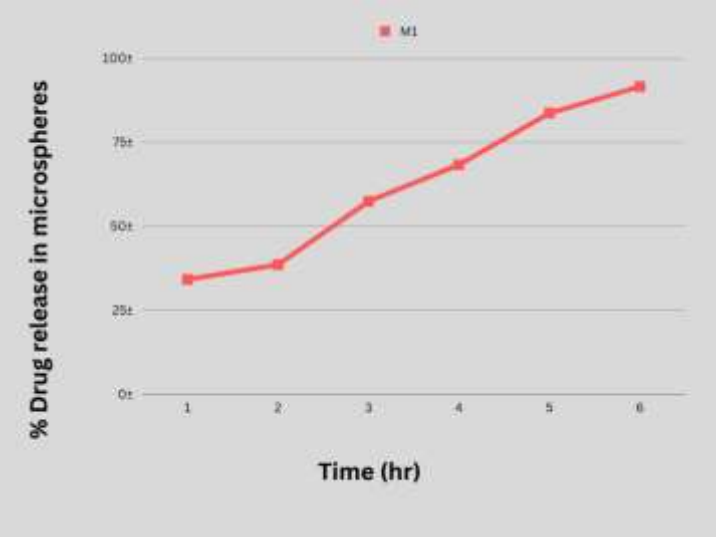


Fig 5. Graphical data of drug release (M1)

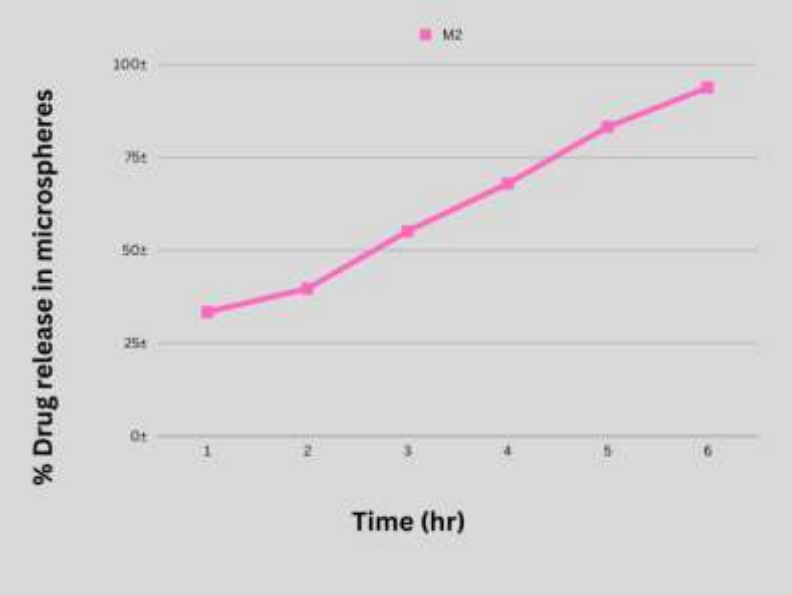


Fig 6. Graphical data of drug release (M2)

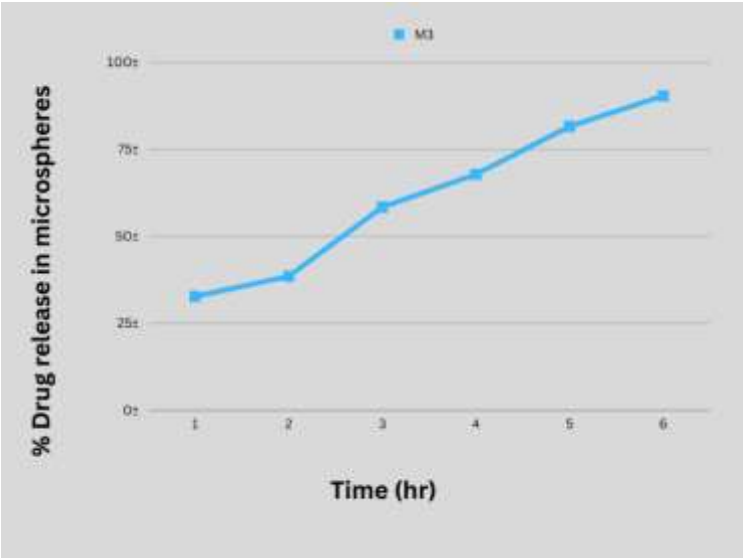


Fig 7. Graphical data of drug release (M3)

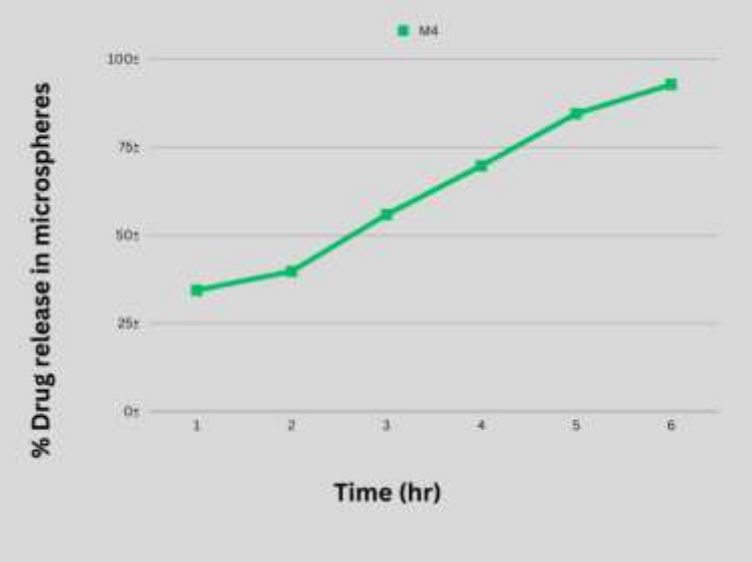


Fig 8. Graphical data of drug release (M4)

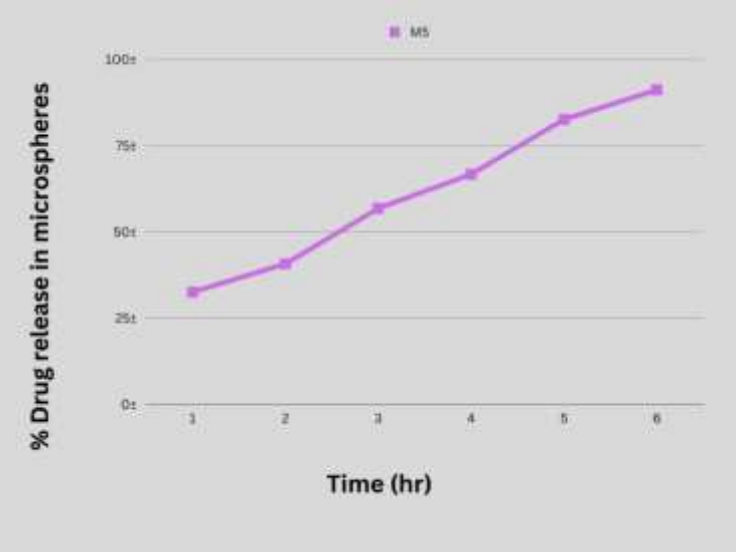


Fig 9. Graphical data of drug release (M5)

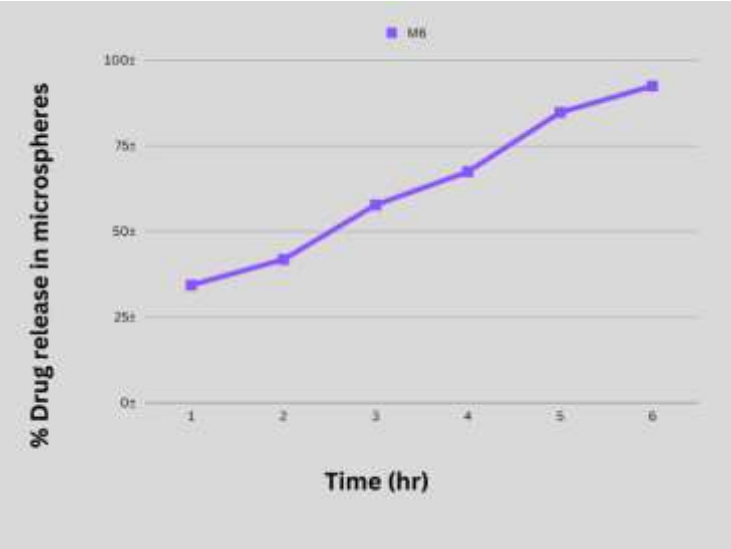


Fig 10. Graphical data of drug release (M6)

Stability

After 1 month storage the formulations were tested for their stability profile. This indicated that all the formulations were found stable with a negligible change in their pH and in-vitro drug release.

However, formulations M3, M4 and M6 were found more stable when compared with others in terms of their characterization parameters.

Stability measures, such as pH, % drug content, and in-vitro drug release, were nearly unchanged after 30 days. Thus, the M1-M6 microspheres exhibited good stability strengths across all of the selected stability parameters, with the M1, M4 and M6 formulations having the most noticeable influence.

In results, microspheres were shown to have remarkable formulization properties throughout the study. It has shown for optimum angle of repose, Carr's Index etc. Microspheres M1 and M4 showed better drug release and stability and thus considered as optimized formulation.

CONCLUSION

Patients from high-risk groups can also benefit from nanocarrier-based delivery methods. Precision medication delivery through inflammatory barriers is a major challenge, but vesicular methods and lipophilic nanocarriers show great promise. nanovesicles that get along well with lipids. Substances like solid lipid nanocarriers and nano emulsions are used to improve drug loading because they stabilise the environment and help with dosage reduction by increasing the solubility of poorly water-soluble drugs. Liposomes have several applications due to their adaptability in formulation, surface modification, and drug encapsulation. However, lipid oxidation can easily break it down because lipids make up the bulk of it. Niosomes are a viable alternative to liposomes due to the surfactants they include, which make them far more stable than lipids.

In conclusion, microspheres of rabeprazole sodium found as optimized formulation in terms of particle size, % drug content, stability etc. It might be effective externally in the cure of fungal infections due to its already proved antibacterial efficacy.

Future aspect

It suggests to fellow researchers to determine the pharmacokinetic profile of formulated microspheres of rabeprazole sodium. It is well known proton pump inhibitor that is utilizing worldwide. It would be a promising dosage form in the coming era of research.

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