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FORMULATION AND EVALUATION OF RABEPRAZOLE MICROSPHERES

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ABSTRACT

Microspheres can be tailored to provide targeted and/or sustained release in different parts of the body, including those of eye, nasal cavity, urinary, colon and gastrointestinal tract, thus offering the possibilities of localized as well as systemic controlled release of drugs. Prolonged release of drugs and a reduction in frequency of drug administration can highly improve the patient compliance. Recent advances in targeted drug delivery and sustained release of drug uses this mechanism even for the delivery of protein and peptide drugs, antigens for vaccination and plasmid DNA for gene therapy. In the present work we have developed the formulation of microspheres for an anti-ulcer drug Rabeprazole which has very less plasma half life 0.85 hrs, hence it is necessary to develop the formulation which will provide the sustained release of the drug thereby reducing the dose of the drug. We developed and characterized the microsphere formulation which improved the efficacy of the drug and hence reduce the side effects. Characterization also showed that there is no drug excipients interaction. The present study was planned to prepare microspheres for sustained release of using Rabeprazole & various cellulose polymers such as Ethyl cellulose, Cellulose acetate phthalate, cellulose acetate by employing solvent evaporation technique. Microspheres were Characterized for the particle size distribution, wall thickness by Scanning electron microscopy (SEM), angle of repose, drug content , bulk density, entrapment efficiency and in vitro dissolution studies. Drug excipients compatibility was determined by FTIR and DTA. Accelerated stability studies were also carried out following ICH Guidelines. SEM shows that microspheres were found spherical in shape and free flowing. The entrapment efficiency and wall thickness was found in between 68.85% & 42.88%, 120.28µ & 72.32µ respectively. The drug release was extended maximum up to 12 hrs with ethyl cellulose. FTIR and DSC results showed Rabeprazole was compatible with excipients. The curve fitting data shows that the drug release followed first order kinetics, Higuchi's and Peppa's plots stated non-fickian diffusion controlled.

KEY WORDS

Rabeprazole, Microspheres, Sustained Release, Cellulose Acetate Phthalate

INTRODUCTION[1, 2]

Microspheres are characteristically free flowing powders consisting of solid spherical particles of size 1-1000µm.

These are made up of polymeric substance in which the drug is dispersed throughout the particle i.e. internal structure is made up of drug matrix and polymeric excipients. New drug delivery technologies are revolutionizing the drug discovery, development and creating R&D focused pharmaceutical industries to increase the momentum of global advancements. In this regard novel drug delivery systems (NDDS) have many benefits, which includes improved therapy by increasing the efficacy and duration of drug activity, increased patient compliance through decreased dosing frequency and convenient routes of administration and improved site specific delivery to reduce unwanted adverse effects. The concept of the advanced drug delivery systems especially those offering a sustained and controlled action of drug to

desired area of effect, attained great appeal for nearly half a century. However, prior to advent of improved alternate methods, drug delivery systems were considered only as a means of getting the drug into the patient's body. Actual practice of controlled release begin with advent of timed release coating to the pills or solid drug particles in order to mask their unacceptable taste or make them more palatable.

Between 1940s and 1960s, the concept of chemical microencapsulation technology begin as an alternative means of delivering drugs. In continued quest for the more refined system, in 1980s polymer/membrane technology came to be known at for front. Further, the process of targeting and site specific delivery with absolute accuracy can be achieved by attaching bioactive molecule to liposome's, bio erodible polymer, implants, monoclonal antibodies and various particulate carriers (E.g., nanoparticles and microspheres, etc.).

The micro particulate delivery systems are considered and accepted as a reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effect(s).The term microcapsule is defined as a spherical particle with size varying from 50nm to 2mm, containing a core substance. Microspheres are, in strict sense, spherical empty particles. However, the terms microcapsules and microspheres are often used synonymously. In addition, some related terms are used as well. For example, essentially "micro beads" and "beads" are used alternatively. Spheres and spherical particles are also used for a large size and rigid morphology. The microsphere are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature, and ideally having a particle size less than 100 mm.

Ideal Characteristics of Drug:

Particle Size Requirement: Lower the molecular weight the drugs having size 150-600 Daltons can easily diffuse through the membrane, but diffusivity (the ability of drug to diffuse through the membrane) is inversely related to molecular size.

There should be no toxic product associated with the final product.

No Stability Problem: The drugs that are unstable in gastro-intestinal environment cannot be administered as oral controlled release formulation because of bioavailability problems e.g. nitroglycerine.

The drug or protein should not be adversely affected by the process.

Therapeutic Range: A candidate drug for controlled drug delivery system should have a wide therapeutic range such that variations in the release rate do not result in a concentration beyond this level.

Therapeutic Index: The ratio of maximum safe concentration to the minimum effective concentration of drug is called as therapeutic index. The release rate of drug with narrow therapeutic index should be such that the plasma concentration attained between the therapeutically safe and effective range. It is necessary because such drugs have toxic concentration nearer to their therapeutic range.

Elimination Half Life: Smaller the half life larger the amount of drug to be incorporated in the controlled release dosage form. Drugs with $t_{1/2}$ in the range of 1 to 4 hours make a good candidate for such a system. E.g. Propranolol.

Plasma Concentration-Response Relationship: Drugs whose pharmacological activity is independent of its concentration are poor candidates for controlled release systems.

Prerequisites for Ideal Micro Particulate Carriers: The material utilized for the preparation of microspheres should ideally fulfill the following prerequisites: Control of content release, Protection of drug, Longer duration of action, Increase of therapeutic efficiency, Reduction of toxicity, Sterilizability, Bioresorbability, Relative stability, Biocompatibility, Water solubility, Polyvalent,

Advantages:

Reliable means to deliver the drug to the target site with specificity, if modified, and to maintain the desired concentration at the site of interest without untoward effects.

Solid biodegradable microspheres have the potential throughout the particle matrix for the controlled release of drug.

The size, surface charge and surface hydrophilicity of microspheres have been found to be important in determining the fate of particles in vivo.

Studies on the macrophage uptake of microspheres have demonstrated their potential in targeting drugs to pathogens residing intracellularly.

Blood flow determination:

Relatively large microspheres (10-15 µm in diameter) are useful for regional blood flow studies in tissues and organs. In most cases the microspheres are injected at desired locations in the circulatory system and eventually lodge in the capillaries. The microspheres and fluorescent dyes they contain are first extracted from the tissue sample, and then fluorescence is quantitated on a Spectro fluorometer or fluorescence micro plate reader. Traditionally, this type of study has been carried out using radiolabelled microspheres; however fluorescent microspheres have been shown.

They facilitate accurate delivery of small quantities of potent drug and reduced concentration of drug at site other than the target organ or tissue. They provide protection for unstable drug before and after administration, prior to their availability at the site of action.They provide the ability to manipulate the in vivo action of the drug, pharmacokinetic profile, tissue distribution and cellular interaction of the drug. They enable controlled release of drug. Ex: narcotic, antagonist, steroid hormones.

Preparation Methods: The choice of the technique mainly depends on the nature of the polymer used, the drug, the intended use and the duration of therapy. The preparation methods should satisfy certain criteria:

1) Solvent evaporation or extraction 2) Spray drying 3) Single emulsion technique 4) Double emulsion technique 5) Polymerization technique 6) Phase separation.

Solvent Evaporation: In this method of preparation the drug and polymer should be soluble in organic solvent (methylene chloride).The solution containing drug and polymer is dispersed in an aqueous phase to form droplets. Continuous mixing and elevated temperature is employed to evaporate more volatile organic solvents and to leave the solid polymer-drug particles suspended in an aqueous medium. The particles are finally filtered and washed thrice with double distilled water.**Fig.No.1**

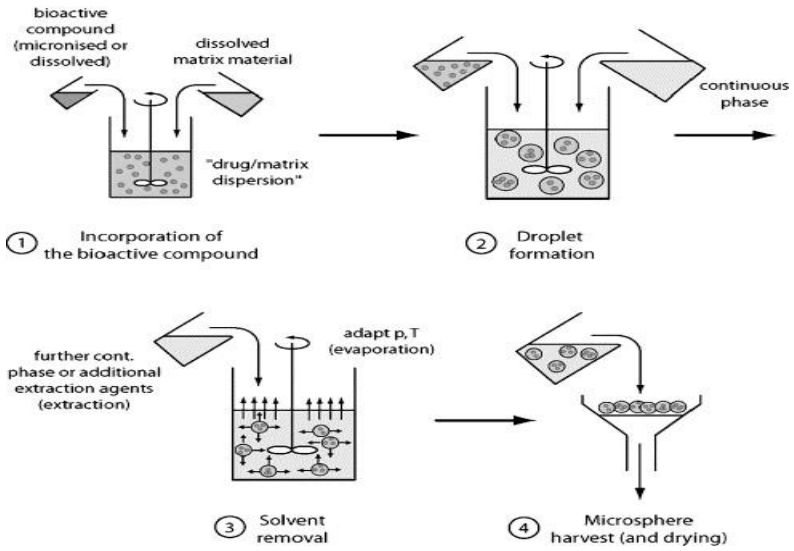


Fig: 1. Solvent evaporation

CHARACTERIZATION:

The characterization of a micro particulate carrier is an important phenomenon which helps to design a suitable carrier for the drug, proteins or antigen delivery. The microspheres have different microstructure that determines the release and stability of the carrier.

Particle Size and Shape: The size and the size distribution of microspheres were measured by particle size analyzer. The shape and surface morphology of the prepared microspheres were examined with scanning electron microscope.

Measurement of Micromeritic Properties: The flow properties of prepared microspheres were

investigated by measuring the bulk density, tapped density and Carr's index.

X-Ray Powder Diffractometry (X-Rd): X-ray powder Diffractometry was carried out to investigate the effect of microencapsulation process on crystallinility of drug.

Angle of Contact: The angle of contact is measured to determine the wetting property of a micro particulate carrier. It determines the nature of microspheres in terms of hydrophilicity or hydrophobicity. This thermodynamic property is specific to solid and affected by the presence of the adsorbed component. The angle of contact is measured at the solid/air/water interface.

Loose Surface Crystal Study: The prepared microspheres were evaluated to observe the excess drug present on the surface of microspheres.

Isoelectric Point: Micro electrophoresis apparatus is used to measure elecrophoretic mobility of microspheres from which isoelectric point can be determine. It can be correlated to surface charge or ion adsorption of microspheres.

Density Determination: Density measured by using a multivolume pychometer.

Electron Spectroscopy for Chemical Analysis: The surface chemistry of the microspheres can be determined using the electron spectroscopy for chemical analysis (ESCA).

Entrapment Efficiency: The entrapment efficiency of the microspheres or the percent entrapment can be determined by allowing washed microspheres to lyse. The lysate is then subjected to the determination of active constituents as per monograph requirement. The percent encapsulation efficiency is calculated using following equation:

% Entrapment = Actual content/Theoretical content x 100

Attenuated Total Reflectance Fourier Transform-Infrared Spectroscopy: FT-IR is used to determine the degradation of the polymeric matrix of the carrier system. The surface of the microspheres is investigated measuring alternated total reflectance (ATR). The IR beam passing through the ATR cell reflected many times through the sample to provide IR spectra mainly of surface material. The ATRFTIR provides information about the surface composition of the microspheres depending upon manufacturing procedures and conditions[3].

In Vitro Methods: There is a need for experimental methods which allow the release characteristics and permeability of a drug through membrane to be determined. For this purpose, a number of in vitro and in vivo techniques have been reported. In vitro drug release studies have been employed as a quality control procedure in pharmaceutical production, in product development etc. Sensitive and reproducible release data derived from physico chemically and hydro dynamically defined conditions are necessary. The influence of technologically defined conditions and difficulty in simulating in vivo conditions has led to development of a number of in vitro release methods for buccal formulations; however no standard in vitro method has yet been developed. Different workers have used apparatus of varying designs and under varying conditions, depending on the shape and application of the dosage form developed

Beaker Method: The dosage form in this method is made to adhere at the bottom of the beaker containing the medium and stirred uniformly using over head stirrer. Volume of the medium used in the literature for the studies varies from 50-500 ml and the stirrer speed from 60-300 rpm.

Interface Diffusion System: This method is developed by Dearden & Tomlinson. It consists of four compartments. The compartment A represents the oral cavity, and initially contained an appropriate concentration of drug in a buffer. The compartment B representing the buccal membrane, contained 1-octanol, and compartment C representing body fluids, contained 0.2 M HCl. The compartment D representing protein binding also contained 1-octanol. Before use, the aqueous phase and 1-octanol were saturated with each other. Samples were withdrawn and returned to compartment A with a syringe.

Modified Keshary Chien Cell: A specialized apparatus was designed in the laboratory. It comprised of a Keshary Chien cell containing distilled water (50ml) at 37°C as dissolution medium. TMDDS (Trans Membrane Drug Delivery System) was placed in a glass tube fitted with a 10 # sieve at the bottom

which reciprocated in the medium at 30 strokes per min.

Dissolution Apparatus: Standard USP or BP dissolution apparatus have been used to study in vitro release profiles using rotating elements, paddle and basket. Dissolution medium used for the study varied from 100- 500 ml and speed of rotation from 50-100 rpm.

Other Methods: Few other methods involving plexi glass sample blocks placed in flasks, agar gel method, Valia-Chein cell USP n2 III dissolution apparatus, etc have also been reported. Although a number of methods have been reported, the ideal method would be one where sink condition is maintained and dissolution time in vitro simulates dissolution time in vivo

In Vivo Methods: Methods for studying the permeability of intact mucosa comprise of techniques that exploit the biological response of the organism locally or systemically and those that involve direct local measurement of uptake or accumulation or penetrate at the surface. Some of the earliest and simple studies of mucosal permeability utilized systemic pharmacological effects produced by drugs after application to the oral mucosa. However the most widely used methods include in vivo studies using animal models, buccal absorption tests, and perfusion chambers for studying drug permeability[4]

Animal Models: Animal models are used mainly for the screening of the series of compounds, investigating the mechanisms and usefulness of permeation enhancers or evaluating a set of formulations. A number of animal models have been reported in the literature, however, very few *in vivo* (animal). Animal models such as the dog, rats, rabbits, cat40, hamster, pigs, and sheep have been reported. In general, the procedure involves anesthetizing the animal followed by administration of the dosage form. In case of rats, the esophagus is ligated to prevent absorption pathways other than oral mucosa. At different time intervals, the blood is withdrawn and analyzed[5].

Buccal Absorption Test: The buccal absorption test was developed by Beckett & Trigg's in 1967. It is a simple and reliable method for measuring the extent of drug loss of the human oral cavity for single and multi component mixtures of drugs. The test has been successfully used to investigate the relative importance of drug structure, contact time, initial drug concentration and pH of the solution while the drug is held in the oral cavity.

In Vitro-In Vivo Correlation: Correlations between in vitro dissolution rates and the rate and extent of availability as determined by blood concentration and or urinary excretion of drug or metabolites are referred to as "in vitro-in vivo correlations". Such correlations allow one to develop product specifications with bioavailability.

In-Vitro Release Studies:

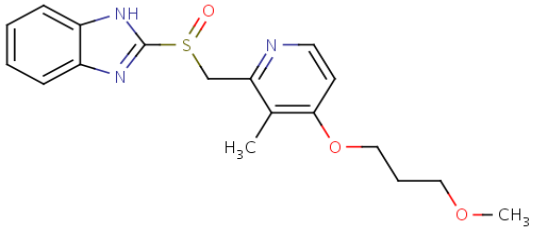
The release rate of floating microspheres was determined in a United States Pharmacopoeia (USP) XXIII basket type dissolution apparatus. A weighed amount of floating microspheres equivalent to 50 mg drug was filled into a hard gelatin capsule (No. 0) and placed in the basket of dissolution rate apparatus. Five hundred milliliters of the SGF containing 0.02% w/v of Tween 20 was used as the dissolution medium. The dissolution fluid was maintained at 37 ± 1° at a rotation speed of 100 rpm. Perfect sink conditions prevailed during the drug release study. 5ml samples were withdrawn at each 30 min interval, passed through a 0.25 µm membrane filter (Millipore), and analyzed using LC/MS/MS method to determine the concentration present in the dissolution medium. The initial volume of the dissolution fluid was maintained by adding 5 ml of fresh dissolution fluid after each withdrawal. All experiments were run in triplicate[6].

In-Vivo Studies:

The in-vivo floating behavior can be investigated by X-ray photography of hollow microspheres loaded with barium sulphate in the stomach of beagle dogs. The in-vitro drug release studies are performed in a dissolution test apparatus using 0.1N hydrochloric acid as dissolution media. The in-vivo plasma profile can be obtained by performing the study in suitable animal models (e.g. beagle dogs) [7.]

RABEPRAZOLE:

Rabeprazole is an antiulcer drug in the class of proton pump inhibitors. It is a pro drug – in the acid environment of the parietal cells it turns into active sulphenamide form. Rabeprazole inhibits the H^+/ K^+ ATPase of the coating gastric cells and dose-dependent oppresses basal and stimulated gastric acid secretion.



Chemical 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**: 359.443, **Category**: Anti-Ulcer

Mechanism of Action:

Rabeprazole belongs to a class of antisecretory compounds (substituted benzimidazole proton-pump inhibitors) that do not exhibit anticholinergic or histamine H2-receptor antagonist properties, but suppress gastric acid secretion by inhibiting the gastric H^+/K^+ATPase (hydrogen-potassium adenosine triphosphatase) at the secretary surface of the gastric parietal cell. Because this enzyme is regarded as the acid (proton) pump within the parietal cell, Rabeprazole has been characterized as a gastric proton-pump inhibitor. Rabeprazole blocks the final step of gastric acid secretion. In gastric parietal cells, Rabeprazole is protonated, accumulates, and is transformed to an active sulfonamide. When studied in vitro, rabeprazole is chemically activated at pH 1.2 with a half-life of 78 seconds.

Physical and Chemical Properties: Color: White to Yellowish Crystalline.Form: Powder, Odor: odorless, State: solid, Bulk density: 1.308g/cm^3 (20 C), Solubility:3.36e-01 g/l, Partition coefficient: 1.83, Dissociation constant: 8.91 (pKa), Melting temperature: 99 to 100°c

Pharmacokinetic parameters: C_{max} (µg/ml): 3.99, $t_{max:}$ 3.5 Hrs, $t_{1/2}$ (half life): 0.7 – 1.5 Hrs, AUC_{0-t} (µg.h/ml): 7.29, $AUC_{0-\infty}$ (µg.h/ml): 7.40.

Uses: Rabeprazole is used for treating ulcers of the stomach and duodenum, erosive or ulcerative gastro esophageal reflux disease (GERD) and Zollinger-Ellison Syndrome (in which there is overproduction of acid caused by tumors). It also is used with antibiotics for eradicating Helicobacter infections of the stomach that, along with acid, are responsible for many ulcers.

Materials Used: Rabeprazole(Nishka labs, Hyderabad)Ethyl cellulose, HPMC, Dichloromethane, Poly vinyl alcohol, Polyethylene glycol, Hydrochloric Acid, Potassium Dihydrogen Orthophosphate, Sodium hydroxide (S.D Fine Chemicals Pvt Ltd Mumbai)

Equipments Used: Magnetic Stirrer (Digisun electronics), Differential Scanning Calorimetry(SII Nano Technology, Japan), Fourier transform infrared spectroscopy(Thermo Fischer), UV-spectrophotometer(Model-1800, Shimadzu, Japan), Particle size analyzer(Micro track)

Preparation of Microspheres[8, 9, 10]: The microspheres were prepared by (o/w) solvent evaporation method, since Rabeprazole is a slightly water–soluble drug. Polymers ethyl cellulose and HPMC were dissolved in 20ml of dichloromethane. These polymers and drug are mixed vigorously to form a clear solution. Then 0.1% of polyethylene glycol was added which acts as a surfactant. Then the above solution was emulsified by adding drop by drop into the aqueous solution containing 160 ml of 0.46% w/v of PVA as an emulsifier. Dichloromethane was removed at 35°C by evaporation. As the solvent was being removed, the emulsifier continued to maintain the oil droplets in their spherical configuration and prevented from aggregating until the solvent was completely removed, and the microspheres were hardened as discrete particles. Finally, the hardened microspheres were washed with distilled water for 5 times and dried. **Fig No: 2**

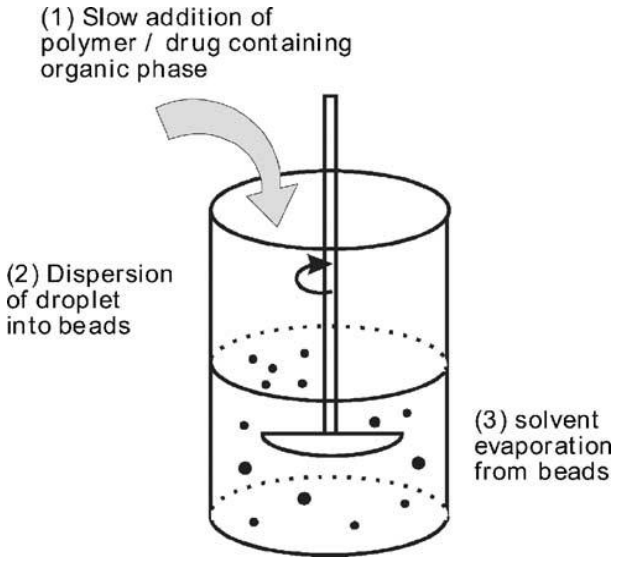


Fig: 2. Process of Microsphere Preparation

Table No-1. Formulation Table for Rabeprazole Microspheres

S.No	Drug (g)	Ethyl Cellulose (g)	HPMC (g)	DCM (ml)	PVA (0.46% w/v)	SPEED (rpm)
1	0.5	2	1	20	160 ml	800
2	0.5	1	2	20	160 ml	800

*Dichloromethane (DCM), *Polyvinyl alcohol (PVA)

Preparation Of 0.1N HCL (pH-1.2): 9ml of hydrochloric acid was dissolved in 1000ml of volumetric flask with distilled water up to the mark**.** Then the pH was adjusted to 1.2.

Preparation Of pH 7.4 Phosphate Buffer: Phosphate buffer was prepared by placing 25 ml of 0.2M potassium dihydrogen orthophosphate solution and 19.55 ml of 0.2N sodium hydroxide solution in a 100 ml volumetric flask and then distilled water was added to make up the volume. The pH was found to be 7.4±0.1.

RESULTS AND DISCUSSION

Micromeritic Properties[11, 12]:

Bulk Density: Accurately weighed microspheres were transferred into 10ml of measuring cylinder and the volume occupied by the powder was noted. The bulk density was calculated in g/mm by the following formula; Bulk density (ρ0) =M/V_0

Where M= mass of the powder, V_0 =volume of the powder.

Angle of Repose: Angle of repose was determined using fixed funnel method. A glass funnel is held in place with a clamp on a ring support over a glass plate. Approximately 1 gm of powder is transferred into funnel keeping the orifice of the funnel blocked by the thumb. When the powder is emptied from funnel, the angle of the heap to the horizontal plane is measured.

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

Carr's Index: Based on the apparent bulk density and the tapped density, the percentage compressibility of the powder mixture was determined by the following formula.

$$\text{Carr's index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Bulk density}} \times 100$$

Hausner's Ratio: Hausner's ratio is an indirect index of ease of measuring the powder flow. It is calculated by the following formula.

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

Lower hausner's ratio (<1.25) indicates better flow properties than higher ones (>1.25).

Table No 2. Micromeritic Properties of Microspheres

Formulation code	DERIVED PROPERTIES		FLOW PROPERTIES		
	Bulk density	Tapped density	Angle Of repose	Carr's index	Hausner's ratio
F1	0.436±0.015	0.513±0.025	26.26±0.305	11.9±0.43	1.12±0.015
F2	0.456±0.015	0.53±0.026	28.06±0.40	13.4±0.55	1.13±0.030

Percentage Yield: The microspheres were evaluated for percentage yield. The yield was calculated by

$$\text{Percentage yield} = \frac{\text{weight of microsphere recovered}}{\text{Weight of drug} + \text{weight of polymer}} \times 100$$

Table No-3.Yield of Microspheres

Formulation code	% yield
F1	93.70±1.28
F2	87.82±2.01

Drug Entrapment[13, 14]**:** Drug loaded microspheres (50mg) were powdered and suspended in 5oml of methanol. The resultant dispersion was kept for half hour in sonicator and filtered. From this above solution 1ml was diluted to 25ml with distilled water and analyzed spectrophotometrically at 240nm.

Table No-4.Entrapment Efficiency

Formulation code	Drug entrapment
F1	87.04±1.92
F2	78.68±2.1

Fourier Transform Infrared (FTIR) Spectral Studies[15, 16]**:** FTIR spectra of the Ethyl Cellulose, HPMC, Rabeprazole and Rabeprazole-loaded microspheres were obtained. In order to investigate the possible reaction between Ethyl cellulose and Rabeprazole, Rabeprazole was treated with Ethyl Cellulose. The ratio (mL/mg) and concentration of Ethyl Cellulose as well as Rabeprazole was kept identical to that used in formulations. The time of exposure was also kept identical to that of microsphere preparation, i.e., 2 h. Then, Rabeprazole was washed with double distilled water. After drying, FTIR spectrum was recorded. The samples were crushed with KBr to get pellets by applying a pressure of 600 kg/cm^2 Spectral scans were taken in the range between 4000 and 500 cm−1 on a Nicolet Model Impact 410, Milwaukee, WI, USA) instrument.**Fig.No.3 to 6**

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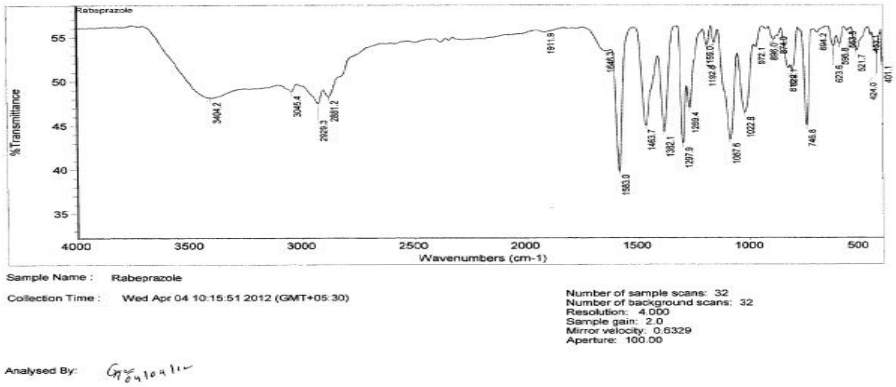

Fig: 3. FTIR OF PURE RABEPRAZOLE

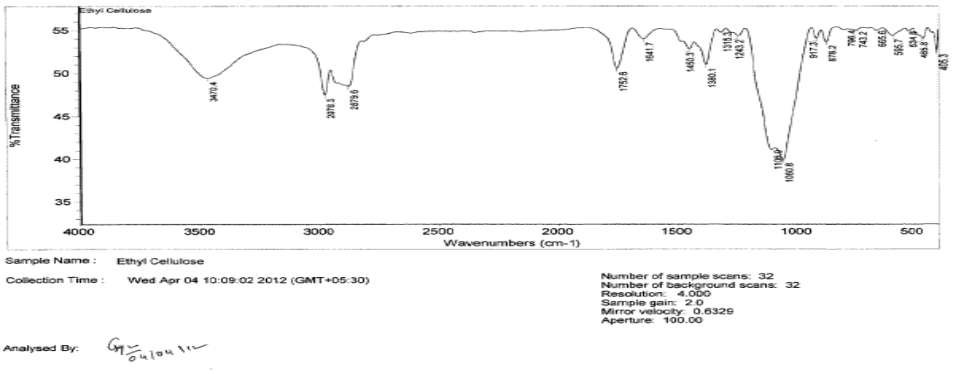

Fig: 4. FTIR OF ETHYL CELLULOSE

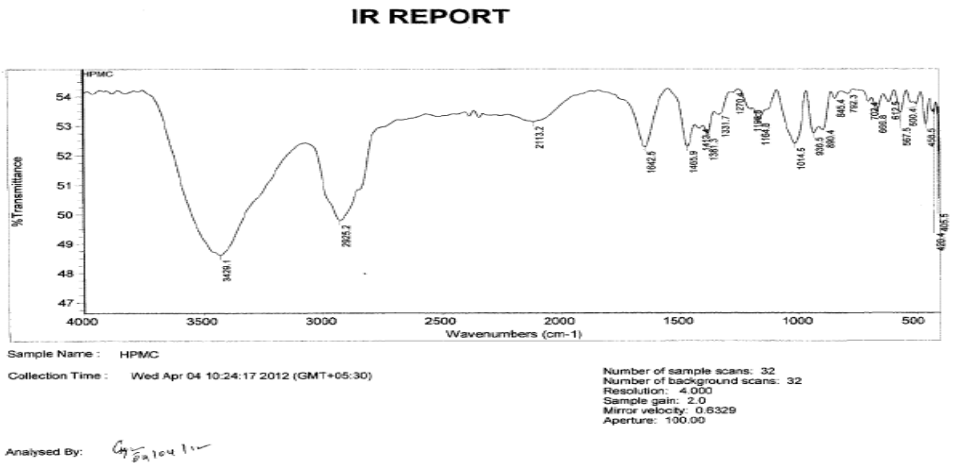

Fig: 5.FTIR OF HPMC

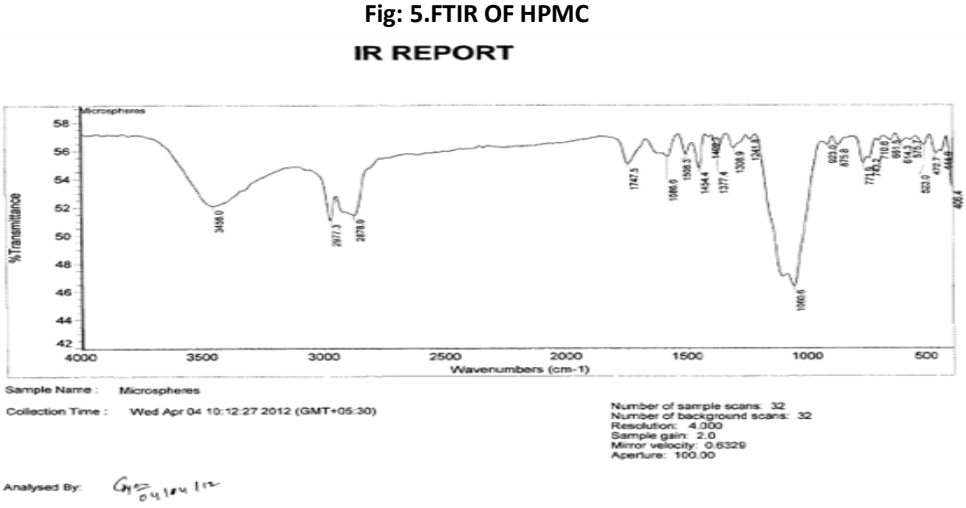

Fig: 6.FTIR OF RABEPRAZOLE MICROSPHERES

Differential Scanning Calorimetry (DSC) Studies[17]**:** Differential scanning calorimetry (DSC) was performed on Ethyl Cellulose, HPMC, Rabeprazole and Rabeprazole-loaded microspheres. DSC

measurements were done on a Rheometric Scientific (DSC-SP, Surrey, UK) by heating the samples from ambient to 400°C at the heating rate of 10°C/min in a nitrogen atmosphere (flow rate, 20 mL/min).

Surface Morphology[18]: The shape and surface topography of the microspheres were studied by scanning electron microscopy (SEM).**Fig No.7**

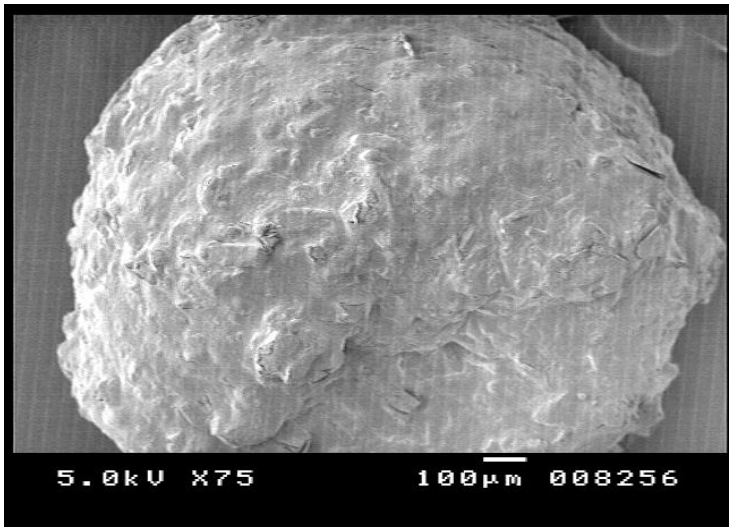

Fig: 7. SEM ANALYSIS

Particle Size Measurements[19]: Particle size was measured by using light scattering technique (Microtrack). Sizes of the microspheres were measured by using a dry sample adapter. Completely dried microspheres were placed on the sample tray in an inbuilt vacuum and compressed air system was used to suspend the particles. The laser obscuration range was maintained between 1 and 2%. The volume mean diameter (V_d) was recorded. The analysis was performed in triplicate and average values are used. **Fig.No: 8**

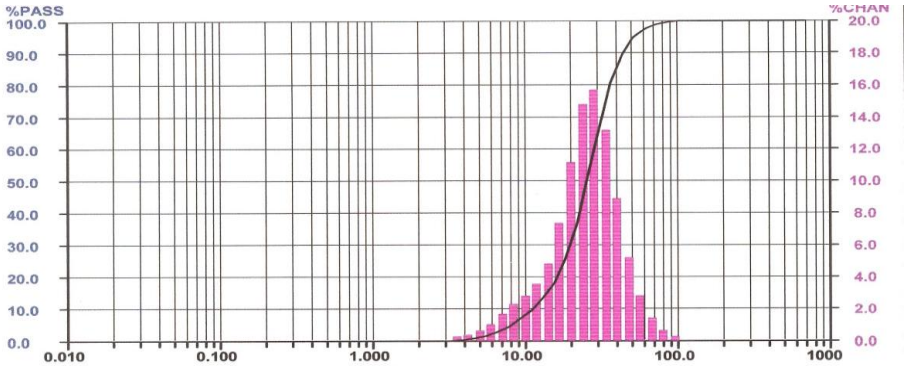

Fig: 8. PARTICLE SIZE ANALYSIS

In Vitro Drug Release[20]: In vitro drug release was investigated in SGF (0.1N HCl, pH 1.2, ionic strength 0.1) for the first 2 h, followed by the SIF [pH 7.4 phosphate buffer (0.05M potassium dihydrogen phosphate), ionic strength 0.09] until complete dissolution. These experiments were performed using dissolution tester equipped with six baskets at the stirring speed of 100 rpm. A weighed quantity of each sample was placed in 500mL of dissolution medium maintained at 37 °C. At regular interval of time i.e. for every hour 2ml of sample was withdrawn and the same amount of replaced with fresh buffer. After filtering the 2ml of sample it was diluted to 10ml with the buffer[21, 22, and 23]. Then the Rabeprazole concentration was determined Spectrophotometrically at 240nm.

Fig.No.9 to 11

Table No-5. In vitro Drug release studies of F1

S.No	Time (hrs)	% cumulative drug release
1	0	0
2	1	4.23±0.36
3	2	14.26± 1.63
4	3	25.52±1.21
5	4	32.06±0.7
6	5	43.12±1.29
7	6	59.51±0.87
8	7	71.65±1.58
9	8	79.58±0.94

Table No-6. In vitro Drug release studies of F2

S.No	Time (hrs)	% cumulative drug release
1	0	0
2	1	7.43±0.26
3	2	18.31± 1.03
4	3	27.52±0.21
5	4	39.06±0.63
6	5	53.18±1.57
7	6	68.51±1.08
8	7	79.65±0.94
9	8	88.74±1.80

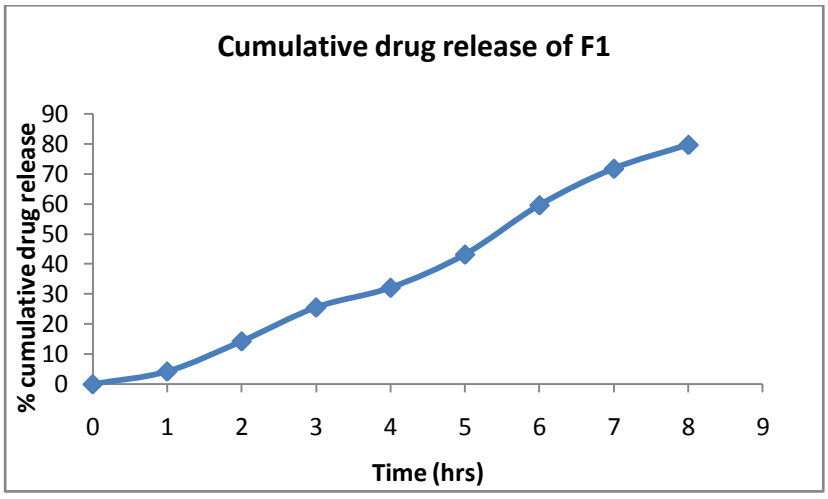

Fig: 9. Cumulative Drug Release of F1

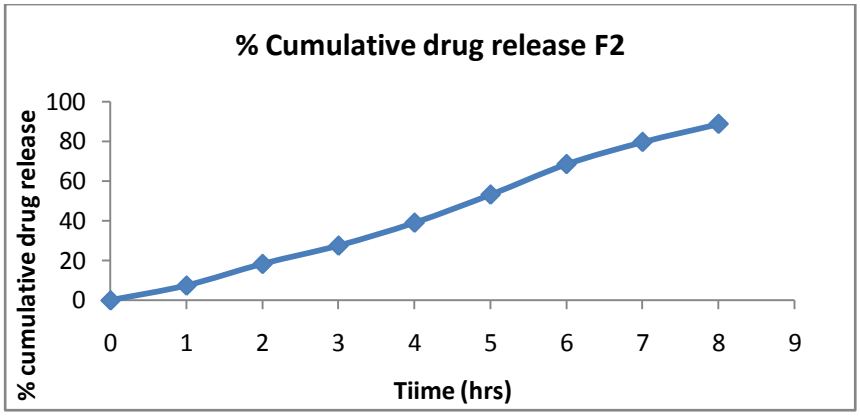

Fig: 10 Cumulative drug release of F2

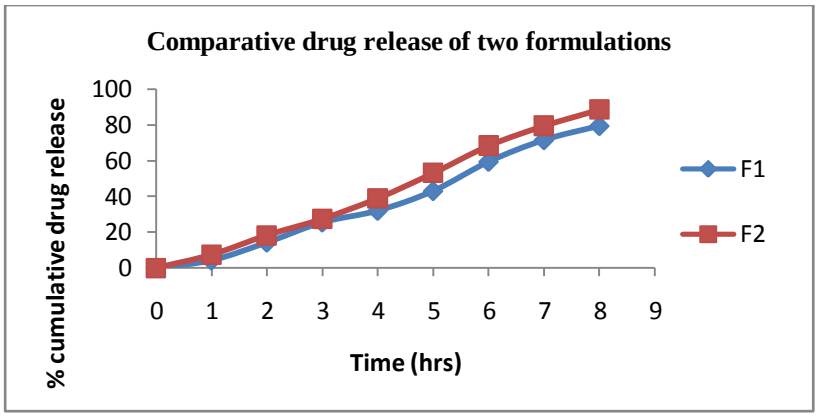

Fig: 11. Comparative drug release of F1 and F2

SUMMARY

The microspheres thus obtained were found to be spherical as given in **Fig 14** and without aggregation. The mean particle size was found in a range of 10 to 100 µm that is represented in **Fig. 21**, smaller size provide better absorption properties to the formulation. The percentage yield of all the formulations was found to be satisfactory. The study was executed with various prepared formulations and the results were tabularized in table. The *in-vitro* drug release profile was presented in **Fig 15 and Fig. 16** the F1 formulation showed a longer duration 8hrs of 79.58% of drug release and F2 showed 88.74% of drug release in 8hrs. The comparison of the two formulations was presented in **Fig.17**. It was noticed that the increase in the amount of ethyl cellulose showed slow release of the drug for a longer period of time.

CONCLUSION

The Rabeprazole loaded microspheres were prepared by emulsion solvent evaporation method using ethyl cellulose and HPMC as polymers. The following conclusions can be drawn from the results obtained: Micromeritic properties of microspheres were within the official range. Encapsulation efficiency was 78 to 87%. % yield of the microspheres was good. DSC studies indicated no chemical interaction between drug and Polymers during encapsulation process. Drug release of the microspheres showed a longer duration up to 8hrs of 80%. Hence microspheres prepared using ethyl cellulose and HPMC as polymers showed promising results and there exist a scope for *in-vivo* evaluation using suitable animal models.

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