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Research Article

FORMULATION DEVELOPMENT AND *IN VITRO*
CHARACTERISATION OF ENTECAVIR MICROSPHERESMasireddy Tejasri*¹, Shaik Zareena Begum²Department Of Pharmaceutics, Princeton College Of Pharmacy, Pocharam, Hyderabad,
Telangana 500088.**Abstract:**

Oral route drug administration is by far the most preferable route for taking medications. However, their short circulating half life and restricted absorption via a defined segment of intestine limits the therapeutic potential of many drugs. Such a pharmacokinetic limitation leads in many cases to frequent dosing of medication to achieve therapeutic effect. Rational approach to enhance bioavailability and improve pharmacokinetic and pharmacodynamics profile is to release the drug in a controlled manner and site specific manner. Microspheres are small spherical particles, with diameters 1 μm to 1000 μm . They are spherical free flowing particles consisting of proteins or synthetic polymers which are biodegradable in nature. There are two types of microspheres; microcapsules and micromatrices, which are described as, Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall. and micromatrices in which entrapped substance is dispersed throughout the matrix. Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Microsphere play an important role to improve bioavailability of conventional drugs and minimizing side effects.

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INTRODUCTION:

Oral route drug administration is by far the most preferable route for taking medications. However, their short circulating half life and restricted absorption via a defined segment of intestine limits the therapeutic potential of many drugs. Such a pharmacokinetic limitation leads in many cases to frequent dosing of medication to achieve therapeutic effect. Rational approach to enhance bioavailability and improve pharmacokinetic and pharmacodynamics profile is to release the drug in a controlled manner and site specific manner. Microspheres are small spherical particles, with diameters 1 μm to 1000 μm . They are spherical free flowing particles consisting of proteins or synthetic

polymers which are biodegradable in nature. There are two types of microspheres; microcapsules and micromatrices, which are described as, Microcapsules are those in which entrapped substance is distinctly surrounded by distinct capsule wall. and micromatrices in which entrapped substance is dispersed throughout the matrix. Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Microsphere play an important role to improve bioavailability of conventional drugs and minimizing side effects. Ideal characteristics of microspheres: ^{1,2,3,4,5}

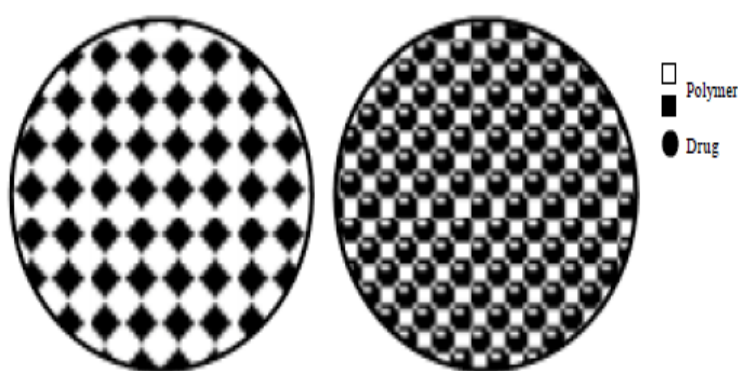


FIG 1.1 : Structure Of Microspheres

Ideal characteristics of microspheres: ⁶

- ✓ The ability to incorporate reasonably high concentrations of the drug.
- ✓ Stability of the preparation after synthesis with clinically acceptable shelf life.
- ✓ Controlled particle size and dispersability in aqueous vehicles for injection.
- ✓ Release of active reagent with a good control over a wide time scale.
- ✓ Biocompatibility with a controllable biodegradability.
- ✓ Susceptibility to chemical modification.

Advantages of microspheres:

1. Particle size reduction for enhancing solubility of the poorly soluble drug.
2. provide constant and prolonged therapeutic effect.
3. provide constant drug concentration in blood there by increasing patient compliance,
4. Decrease dose and toxicity.
5. Protect the drug from enzymatic and photolytic cleavage hence found to be best for drug delivery of protein.
6. Reduce the dosing frequency and thereby improve the patient compliance
7. Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.

8. Microsphere morphology allows a controllable variability in degradation and drug release.

9. Convert liquid to solid form & to mask the bitter taste.

10. Protects the GIT from irritant effects of the drug.

11. Biodegradable microspheres have the advantage over large polymer implants in that they do not require surgical procedures for implantation and removal.

12. Controlled release delivery biodegradable microspheres are used to control drug release rates thereby decreasing toxic side effects, and eliminating the inconvenience of repeated injections.

Limitation:

Some of the disadvantages were found to be as follows

1. The costs of the materials and processing of the controlled release preparation, are substantially higher than those of standard formulations.
2. The fate of polymer matrix and its effect on the environment.
3. The fate of polymer additives such as plasticizers, stabilizers, antioxidants and fillers.
4. Reproducibility is less.
5. Process conditions like change in temperature, pH, solvent addition, and evaporation/agitation

may influence the stability of core particles to be encapsulated.

6. The environmental impact of the degradation products of the polymer matrix produced in response to heat, hydrolysis, oxidation, solar radiation or biological agents.

Types of microspheres:

1. Bioadhesive microspheres
2. Magnetic microspheres
3. Floating microspheres
4. Radioactive microspheres
5. Polymeric microspheres
 - i) Biodegradable polymeric microspheres
 - ii) Synthetic polymeric microspheres

1. Bioadhesive microspheres: ^{7,8}

Adhesion can be defined as sticking of drug to the membrane by using the sticking property of the water soluble polymers. Adhesion of drug delivery device to the mucosal membrane such as buccal, ocular, rectal, nasal etc. can be termed as bio adhesion. These kinds of microspheres exhibit a prolonged residence time at the site of application and causes intimate contact with the absorption site and produces better therapeutic action.

2. Magnetic microspheres: ^{9,10}

This kind of delivery system is very much important which localises the drug to the disease site. In this larger amount of freely circulating drug can be replaced by smaller amount of magnetically targeted drug. Magnetic carriers receive magnetic responses to a magnetic field from incorporated materials that are used for magnetic microspheres are chitosan, dextran etc. The different types of

- a. Therapeutic magnetic microspheres used to deliver chemotherapeutic agent to liver tumour. Drugs like proteins and peptides can also be targeted through this system.
- b. Diagnostic microspheres, used for imaging liver metastases and also can be used to distinguish bowel loops from other abdominal structures by forming nano size particles supramagnetic iron oxides.

3. Floating microspheres: ^{11,12,13}

In floating types the bulk density is less than the gastric fluid and so remains buoyant in stomach without affecting gastric emptying rate. The drug is released slowly at the desired rate, and the system is found to be floating on gastric content and increases gastric residence and increases fluctuation in plasma concentration. Moreover it also reduces chances of dose dumping. It produces prolonged therapeutic effect and therefore reduces dosing frequencies. Drug (ketoprofen) is given in the form of floating microspheres.

4. Radioactive microspheres: ¹⁴

Radio embolization therapy microspheres sized 10-30 nm are of larger than the diameter of the capillaries and gets trapped in first capillary bed when they come across. They are injected in the arteries that leads them to tumour of interest so all these conditions radioactive microspheres deliver high radiation dose to the targeted areas without damaging the normal surrounding tissues. It differs from drug delivery system, as radio activity is not released from microspheres but acts from within a radioisotope typical distance and the different kinds of radioactive microspheres are α emitters, β emitters, γ emitters.

5. Polymeric microspheres:

The different types of polymeric microspheres can be classified as follows and they are biodegradable polymeric microspheres and Synthetic polymeric microspheres.

i) Biodegradable polymeric microspheres: ¹⁵

Natural polymers such as starch are used with the concept that they are biodegradable, biocompatible, and also bio adhesive in nature. Biodegradable polymers prolongs the residence time when contact with mucous membrane due to its high degree of swelling property with aqueous medium, results gel formation. The rate and extent of drug release is controlled by concentration of polymer and the release pattern in a sustained manner. The main drawback is, in clinical use drug loading efficiency of biodegradable microspheres is complex and is difficult to control the drug release. However they provide wide range of application in microsphere based treatment.

ii) Synthetic polymeric microspheres: ¹⁶

Synthetic polymeric microspheres are widely used in clinical application, moreover that also used as bulking agent, fillers, embolic particles, drug delivery vehicles etc. and proved to be safe and biocompatible but the main disadvantage of these kind of microspheres, are tend to migrate away from injection site and lead to potential risk, embolism and further organ damage.

Method of preparation:

1. Spray Drying
2. Solvent Evaporation
3. Single emulsion technique
4. Double emulsion technique
5. Phase separation coacervation technique
6. Spray drying and spray congealing
7. Solvent extraction
8. Quassi emulsion solvent diffusion:

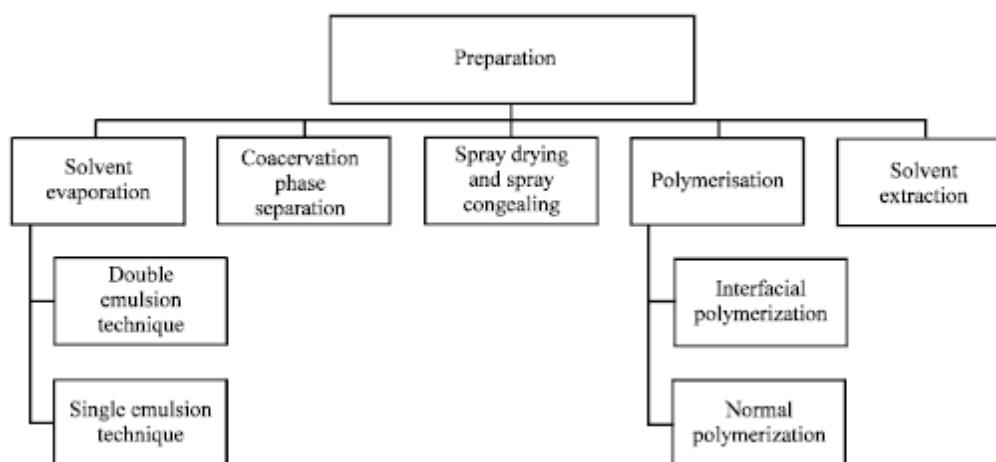


FIG 1.2 : Method of preparation of microspheres

1. Spray Drying:¹⁷

In Spray Drying technique, the polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution with high-speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leading the formation of the microspheres in a size range 1-100 μ m. Micro particles are separated from hot air by means of the cyclone separator while the trace of solvent is removed by vacuum drying. One of the major advantages of this process is feasibility of operation under aseptic conditions.

2. Solvent Evaporation:

This process is carried out in a liquid manufacturing vehicle phase. The microcapsule coating is dispersed in a volatile solvent which is immiscible with the liquid manufacturing vehicle phase. A core material to be microencapsulated is dissolved or dispersed in the coating polymer solution. With agitation the core material mixture is dispersed in the liquid manufacturing vehicle phase to obtain the appropriate size microcapsule. The mixture is then heated if necessary to evaporate the solvent for the polymer of the core material to disperse in the polymer solution, polymer shrinks around the core. If the core material is dissolved in

the coating polymer solution, matrix – type microcapsules are formed. The core materials may be either water soluble or water insoluble materials. Solvent evaporation involves the formation of an emulsion between polymer solution and an immiscible continuous phase whether aqueous (o/w) or non-aqueous.

3. Single emulsion technique:

The micro particulate carriers of natural polymers i.e. those of proteins and carbohydrates are prepared by single emulsion technique. The natural polymers are dissolved or dispersed in aqueous medium followed by dispersion in non-aqueous medium like oil. In the next step, the cross linking of the dispersed globule is carried out. The cross linking can be achieved either by means of heat or by using the chemical cross linkers. The chemical cross linking agents used are glutaraldehyde, formaldehyde, acid chloride etc. Heat denaturation is not suitable for thermolabile substances. Chemical cross linking suffers the disadvantage of excessive exposure of active ingredient to chemicals if added at the time of preparation and then subjected to centrifugation, washing, separation³ The nature of the surfactants used to stabilize the emulsion phases can greatly influence the size, size distribution, surface morphology, loading, drug release, and bio performance of the final multiparticulate product.

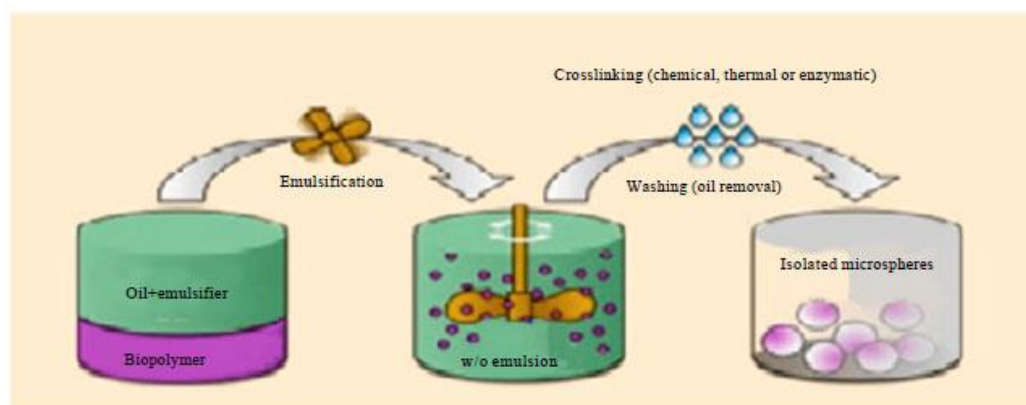


FIG 1.3 : Single emulsion technique

4. Double emulsion technique:

Double emulsion method of microspheres preparation involves the formation of the multiple emulsions or the double emulsion of type w/o/w and is best suited for water soluble drugs, peptides, proteins and the vaccines. This method can be used with both the natural as well as synthetic polymers. The aqueous protein solution is dispersed in a lipophilic organic continuous phase. This protein solution may contain the active constituents. The continuous phase is generally consisted of the polymer solution that eventually encapsulates of the protein contained in dispersed aqueous phase. The primary emulsion is subjected

then to the homogenization or the sonication before addition to the aqueous solution of the poly vinyl alcohol (PVA). This results in the formation of a double emulsion. The emulsion is then subjected to solvent removal either by solvent evaporation or by solvent extraction. A number of hydrophilic drugs like luteinizing hormone releasing hormone (LH-RH) agonist, vaccines, proteins/peptides and conventional molecules are successfully incorporated into the microspheres using the method of double emulsion solvent evaporation/ extraction.

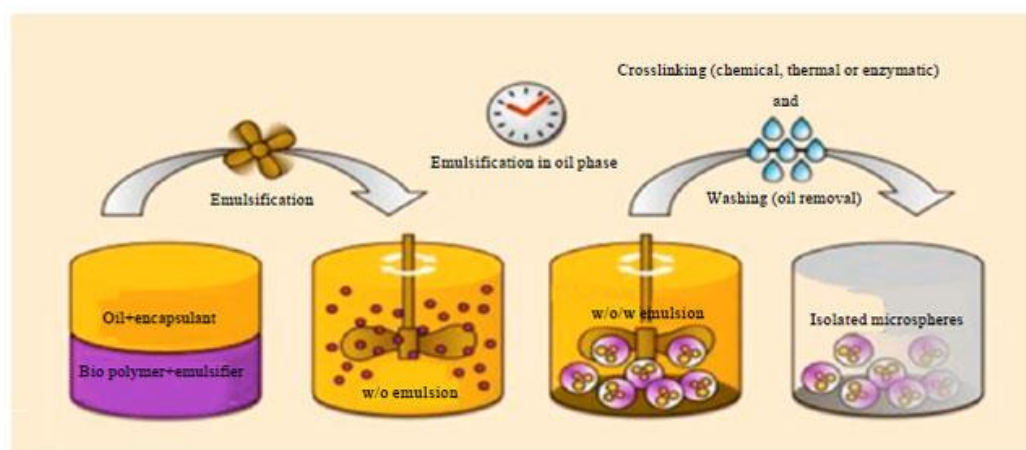


FIG 1.4 : Double emulsion technique

5. Phase separation coacervation technique:^{18,19}

This process is based on the principle of decreasing the solubility of the polymer in organic phase to affect the formation of polymer rich phase called the coacervates. In this method, the drug particles are dispersed in a solution of the polymer and an incompatible polymer is added to the system which makes first polymer to phase separate and engulf the drug particles. Addition of non-solvent results in the solidification of polymer. Poly lactic acid (PLA) microspheres have been prepared by this method by using butadiene as incompatible polymer. The process variables are very important

since the rate of achieving the coacervates determines the distribution of the polymer film, the particle size and agglomeration of the formed particles. The agglomeration must be avoided by stirring the suspension using a suitable speed stirrer since as the process of microspheres formation begins the formed polymerize globules start to stick and form the agglomerates. Therefore the process variables are critical as they control the kinetic of the formed particles since there is no defined state of equilibrium attainment.

6. Spray drying and spray congealing:

These methods are based on the drying of the mist of the polymer and drug in the air. Depending upon the removal of the solvent or cooling of the solution, the two processes are named spray drying and spray congealing respectively. The polymer is first dissolved in a suitable volatile organic solvent such as dichloromethane, acetone, etc. The drug in the solid form is then dispersed in the polymer solution under high speed homogenization. This dispersion is then atomized in a stream of hot air. The atomization leads to the formation of the small droplets or the fine mist from which the solvent evaporates instantaneously leading the formation of

the microspheres in a size range 1-100 μm . Microparticles are separated from the hot air by means of the cyclone separator while the traces of solvent are removed by vacuum drying. One of the major advantages of the process is feasibility of operation under aseptic conditions. The spray drying process is used to encapsulate various penicillins. Thiamine mononitrate and sulphathiazole are encapsulated in a mixture of mono- and diglycerides of stearic acid and palmitic acid using spray congealing. Very rapid solvent evaporation, however leads to the formation of porous microparticles.

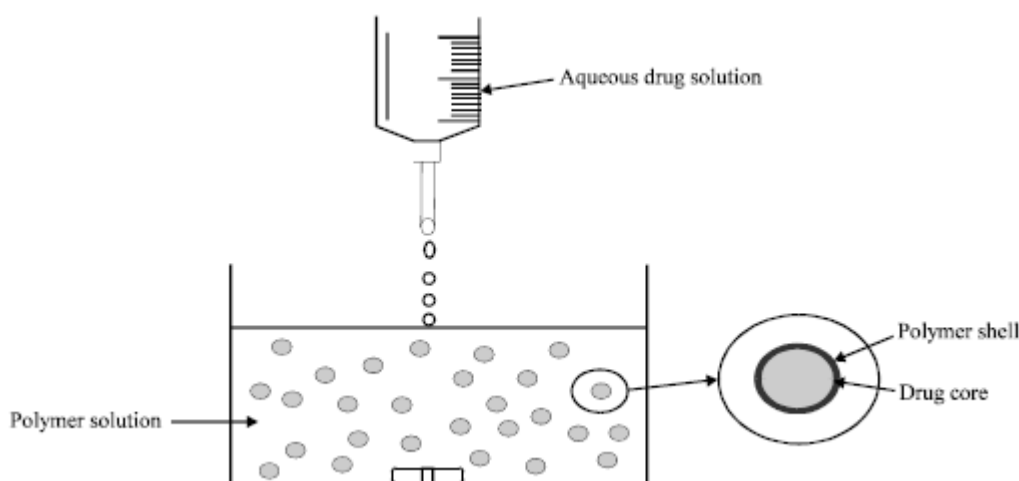


FIG 1.5 : Coacervation phase separation method

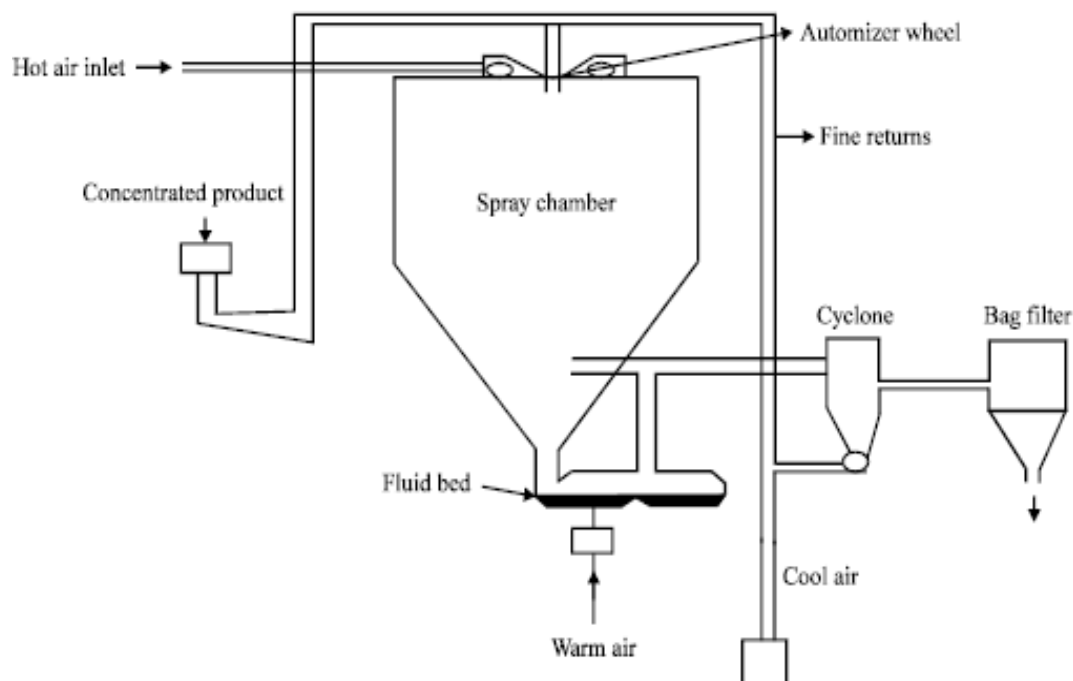


FIG 1.5 : Spray drying and spray congealing

7. Solvent extraction:

Solvent evaporation method is used for manufacturing of microparticles, involves removal

of the organic phase by extraction of the or non aqueous solvent. This method involves water miscible organic solvents as isopropanol. Organic

phase can be removed by extraction with water. This process decreases the hardening time for the microspheres. One variation of the process involves direct incorporation of the drug or protein to polymer organic solution. Rate of solvent removal by extraction method depends on the temperature of water, ratio of emulsion volume to the water and solubility profile of polymer.

8. Quasi emulsion solvent diffusion:

A novel quasi-emulsion solvent diffusion method to manufacture the controlled release microspheres of drugs with acrylic polymers has been reported in the literature. Microspheres can be manufactured by a quasi emulsion solvent diffusion method using an external phase containing distilled water and polyvinyl alcohol. The internal phase consists of drug, ethanol and polymer. The concentration of polymer is in order to enhance plasticity. At first, the internal phase is manufactured at 60°C and then added to the external phase at room temperature. After emulsification process, the mixture is continuously stirred for 2 hours. Then the mixture can be filtered to separate the microspheres. The product is then washed and dried by vacuum oven at 40°C for a day.

Polymerization techniques: ^{20,21}

The polymerization techniques conventionally used for preparing the microspheres are mainly classified as:

I. Normal polymerization

II. Interfacial polymerization.

Both are carried out in liquid phase.

I. Normal polymerization:

It is carried out by using different techniques as bulk, suspension, precipitation, emulsion and micellar polymerization methods. In bulk, a monomer or a combin.¹¹

METHODOLOGY PREPARATION OF 0.1N HCl (pH 1.2):

CHARACTERIZATION OF MICROSPHERES:

INGREDIENTS (MG)	FORMULATIONS								
	E1	E2	E3	E4	E5	E6	E7	E8	E9
Entecavir	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
PLGA	5	10	15	-	-	-	-	-	-
Ethyl cellulose	-	-	-	5	10	15	-	-	-
HPMC K4M	-	-	-	-	-	-	5	10	15
Dichloro methane (mL)	10	10	10	10	10	10	10	10	10
Methanol (mL)	30	30	30	30	30	30	30	30	30
Sodium lauryl sulphate (mg)	20	20	20	20	20	20	20	20	20

Micromeritic properties

The microspheres were characterized by their micromeritic properties such as Particle size, Bulk

Take 8.6ml of HCl in a 1000ml volumetric flask and make up the volume with distilled water

Preparation Of Standard Calibration Curve Of Entecavir:

- ✓ 10 mg of Entecavir was accurately weighed and dissolved in 10ml of methanol (Stock Solution – I) to get a concentration of 1000 µg/ml.
- ✓ From the stock solution- I, 1ml of aliquots was taken and suitably diluted with 0.1N HCl (Stock Solution-II) to get concentrations of 100µg/ml.
- ✓ From the stock solution- II, aliquots were taken and suitably diluted with 0.1N HCl (pH 1.2) to get concentrations in the range of 2 to 10µg/ml. The absorbance of these samples were analyzed by using UV-Visible Spectrophotometer at 270nm against reference solution 0.1N HCl (pH 1.2). the procedure repeated to pH 6.8 phosphate buffer and pH 7.4 phosphate buffer.

METHOD OF PREPARATION

SOLVENT EVAPORATION METHOD:

Entecavir microspheres were prepared using PLGA, Ethyl cellulose and HPMC K4M and distilled water as continuous phase by solvent evaporation technique. Initially dichloromethane (DCM) and methanol was mixed uniformly at room temperature, then PLGA, Ethyl cellulose and HPMC K4M in various proportions was dissolved in the above solution. To this mixture, a drug solution corresponding was added and mixed thoroughly and injected drop wise in to the continuous phase consisting of 100mL of 0.2% (w/v) SLS (sodium lauryl sulphate) at 250 rpm. The microspheres obtained was washed for 2-3 times with distilled water and dried at room temperature. Different concentrations and ratios of polymers used in the formulation of microspheres are mentioned in Table.

density, Tapped density, Compressibility index, Hausners ratio and Angle of repose.

Bulk density

In this method floating microspheres are transferred to a measuring cylinder and is tapped manually till a constant volume is obtained. This volume is bulk volume and it includes true volume of the powder and the void space among the microspheres.

Tapped density

In this method floating microspheres were transferred to a measuring cylinder & tapped for

$$\text{Bulk density} = \frac{\text{Mass of microspheres}}{\text{Bulk volume}}$$

Hausners ratio

Hausners ratio of microspheres was determined by comparing tapped density to bulk density using the equation

Hausner ratio = tapped density / bulk density

Angle of repose

Angle of repose (θ) of the microspheres, which measures the resistance to particle flow, was determined by a fixed funnel method⁴. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the heap of the blends. Accurately weighed microspheres were allowed to pass through the funnel freely on to the surface. The height and radius of the powder cone was

100 times. After tapping volume of microspheres was visually examined. The ratio of mass of microspheres to volume of microspheres after tapping gives tapped density floating microspheres. Percent Compressibility index was determined by using the formula,

$$\text{Carr's Index} = \frac{(\text{tapped density} - \text{bulk density}) \times 100}{\text{tapped density}}$$

measured and angle of repose was calculated using the following equation.

$$\theta = \tan^{-1} h / r$$

Here,

θ - Angle of repose

h - Height of granules above the flat surface

r - Radius of the circle formed by the granule heap.

Percentage yield

The percentage of production yield was calculated from the weight of dried microspheres recovered from each batch and the sum of the initial weight of starting materials. The percentage yield was calculated using the following formula:

$$\% \text{ Yield} = \frac{\text{Practical mass (Microspheres)}}{\text{Theoretical mass (Polymer + Drug)}} \times 100$$

Drug entrapment efficiency:

Weighed amount of microspheres (100 mg) with phosphate buffer pH 7.4 (10 ml) was added in a vial. The solution was stirred vigorously for 24 hours with mechanical stirrer. Supernatant was

collected by centrifugation and drug content in supernatant was determined by using UV spectrophotometer at wavelength 260nm. The amount of drug entrapped in the microspheres was calculated by the following formula,

$$\% \text{ Drug Entrapment Efficiency} = \frac{\text{Experimental Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

Swelling study:

Swelling ratio of different dried microspheres were determined gravimetrically in simulated gastric fluid pH 1.2. The microspheres were removed

periodically from the solution, blotted to remove excess surface liquid and weighed on balance. Swelling ratio (% w/v) was determined from the following relationship:

$$\text{Swelling ratio} = \frac{(W_t - W_0)}{W_0} \times 100$$

Where W_0 & W_t are initial weight and Final weight of microspheres respectively

In vitro drug release study:

The dissolution studies were performed in a fully calibrated eight station dissolution test apparatus

($37 \pm 0.5^\circ\text{C}$, 50 rpm) using the USP type – I rotating basket method in simulated gastric fluid pH 1.2 (900ml) for 2 hours then replace the media

with pH 6.8 phosphate buffer for 3 hours, then replace the media with pH 7.4 Phosphate buffer. A quantity of accurately weighed microspheres equivalent to 100mg Sumatriptan Succinate each formulation was employed in all dissolution studies. Aliquots of sample were withdrawn at predetermined intervals of time and analyzed for drug release by measuring the absorbance at 270nm. At the same time the volume withdrawn at each time intervals were replenished immediately with the same volume of fresh pre-warmed simulated gastric fluid pH 1.2 maintaining sink conditions throughout the experiment.

In Vitro drug release kinetics

The release data obtained was fitted into various mathematical models. The parameters 'n' and time

component 'k', the release rate constant and 'R', the regression coefficient were determined by Korsmeyer-Peppas equation to understand the release mechanism.

To examine the release mechanism of Sumatriptan Succinate from the microspheres, the release data was fitted into Peppas's equation,

$$M_t / M_\infty = K t^n$$

Where, M_t / M_∞ is the fractional release of drug, 't' denotes the release time, 'K' represents a constant incorporating structural and geometrical characteristics of the device, 'n' is the diffusional exponent and characterize the type of release mechanism during the release process.

Table 7.2 : In-Vitro drug release kinetics

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
$0.5 < n < 1.0$	Anomalous transport or non-Fickian	t^{n-1}
1.0	Case-II transport	Zero-order release
Higher than 1.0	Super Case-II transport	t^{n-1}

If $n < 0.5$, the polymer relaxation does not affect the molecular transport, hence diffusion is Fickian. If $n > 0.5$, the solid transport will be non-Fickian and will be relaxation controlled.

Other equations to study the drug release kinetics from dosage forms

a. Zero Order

$$\% R = kt$$

This model represents an ideal release in order to achieve prolonged pharmacological action. This is applicable to dosage forms like transdermal systems, coated forms, osmotic systems, as well as Matrix tablets containing low soluble drugs.

b. First Order

$$\text{Log (fraction unreleased)} = kt/2.303$$

The model is applicable to hydrolysis kinetics and to study the release profiles of pharmaceutical dosage forms such as those containing water soluble drugs in porous matrices.

c. Matrix (Higuchi Matrix)

$$\% R = kt^{0.5}$$

This model is applicable to systems with drug dispersed in uniform swellable polymer matrix as in case of matrix tablets with water soluble drug.

d. Peppas Korsmeyer Equation

$$\% R = kt^n$$

$$\log \% R = \log k + n \log t$$

This model is widely used when release mechanism is well known or when more than one type of release phenomenon could be involved. The 'n' values could be used to characterize different release mechanisms as.

Fourier Transform Infrared (FTIR) spectroscopy:

The physical properties of the physical mixture were compared with those of plain drug. Samples were mixed thoroughly with 100mg potassium bromide IR powder and compacted under vacuum at a pressure of about 12 psi for 3 minutes. The resultant disc was mounted in a suitable holder in Agilent spectrophotometer and the IR spectrum was recorded from 4000 cm^{-1} to 500 cm^{-1} . The resultant spectrum was compared for any spectrum changes.

SEM (Scanning Electron Microscope) studies:

The surface morphology of the layered sample was examined by using SEM (JEOL Ltd., Japan). The small amount of powder was manually dispersed onto a carbon tab (double adhesive carbon coated tape) adhered to an aluminum stubs were coated with a thin layer (300Å) of gold by employing POLARON - E 3000 sputter coater. The samples

were examined by SEM with direct data capture of the images on to a computer.

RESULTS AND DISCUSSION:

PREFORMULATION STUDIES

8.1.1. SPECTROSCOPIC STUDIES

Determination of $\lambda_{\max}$

A solution of 10 μ g/ml of Entecavir was scanned in the range of 200 to 400nm. The drug exhibited a $\lambda_{\max}$ at 270 nm in simulated gastric fluid pH 1.2 and pH 7.4 phosphate buffer respectively. Correlation between the concentration and absorbance was

found to be near to 0.998, with a slope of 0.028 and intercept of 0.004.

Calibration curve of Entecavir in simulated gastric fluid pH 1.2

Table 8.1 shows the calibration curve data of Entecavir in simulated gastric fluid pH 1.2 at 270 nm Fig.8.1 shows the standard calibration curve with a regression value of 0.997, slope of 0.058 and intercept of 0.017 in simulated gastric fluid pH 1.2. The curve was found to be linear in the concentration range of 2-10 μ g/ml.

Table 8.1: Calibration curve data for Entecavir in simulated gastric fluid pH 1.2

Concentration (μ g /ml)	Absorbance
0	0
2	0.149
4	0.255
6	0.375
8	0.478
10	0.591

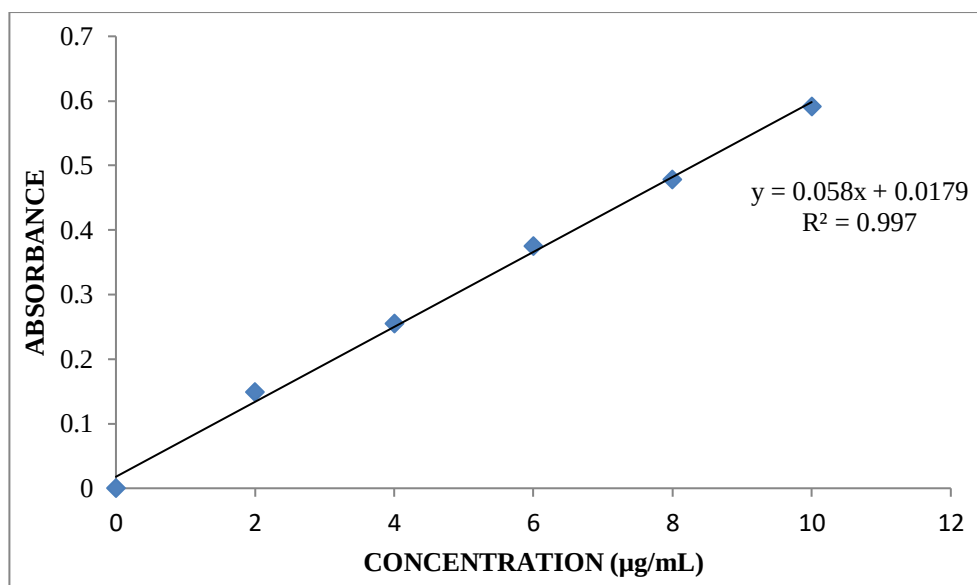


Figure 8.1: Standard graph Of Entecavir in simulated gastric fluid pH 1.2

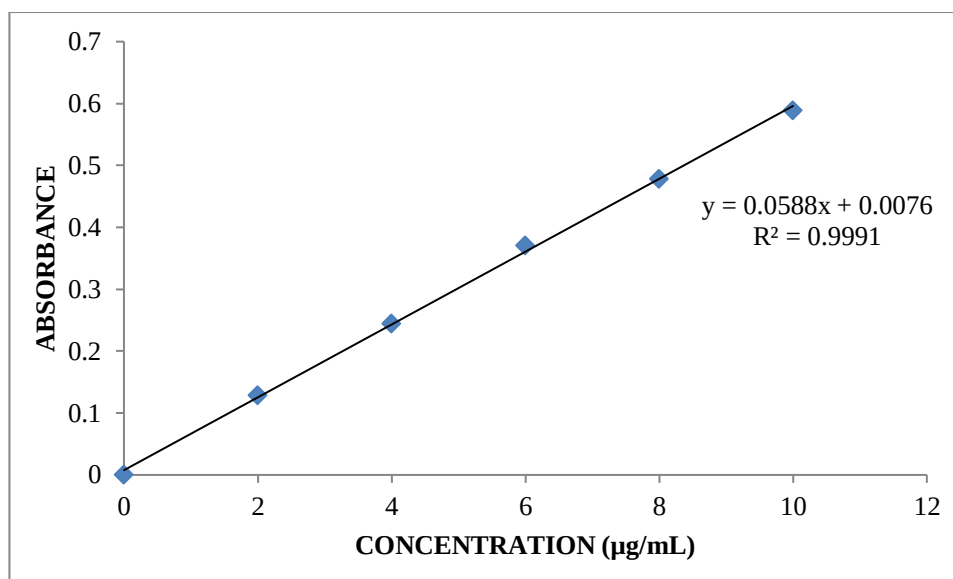
Calibration curve of Entecavir in pH 7.4 phosphate buffer

Table 8.2 shows the calibration curve data of Entecavir in pH 7.4 phosphate buffer at 262nm. Fig. 8.2 shows the standard calibration curve with a

regression value of 0.999, slope of 0.058 and intercept of 0.007 in simulated gastric fluid Ph 1.2. The curve was found to be linear in the concentration range of 2-10 μ g/ml.

Table 8.2: Calibration curve data for Entecavir in pH 7.4 phosphate buffer

Concentration ($\mu\text{g/ml}$)	Absorbance
0	0
2	0.129
4	0.244
6	0.371
8	0.478
10	0.589

**Figure 8.2: Standard graph Of Entecavir in pH 7.4 phosphate buffer**

Evaluation and characterisation of microspheres

Micrometric Properties

The mean size increased with increasing polymer concentration which is due to a significant increase in the viscosity, thus leading to an increased droplet size and finally a higher microspheres size. Microspheres containing PLGA as a polymer had a size range of 312.14 μm to 3.32.41 μm . microspheres containing Ethyl cellulose as polymer exhibited a size range between 310.15 μm to 341.65 μm . Microspheres containing HPMC K4M as copolymer had a size range of 309.54 μm to 325.14 μm .

The particle size data is presented in Tables 8.3 and displayed in Figures. The effect of drug to polymer ratio on particle size is displayed in Figure. The particle size as well as % drug

entrapment efficiency of the microspheres increased with increase in the polymer concentration.

The bulk density of formulation E1 to E9 containing PLGA, Ethyl cellulose and HPMC K4M formulation was in the range of 0.277 ± 0.2 to $0.625 \pm 0.1 \text{ gm./cm}^3$ (as shown in table 8.3), tapped density 0.312 ± 0.2 to 0.833 ± 0.1 and Hausners ratio 1.095 to 1.333.

The carr's index of formulation E1 to E9 containing different grades of PLGA, Ethyl cellulose and HPMC K4M 8.695 to 25.00 respectively. The angle of repose of formulation E1 to E9 containing PLGA, Ethyl cellulose and HPMC K4M formulation was in the range <28.3 respectively(as shown in table 8.3) The values of carr's index and angle of repose indicate good flow properties.

Table 8.3: Micromeritic property of floating microspheres of Entecavir

Formulation code	Mean partical size	Bulk density ((gm./cm ³))	Tapped density (gm./cm ³)	Hauseners ratio	Carr's index	Angle of repose
E1	312.14	0.434 ± 0.2	0.476 ± 0.3	1.095	8.695	23.2 ± 0.2
E2	3.25.95	0.277 ± 0.2	0.312 ± 0.2	1.133	11.11	25.2 ± 0.1
E3	3.32.41	0.588 ± 0.3	0.666 ± 0.4	1.333	11.76	27.1 ± 0.1
E4	310.15	0.521 ± 0.3	0.631 ± 0.3	1.121	17.39	24.4 ± 0.4
E5	320.96	0.625 ± 0.1	0.833 ± 0.1	1.333	25.00	28.3 ± 0.4
E6	341.65	0.476 ± 0.3	0.526 ± 0.2	1.105	9.52	25.1 ± 0.1
E7	325.14	0.416 ± 0.2	0.476 ± 0.3	1.142	12.50	26.7 ± 0.4
E8	310.69	0.384 ± 0.4	0.434 ± 0.3	1.130	11.53	26.0 ± 0.3
E9	309.54	0.555 ± 0.1	0.714 ± 0.1	1.285	22.22	26.6 ± 0.2

PERCENTAGE YIELD

It was observed that as the polymer ratio in the formulation increases, the product yield also increases. The low percentage yield in some formulations may be due to blocking of needle and wastage of the drug- polymer solution, adhesion of polymer solution to the magnetic bead and microspheres lost during the washing process. The percentage yield was found to be in the range.

DRUG ENTRAPMENT EFFICIENCY

Percentage Drug entrapment efficiency of Entecavir ranged from 95.24 to 99.76 % for microspheres containing PLGA, Ethyl cellulose and HPMC K4M polymer, The drug entrapment efficiency of the prepared microspheres increased progressively with an increase in proportion of the respective polymers. Increase in the polymer concentration increases the viscosity of the dispersed phase. The particle size increases exponentially with viscosity. The higher viscosity of the polymer solution at the highest polymer concentration would be expected to decrease the diffusion of the drug into the external phase which would result in higher entrapment efficiency. The % drug entrapment efficiency of the prepared microspheres is displayed in Table 8.4, and displayed in Figures.

Table 8.4: Percentage yield and percentage drug entrapment efficiency of the prepared microspheres

S.No.	Formulation code	% yield	Drug Content (mg)	% Drug entrapment efficiency
1	E1	90.56	97.14	73.14
2	E2	93.91	98.65	86.91
3	E3	95.21	99.76	90.72
4	E4	92.47	98.14	96.58
5	E5	96.14	96.52	98.45
6	E6	97.35	99.34	91.87
7	E7	95.41	95.24	89.72
8	E8	93.11	97.53	91.51
9	E9	90.48	99.21	95.82

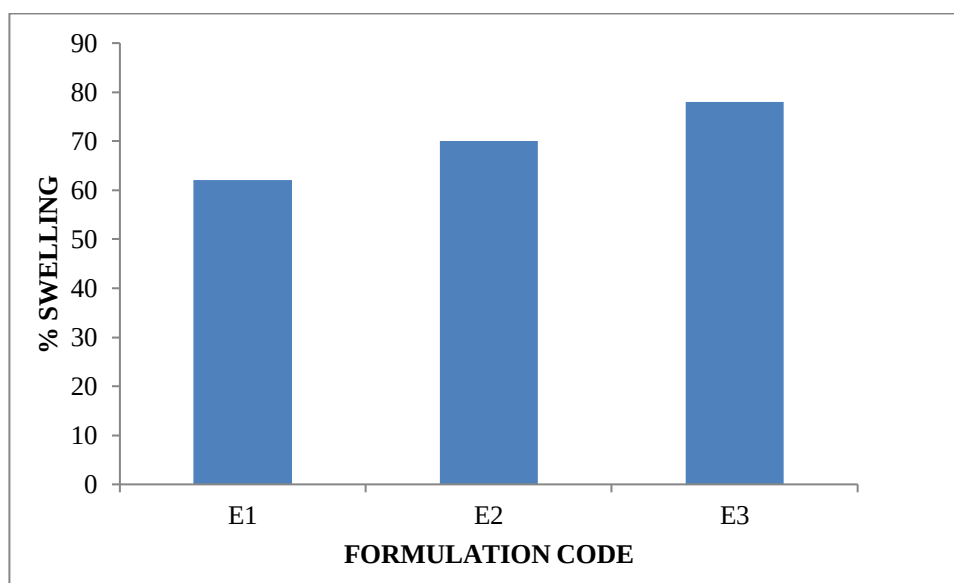
Swelling studies

The swelling ratio is expressed as the percentage of water in the hydrogel at any instant during swelling. Swell ability is an important characteristic as it affects mucoadhesion as well as drug release profiles of polymeric drug delivery systems. Swellability is an indicative parameter for rapid availability of drug solution for diffusion with greater flux. Swellability data revealed that amount of polymer plays an important role in solvent transfer. It can be concluded from the data shown in Table 8.5 that with an increase in polymer concentration, the percentage of swelling also increases. Thus we can say that amount of

polymer directly affects the swelling ratio. As the polymer to drug ratio increased, the percentage of swelling increased from 29 to 60% for microspheres containing sodium alginate as polymer, 59 to 73% for microspheres containing Chitosan as polymer. The percentage of swelling of the prepared microspheres is displayed in Figures. Containing Eudragit as polymer. The percentage of swelling of the prepared microspheres is displayed in Figures. The effect of drug to polymer ratio on percentage swelling is displayed in Figure Table 8.5: Percentage swelling of the prepared microspheres.

Table 8.5 : Swelling studies

S.NO.	FORMULATION CODE	INITIAL (Wt)	FINAL (Wt)	PERCENTAGE SWELLING
1	E1	10	12.45	62
2	E2	10	11.62	70
3	E3	10	13.58	78
4	E4	10	12.45	75
5	E5	10	13.95	79
6	E6	10	14.86	83
7	E7	10	10.59	60
8	E8	10	11.75	65
9	E9	10	12.96	76

**Figure 8.3 : Percentage swelling of microspheres containing PLGA**

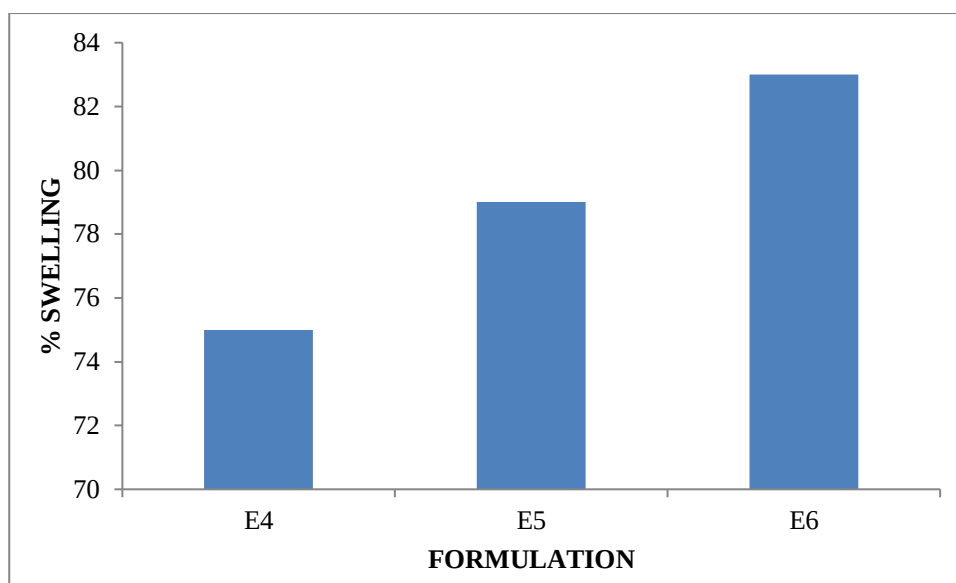


Figure 8.4 : Percentage swelling of microspheres containing Ethyl cellulose

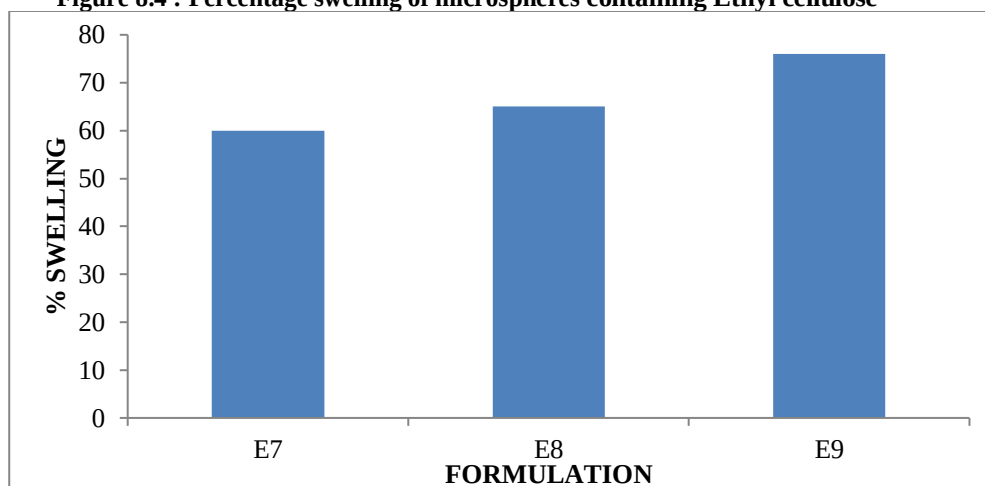


Figure 8.5 : Percentage swelling of microspheres containing HPMC K4M

IN VITRO MUCOADHESION TEST

As the polymer to drug ratio increased, microspheres containing sodium alginate exhibited % mucoadhesion ranging from 69 to 91%, microspheres containing Chitosan exhibited % mucoadhesion ranging from 76 to 91% and microspheres containing Eudragit exhibited %

mucoadhesion ranging from 58 to 79%. The results of in-vitro mucoadhesion test are compiled in Table 8.6. Effect of polymer proportion on % mucoadhesion is depicted in Figures 8.6 to 8.8 and comparative depiction of % mucoadhesion is depicted in Fig. 8.6. Table 8.6: Percentage mucoadhesion of the prepared microspheres.

Table 8.6 : In Vitro Mucoadhesion Test of all Formulations

S.NO.	FORMULATION CODE	No. OF MICROSPHERES		PERCENTAGE MUCOADHESION
		INITIAL	FINAL	
1	E1	20	15.48	61
2	E2	20	11.85	58
3	E3	20	15.14	70
4	E4	20	17.96	93
5	E5	20	20.71	95
6	E6	20	16.17	39
7	E7	20	16.80	93
8	E8	20	11.58	86
9	E9	20	17.21	78

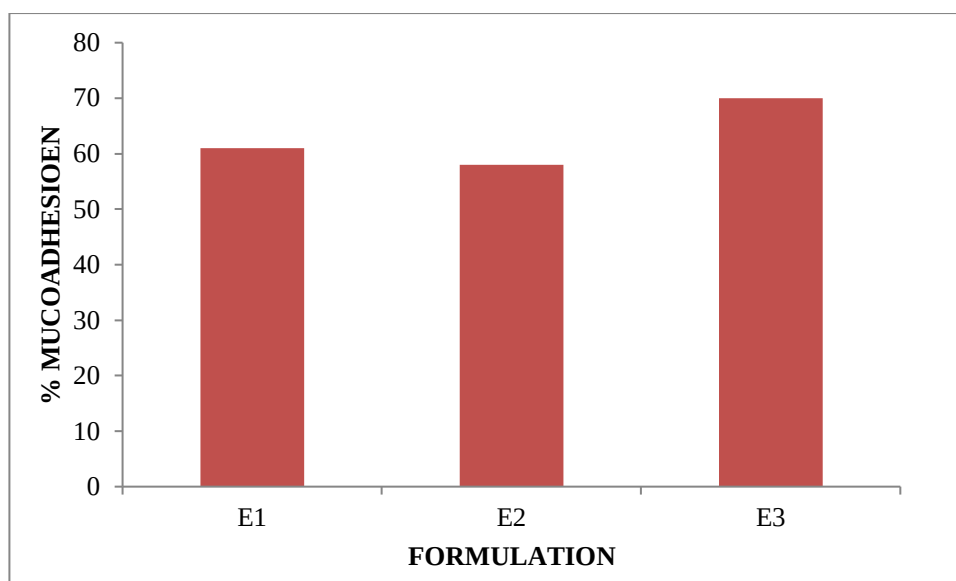


Figure 8.6: Percentage mucoadhesion of microspheres containing PLGA

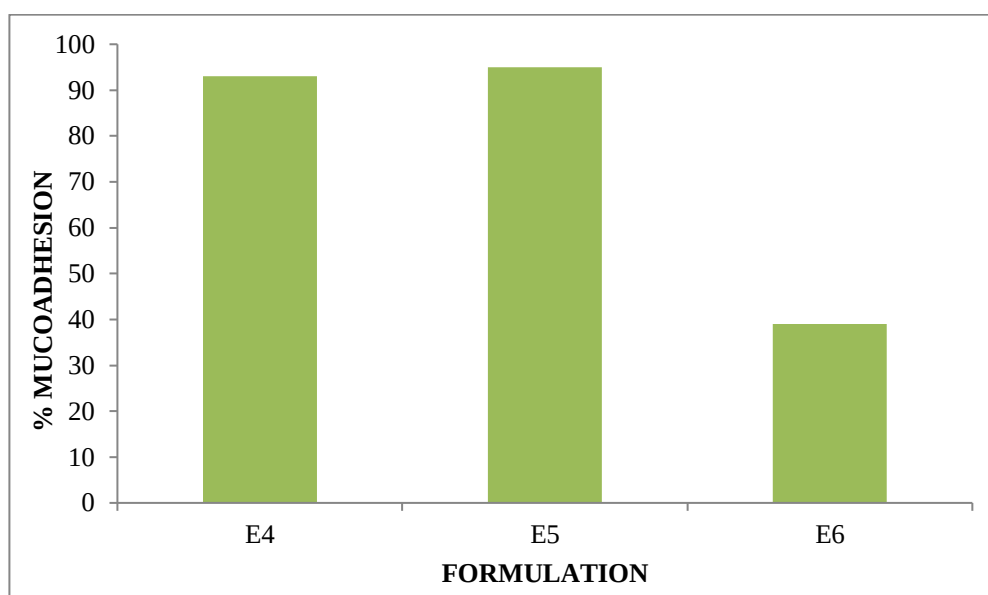


Figure 8.7 : Percentage mucoadhesion of microspheres containing Ethyl cellulose

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